REVIEW ARTICLE

MECHANISMS OF DISEASE

Cystic Fibrosis

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Advances in the care of patients with cystic fibrosis have improved survival, and as a result, patients with the disease now often live beyond the third decade.[1] In addition, developments in the understanding of the genetics and molecular mechanisms of cystic fibrosis have led to new targets for treatment and an increasingly hopeful outlook. This review summarizes the mechanisms underlying the disease, the sequelae stemming from the absence of a functioning cystic fibrosis transmembrane conductance regulator (CFTR), and the therapeutic strategies devised to correct these abnormalities. Progress in the supportive care of patients with the disease has been reviewed elsewhere.[2-4]

HISTORICAL BACKGROUND

In 1949, Lowe et al. postulated that cystic fibrosis must be caused by a defect in a single gene (and therefore a single protein) on the basis of the autosomal recessive pattern of inheritance of the disease.[5] The characterization of the molecular mechanism, therefore, included early attempts to identify the causative protein. High levels of salt in the sweat of patients with cystic fibrosis suggested an abnormality in fluid and electrolyte transport in the sweat gland, and Quinton established that sweat ducts in such patients are impermeable to chloride.[6] Studies of nasal epithelium and subsequent membrane patch-clamp analysis of epithelial cells from the airways of patients with cystic fibrosis provided conclusive evidence of a defect in chloride permeability of plasma membranes in the lung.[7-10] These findings, which were confirmed by several laboratories worldwide, led to the hypothesis that a defective chloride channel situated in the apical membranes of the lung surface or glandular epithelium accounts for respiratory failure and that this abnormality could explain the other clinical manifestations of cystic fibrosis.

Soon after the discovery of abnormal chloride transport in cystic fibrosis, Collins, Riordan, Tsui, and colleagues identified the gene that is responsible for the disease with the use of linkage-based techniques, independently of any prior knowledge of the structure of the cystic fibrosis protein.[11-13] With a measure of circumspection, they named the gene product CFTR, since the predicted protein sequence did not resemble other ion channels. This name posed a problem for those who thought that a chloride channel was primarily responsible for the clinical manifestations of cystic fibrosis.

STRUCTURE AND FUNCTION OF CFTR

The hypothesized structure of CFTR placed it squarely in the ATP-binding cassette (ABC), or traffic ATPase, gene family. As is characteristic of this gene family, CFTR protein contains 2 ATP-hydrolysis domains (also termed nucleotide-binding domains) and 12 membrane-spanning alpha helixes (Fig. 1). ABC proteins were known to function as mediators of organic solute transport and included, for example, the genes that encode multidrug resistance (e.g., *MDRs*, or P-glycoprotein genes), a gene that encodes

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chloroquine resistance in *Plasmodium falciparum*, and a number of prokaryotic and eukaryotic small nutrient and molecular transporters.[14] Because there was no reason to suspect that the cystic fibrosis chloride channel should require ATP hydrolysis, and since the ABC protein family was not characteristic of other ion transporters, the possibility was left open that CFTR might indirectly regulate cellular chloride permeability by other means, rather than act as a chloride channel itself.

These findings caused a measure of scientific drama that was ultimately resolved in two ways. First, Welsh and colleagues showed that the expression of *CFTR* in cells that lacked chloride channels led to the appearance of a new chloride permeability pathway.[15] In a formal sense, these studies still did not distinguish between the role of CFTR as a chloride channel and its role as a regulator of anion conductance that was unmasked by the expression of *CFTR* in cells lacking chloride channels. The subsequent observations that point mutations in the *CFTR* gene caused subtle alterations in ion-channel selectivity, single-channel conductance, channel gating, and other properties of CFTR supported a direct role of the CFTR protein as a chloride channel. Bear et al. made a second major contribution when they synthesized CFTR in cell-free systems, reconstituted the polytopic membrane protein in lipid bilayers,[16] and definitively established that purified CFTR could act (at least in part) as an ion channel regulated by cyclic AMP (cAMP). These experiments thus reconciled a scientific debate concerning the relationship between CFTR and epithelial ion transport.

Numerous laboratories have now established that CFTR conducts chloride across the cell membrane and is regulated by protein kinase A (PKA) in a cAMP-dependent fashion. The PKA sites within the protein serve as targets for phosphorylation. ATP hydrolysis is mediated by nucleotide-binding domains within the full-length ion channel. A number of other cellular functions have been ascribed to CFTR: it down-regulates transepithelial sodium transport, in particular the epithelial sodium channel[7,17]; it also regulates calcium-activated chloride channels and potassium channels and may serve important functions in exocytosis and the formation of molecular complexes in the plasma membrane.

In the apical plasma membrane, CFTR is part of a multiprotein assembly. The final three amino acids (threonine, arginine, and leucine) anchor the protein to PDZ-type receptors (PDZ domains occur in intracellular signaling proteins and other proteins associated with the plasma membrane), in close proximity to a number of membrane receptors, ion channels, and the cytoskeleton.[18] Thus, it appears that the role of CFTR in epithelial cells may extend well beyond chloride permeability. In human cystic fibrosis, and in mice with targeted deletions of the *Cftr* gene,[19] the absence of CFTR influences the expression of several other gene products, including proteins important in inflammatory responses, maturational processing, ion transport, and cell signaling. These other proteins are potential modifiers of the cystic fibrosis phenotype and may help explain the substantial differences in clinical severity among patients with the same mutations in *CFTR*.

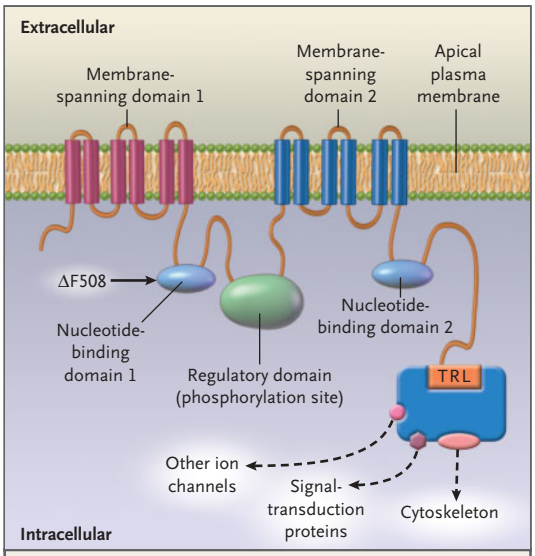

Figure 1. Hypothesized Structure of CFTR.

The protein contains 1480 amino acids and a number of discrete globular and transmembrane domains. Activation of CFTR relies on phosphorylation, particularly through protein kinase A but probably involving other kinases as well. Channel activity is governed by the two nucleotide-binding domains, which regulate channel gating. The carboxyl terminal (consisting of threonine, arginine, and leucine [TRL]) of CFTR is anchored through a PDZ-type–binding interaction with the cytoskeleton and is kept in close approximation (dashed arrows) to a number of important proteins. These associated proteins influence CFTR functions, including conductance, regulation of other channels, signal transduction, and localization at the apical plasma membrane. Each membrane-spanning domain contains six membrane-spanning alpha helixes, portions of which form a chloride-conductance pore. The regulatory domain is a site of protein kinase A phosphorylation. The common ΔF508 mutation occurs on the surface of nucleotide-binding domain 1.

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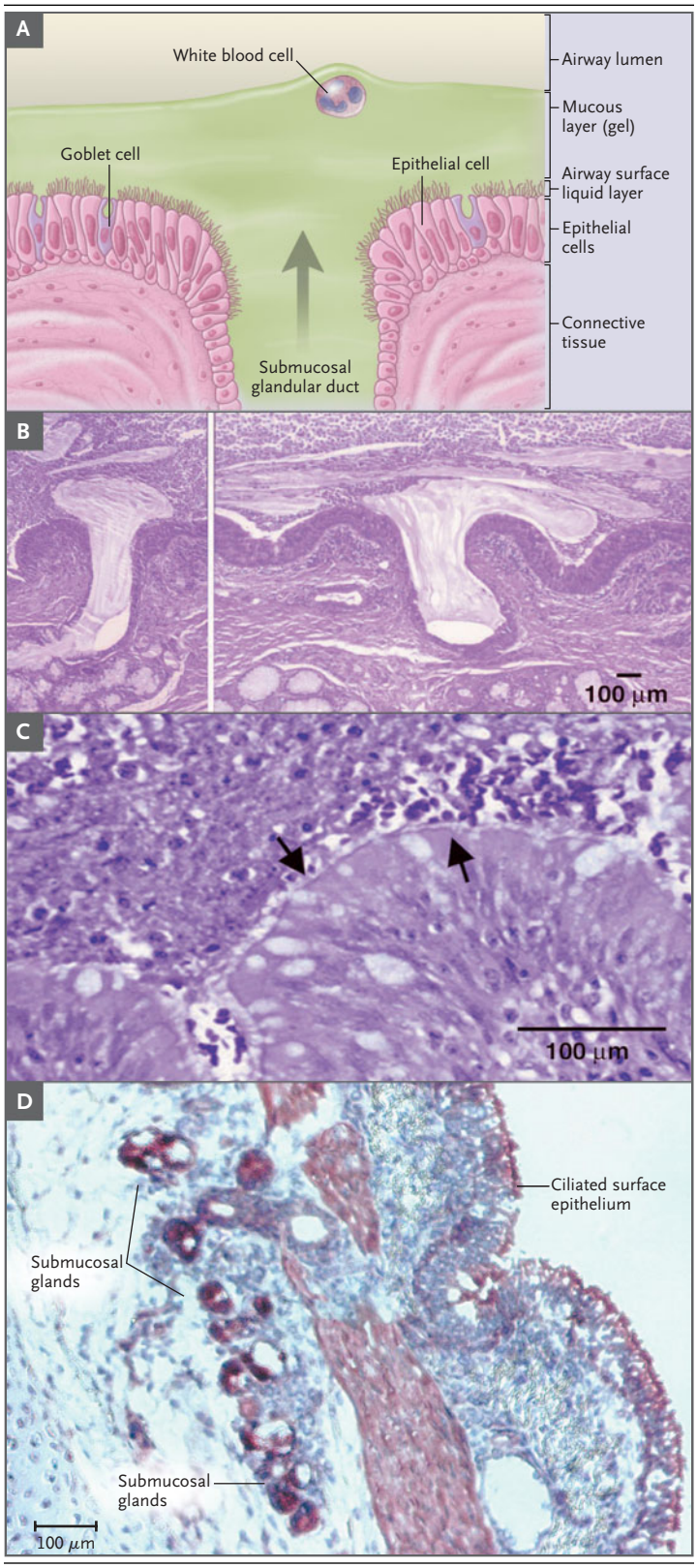

PATHOBIOLOGIC FEATURES OF ORGAN SYSTEM DISEASE

Mucosal obstruction of exocrine glands is the chief contributor to morbidity and mortality in patients with cystic fibrosis (Fig. 2). In the human lung, thick, tenacious secretions obstruct the distal airways and submucosal glands, which express CFTR.[20] Ductular dilatation of these glands (associated with blockage by mucus) and the plastering of airway surfaces by thick, viscous, neutrophil-dominated mucopurulent debris are among the pathological hallmarks of the disease. Glandular hyperplasia in submucosal regions is prominent and surrounded by peribronchiolar inflammation and scar tissue. Submucosal gland ducts are inconspicuous in the lungs of patients without cystic fibrosis, whereas luminal dilatation by mucus is one of the earliest discernible changes in the lungs of newborns and children with cystic fibrosis. Pathogens such as *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Staphylococcus aureus*, and *Haemophilus influenzae* become well established within firmly fixed airway secretions in patients with cystic fibrosis and are not effectively eradicated. *P. aeruginosa*, for example, specifically adapts to the pulmonary microenvironment in patients with cystic fibrosis through the formation of macrocolonies (or biofilms) and the production of a capsular polysaccharide (an alginate product) that inhibits penetration by anti-

Figure 2. Extrusion of Mucus Secretion onto the Epithelial Surface of Airways in Cystic Fibrosis.

Panel A shows a schematic of the surface epithelium and supporting glandular structure of the human airway. In Panel B, the submucosal glands of a patient with cystic fibrosis are filled with mucus, and mucopurulent debris overlies the airway surfaces, essentially burying the epithelium. Panel C is a higher-magnification view of a mucus plug tightly adhering to the airway surface, with arrows indicating the interface between infected and inflamed secretions and the underlying epithelium to which the secretions adhere. (Both Panels B and C were stained with hematoxylin and eosin, with the colors modified to highlight structures.) Infected secretions obstruct airways and, over time, dramatically disrupt the normal architecture of the lung. In Panel D, CFTR is expressed in surface epithelium and serous cells at the base of submucosal glands in a porcine lung sample, as shown by the dark staining, signifying binding by CFTR antibodies to epithelial structures (aminoethylcarbazole detection of horseradish peroxidase with hematoxylin counterstain).

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microbial agents and confers the mucoid phenotype.[21,22]

Pulmonary inflammation is another major cause of the decline in respiratory function in patients with cystic fibrosis and may precede the onset of chronic infection. Elevated levels of interleukin-8, interleukin-6, tumor necrosis factor α, and leukotriene B_4, along with reduced levels of antiinflammatory cytokines and proteases, have been found in the airways of patients with cystic fibrosis. Toll-like receptors, which recognize a variety of inflammatory mediators (including neutrophil elastase, bacterial lipopolysaccharide, and other microbial products), mediate inflammatory effects in part by activating the transcription factor nuclear factor-κB, which governs a molecular pathway that induces the production of inflammatory proteins and cytokines.[23-26] Recently, an elevated ratio of arachidonic acid to docosahexaenoic acid was found in mucosal scrapings from patients with cystic fibrosis, as compared with scrapings from normal persons and from patients with inflammatory bowel disease; thus, the altered ratio cannot be explained by systemic inflammation alone.[27] These and other inflammatory mediators such as mannose-binding protein and alpha$_1$-antitrypsin influence the progression of lung disease.[27-32]

The absence of normal CFTR activity in patients with cystic fibrosis also engenders obstruction in other organs. Mucinous impaction and thick concretions within pancreatic ducts lead to chronic fibrosis, fatty replacement of the gland, or both and to formes frustes of the disease in a large subgroup of patients with a previous diagnosis of idiopathic or alcoholic pancreatitis.[33,34] Nearly 10 percent of patients with the disease are born with intestinal obstruction (meconium ileus), a fatal condition if left untreated. Men with cystic fibrosis are frequently infertile because of glandular obstruction of the vas deferens in utero, which causes involution of the wolffian duct, vas deferens, and associated structures. *CFTR* mutations can also cause infertility in otherwise normal men as a result of the cystic fibrosis variant, called congenital bilateral absence of the vas deferens.[35] Similar obstruction of bile canaliculi frequently causes hepatic damage and, in some patients, overt cirrhosis.

MECHANISM UNDERLYING THE SWEAT-GLAND ABNORMALITY

There is widespread agreement that defects in ion transport, salt homeostasis, or both are intimately linked to organ damage in cystic fibrosis. The precise molecular basis for this connection, however, is unknown. Conversely, sweat glands in patients with cystic fibrosis, which usually do not become obstructed or show major pathologic abnormalities, have pronounced abnormalities in sodium chloride homeostasis that are well understood. In human sweat glands, primary secretion elaborated in the glandular coil is modified as it traverses the sweat duct, before emerging on the surface of the skin. Under normal conditions, sodium (followed by chloride counter-ion) is avidly reabsorbed from the ductular lumen, primarily through apical sodium channels and CFTR (Fig. 3). In patients with cystic fibrosis, the absence of functioning CFTR restricts reabsorption of chloride, thereby limiting the amount of salt that can be reclaimed. Because there is no other pathway for effective chloride reabsorption in the duct, sodium is also poorly absorbed, and sweat emerging on the skin surface contains a high level of salt. By the same token, in cystic fibrosis, the transepithelial potential difference across the sweat duct (the lumen-negative transepithelial voltage) is two to three times the normal value. The increased lumen-negative surface charge is caused by an inability to reabsorb chloride despite the continued existence of pathways for sodium uptake.

BIOELECTRIC MEASUREMENTS IN THE LUNG

Abnormalities of salt and fluid metabolism have also been examined in vivo in human and murine lungs. Knowles et al. popularized a method for measuring the transepithelial potential difference across nasal and lower airways in human subjects with the use of mucosal superperfusion.[36] This in vivo bioelectric measurement reveals virtually pathognomonic defects and can be used as a diagnostic test for cystic fibrosis. For example, under basal conditions, the transepithelial potential difference due to sodium uptake is two to three times as great (lumen negative) in patients with cystic fibrosis as in persons without the disease.

A traditional way of explaining airway mucosal obstruction (the low-volume model) argues persuasively that pulmonary surface epithelium in cystic fibrosis behaves in a fashion essentially opposite that of the sweat duct (Fig. 4). In this model, the absence of CFTR leads to overactivity of sodium absorption through the epithelial sodium channel (Fig. 4D). The mucosal surface has a more nega-

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tive charge because overall chloride permeability is less than that of sodium. However, because Cl^- permeability (through non-CFTR Cl^- uptake pathways) is thought to be available in the lung, the result is a relative increase in the absorption of sodium, chloride, and fluid. This increase causes the dehydration of airway surfaces and defective mucociliary transport. Evidence in favor of the low-volume model derives from compelling in vitro experiments and a mouse model characterized by excessive functioning of the epithelial sodium channel with a lung phenotype similar to that in human cystic fibrosis.[37]

An alternative hypothesis (the high-salt model) contends that the airway epithelial surface in patients with cystic fibrosis behaves similarly to sweat ducts, in that CFTR is the major pathway for counter-ion absorption (Fig. 4C). The model was advanced in part to help unify bioelectric findings in the disease. When CFTR is absent, chloride cannot be reabsorbed — a situation that again resembles that of sweat-gland epithelium in cystic fibrosis. Consequently, the transepithelial potential difference becomes hyperpolarized: the inability of Cl^- ions to follow Na^+ results in a more negatively charged mucosal surface. One interpretation of this hypothesis is that an elevated level of sodium chloride in the airway-surface liquid would inactivate endogenous antimicrobial peptides and thereby predispose patients with the disease to bacterial infections with pathogens such as *P. aeruginosa*.[38]

When normal airway mucosa is superperfused with a solution designed to open CFTR or induce luminal chloride secretion, additional changes in the potential difference occur. These are compatible with chloride secretion (i.e., an increase in the lumen-negative potential difference) in normal persons but not in those with cystic fibrosis. Therefore, regardless of which model of sodium chloride reabsorption (low-volume or high-salt) better accounts for certain aspects of pulmonary insufficiency in cystic fibrosis, there is a strong consensus that in patients with the disease, the airways lack the normal ability to secrete chloride through CFTR.

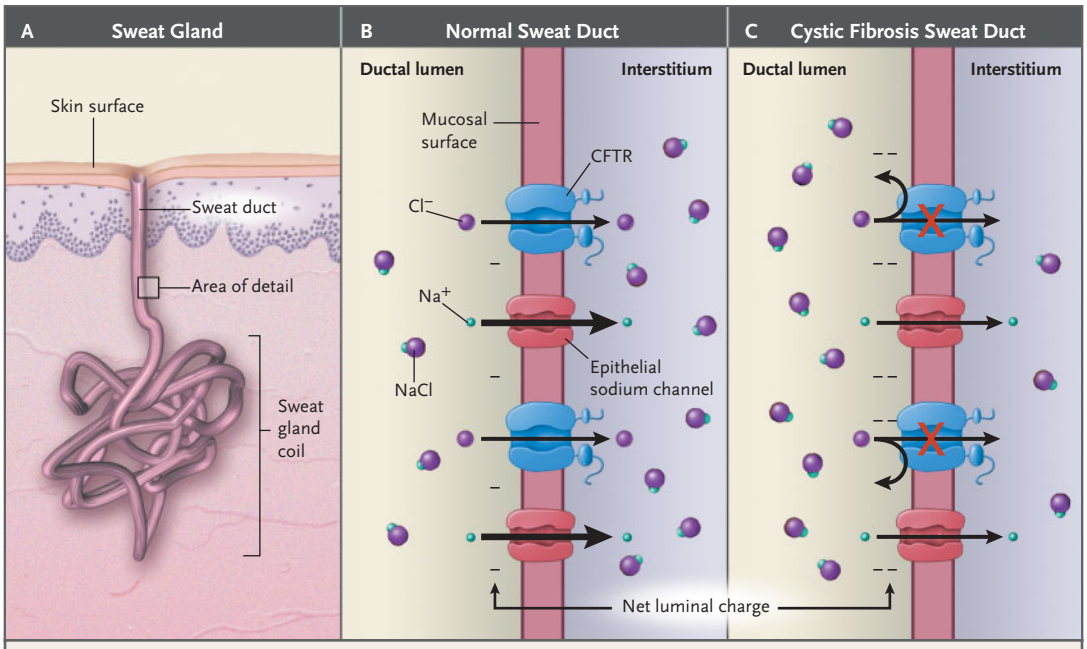

Figure 3. Mechanism Underlying Elevated Sodium Chloride Levels in the Sweat of Patients with Cystic Fibrosis.

Sweat ducts (Panel A) in patients with cystic fibrosis differ from those in people without the disease in the ability to reabsorb chloride before the emergence of sweat on the surface of the skin. A major pathway for Cl^- absorption is through CFTR, situated within luminal plasma membranes of cells lining the duct (i.e., on the apical, or mucosal, cell surface) (Panel B). Diminished chloride reabsorption in the setting of continued sodium uptake leads to an elevated transepithelial potential difference across the wall of the sweat duct, and the lumen becomes more negatively charged because of a failure to reabsorb chloride (Panel C). The result is that total sodium chloride flux is markedly decreased, leading to increased salt content. The thickness of the arrows corresponds to the degree of movement of ions.

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Compounds such as adenosine, genistein, and phosphodiesterase inhibitors augment residual chloride secretion by mutant CFTR channels and form the basis of clinical experiments to restore function in patients with cystic fibrosis. A unified model of organ-system pathophysiology emanates naturally from a universally acknowledged defect in chloride secretion in the disease.

GLANDULAR SECRETION AND SMALL-AIRWAY PHYSIOLOGY

Abnormalities in sodium chloride absorption or antimicrobial peptide function that are invoked to explain specific pulmonary defects in cystic fibrosis are unlikely to contribute to disease-related damage in other organs. In the pancreas, there is no evidence of either sodium hyperabsorption or overactivity of the epithelial sodium channel, and abnormalities of antimicrobial peptides are not believed to contribute to exocrine pancreatic insufficiency in cystic fibrosis. In the intestine, neither immune alterations nor increased susceptibility to clinically apparent infection has been implicated. In tissues in which sodium transport has been examined (e.g., in samples of rectal mucosa and proximal ileum from patients with cystic fibrosis and in the obstructed intestine in the mouse model of the disease[39]), the activity of sodium-uptake pathways is variable and in some cases is decreased. By contrast, virtually every tissue that is affected by the disease is defective in chloride and fluid secretion. It is generally believed that in pancreatic ducts, the failure of the release of anions (including those of chloride and bicarbonate) due to CFTR dysfunction and deficient fluid secretion impairs flushing

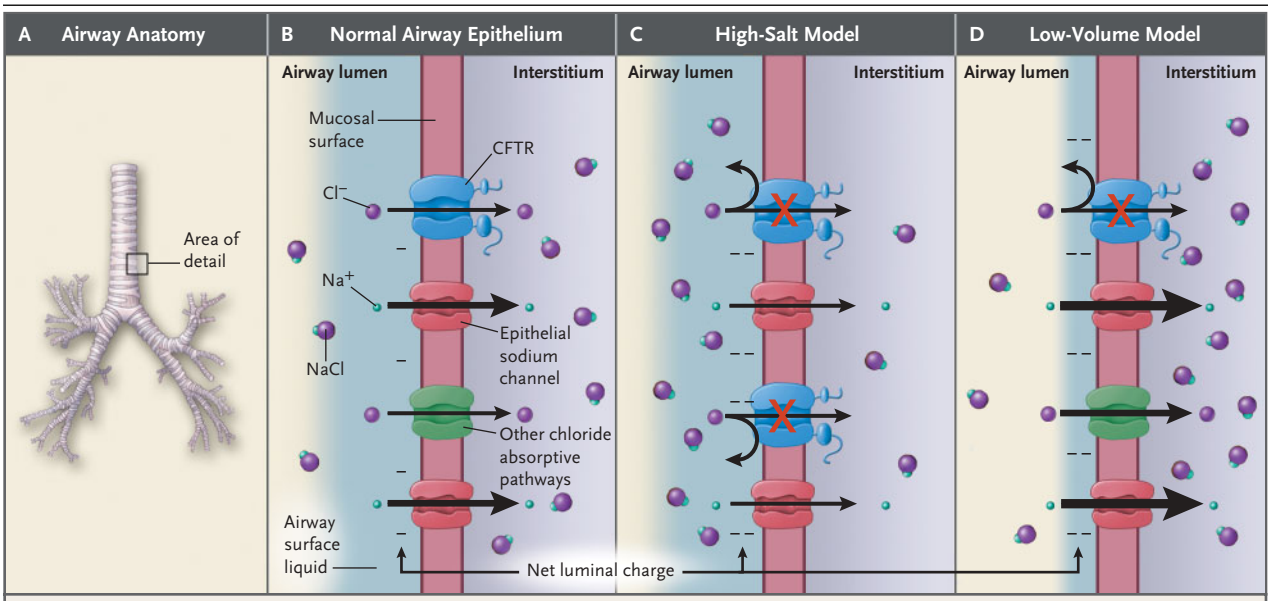

Figure 4. Models Explaining the Transepithelial Potential Difference across the Airway Epithelium in Cystic Fibrosis.

Under normal conditions, sodium chloride is absorbed from the airways (Panel A). The first step of this process uses sodium and chloride absorptive pathways present in the luminal (apical) membranes of airway-surface epithelial cells, designated as the mucosal surface (Panel B). In a bioelectric assay (a measurement of the transepithelial potential difference), the lumen is negative in part because of the relative impermeability of chloride as compared with sodium. The relative contribution of CFTR and other non-CFTR Cl^- permeability pathways is not known. The transepithelial potential difference is markedly hyperpolarized (i.e., the lumen is much more negatively charged) in cystic fibrosis. Two models have been proposed to explain this difference. In the high-salt model (Panel C), the situation resembles that of the sweat duct, in which the absence of CFTR leads to the inability to reabsorb chloride ion from airway-surface liquid. Because of the continued activity of sodium ion reabsorption, which is dependent on epithelial sodium channels, the airway surface negativity is increased (lumen-negative). According to this model, although a large charge separation is observed (with positively charged sodium ions moving across the airway wall and negatively charged chloride ions remaining behind), the net sodium chloride reabsorption decreases because of the inability to reabsorb chloride counter-ions. In the low-volume model (Panel D), both sodium and chloride are hyperabsorbed. The airways of patients with cystic fibrosis are slightly less permeable to chloride ions than they are to sodium ions, a process that leads to an increased transepithelial potential difference. This model predicts a depletion in the volume of airway-surface liquid (shown in blue). The thickness of the arrows corresponds to the degree of movement of ions.

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of the exocrine glands, causing an accumulation of mucus, obstruction, and consequent end-organ damage. A pronounced abnormality in chloride secretion by intestinal tissues has been well documented in cystic fibrosis and provides a compelling explanation for end-organ dysfunction. Similar arguments can be made regarding the hyperviscous secretions in the liver, vas deferens, and other glands in cystic fibrosis.

New tools have emerged for testing the hypothesis that defective chloride secretion in airways is also a fundamental cause of pulmonary failure in cystic fibrosis. The finding of dilated glandular structures (which occurs very early in cystic fibrosis–associated lung disease) implicates abnormal clearance of glandular mucus from the ducts (a process reminiscent of the situation in the pancreas, liver, and intestine in cystic fibrosis). The levels of CFTR in airway serous glandular cells are among the highest of any cell type in the body (Fig. 2D).[20] In the small airways, where surface epithelial cells contribute to the production of mucus, an analogous defect in chloride and fluid secretion could result in decreased surface liquid in airways and similar mucosal obstruction.

These considerations have led to the idea that lung disease in cystic fibrosis is primarily attributable to a failure of CFTR-dependent flushing of mucous secretion from the glands, small airways, or both. Joo et al. have directly measured glandular secretions in lung tissues in cystic fibrosis.[40,41] Their studies suggest fundamental abnormalities in the mobilization and clearance of mucus from submucosal glands (a process that is also crucial in the distal small airways). Similar conclusions have been reached by Verkman and colleagues.[42,43] Attempts to activate CFTR-independent chloride conductance with secretagogues such as uridine 5′-triphosphate or related compounds (which may restore or enhance mucociliary clearance) have yielded encouraging results in clinical trials.[44,45] Models based on defects in glandular and airway secretion as primary contributors to respiratory decline therefore continue to represent a vital and unifying means for understanding pulmonary destruction due to defects in CFTR.

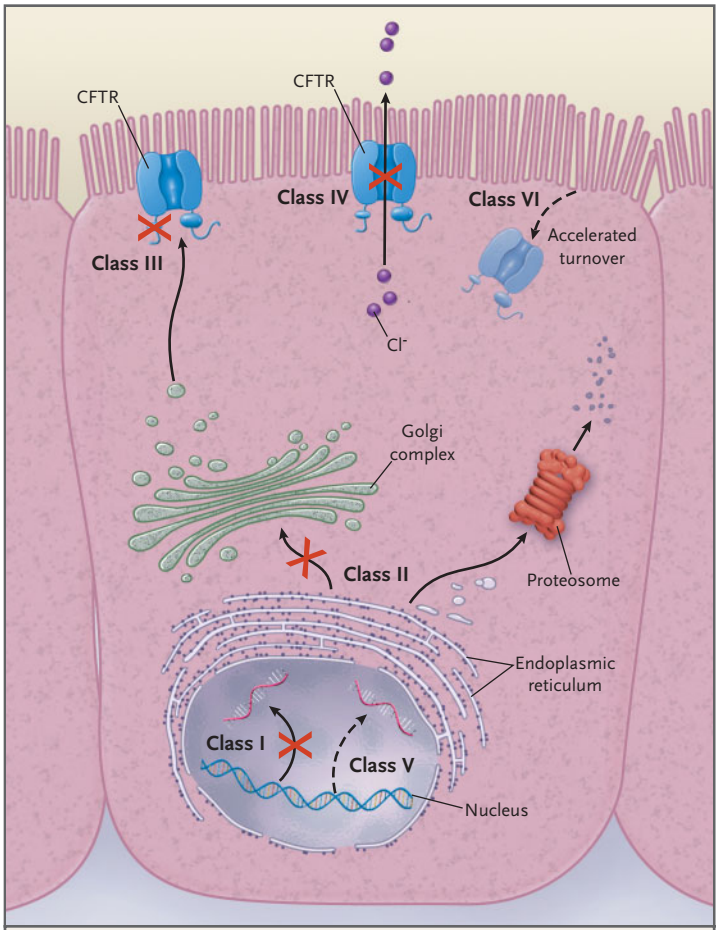

Figure 5. Categories of *CFTR* Mutations.

Classes of defects in the *CFTR* gene include the absence of synthesis (class I); defective protein maturation and premature degradation (class II); disordered regulation, such as diminished ATP binding and hydrolysis (class III); defective chloride conductance or channel gating (class IV); a reduced number of CFTR transcripts due to a promoter or splicing abnormality (class V); and accelerated turnover from the cell surface (class VI).[46]

MUTATIONS IN *CFTR* AND THEIR CONSEQUENCES

The *CFTR* gene encompasses approximately 180,000 base pairs on the long arm of chromosome 7. The protein contains 1480 amino acids (Fig. 1). More than 1000 disease-associated mutations have been described in the coding sequence, messenger RNA splice signals, and other regions. These mutations can be classified on the basis of the mechanism by which they are believed to cause disease (Fig. 5). The most common mutation, which is termed ΔF508 and is present in approximately 70 percent of defective *CFTR* alleles and in 90 percent of patients with cystic fibrosis in the United States, is categorized as a class II defect. CFTR with the ΔF508 mutation lacks a phenylalanine (F) residue at position 508. The defective protein retains substantial chloride-channel function in cell-free lipid mem-

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branes. When synthesized by the normal cellular machinery, however, the protein is rapidly recognized as misfolded and is degraded shortly after synthesis, before it can reach its crucial site of action at the cell surface. Like ΔF508, several other clinically important mutations — such as N1303K, G85E, and G91R — lead to misfolded CFTR protein that is prematurely degraded.

About 5 to 10 percent of *CFTR* mutations are due to premature truncation or nonsense alleles (designated by "X," such as G542X, a class I mutation). As a result of a genetic founder effect, prematurely truncated CFTR is particularly prevalent among persons of Ashkenazi Jewish descent. Other *CFTR* mutations encode properly processed, full-length CFTR protein that lacks normal ion-channel activity. For example, the G551D mutation (class III) is believed to possess little or no chloride-channel function in vivo because of abnormal function of a nucleotide-binding domain, resulting in disordered regulation. The A455E mutation (class IV) exhibits only partial CFTR ion-channel activity, a feature that probably explains a less severe pulmonary phenotype.[47] Other mutation classes include reduced numbers of CFTR transcripts (class V) and defective CFTR stability at the cell surface (class VI).[48-50]

INTERVENTIONS TAILORED TO SPECIFIC CFTR DEFECTS

Insight into the cellular consequences of defective CFTR suggests a role for tailored therapies, a predominant theme in clinical research on cystic fibrosis. For example, robotic drug screening of more than a million random compounds has led to the discovery of compounds that correct the ΔF508 abnormality by restoring the mutant protein to its normal position at the cell surface (thereby partially restoring chloride-channel function).[51,52] The recently elucidated crystal structure of nucleotide-binding domain 1 localizes the crucial phenylalanine 508 residue to a loop on the external surface of the domain. Because of this structure, drug-screening laboratories can test the specificity of ΔF508-correcting compounds by co-crystallization with the CFTR protein.[53] Curcumin, a nontoxic compound and the major constituent of the spice tumeric, has been shown to correct ΔF508 processing in a number of in vitro model systems and prolong life in mice that are homozygous for the ΔF508 mutation.[54,55] New compounds that correct this fundamental processing abnormality in CFTR should undergo clinical testing in the near future.

A number of agents have also been shown to suppress premature stop codons in *CFTR* (class I) mutations, including the surprising finding that ribosomally active drugs such as gentamicin may be capable of correcting premature stop codons in human subjects.[56,57] This same concept has been applied to other diseases caused by premature stop codons, including Duchenne's muscular dystrophy, Hurler's syndrome, and disorders resulting from mutations in the p53 tumor-suppressor gene.[58-60] High-throughput screening programs specifically designed to identify drugs that activate residual CFTR activity (class III and IV mutations) have also been successful.[61-64]

SUMMARY

Important advances have improved our understanding of the role of the CFTR protein in the progression of suppurative pulmonary failure in cystic fibrosis. These discoveries are ushering in a new era of translational research that incorporates specific therapeutic targets and new cellular pathways. Progress in research on cystic fibrosis will continue to rely on an improved understanding of CFTR function and its relationship to mucociliary clearance, airway secretion, and other ion channels. Clinical advances directed at the correction of CFTR function predict an optimistic future for patients with cystic fibrosis and their families.

Dr. Sorscher is coinventor of a method of making and using human papillomavirus vectors for transduction of host cells and a coinventor of a method of activating chloride secretion. Patents on these methods are unlicensed and held by the University of Alabama.

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