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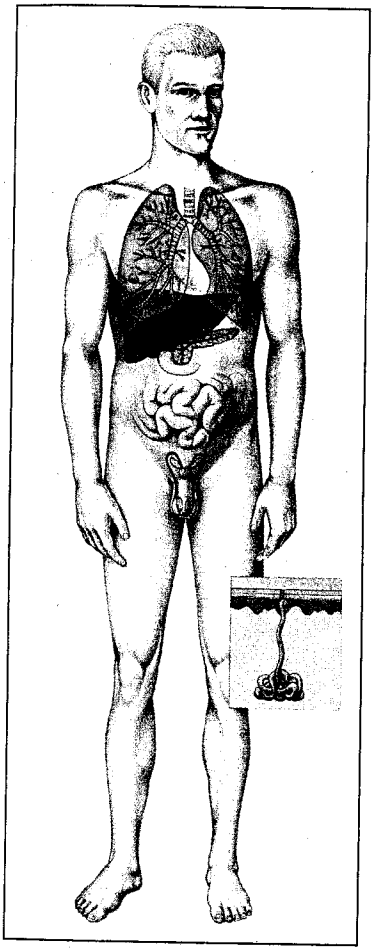


Cystic Fibrosis by Michael J. Welsh and Alan E. Smith

Before reading this article, review pages 418-419 and pages 466-467 in Lehninger Principles of Biochemistry, 3e.

Cystic fibrosis (CF) is the most common fatal genetic disease among Caucasians of European descent. Five percent of this population carries the single recessive gene that is responsible for CF, and about 30,000 people in the United States have CF. The disease affects the epithelial cells of exocrine glands that produce sweat, saliva, gastric and pancreatic secretions, and semen, and creates problems in all of the involved organs. About a decade ago, the gene responsible for CF was discovered and found to encode a plasma membrane protein, a regulated ion channel for chloride ion, which was named the cystic fibrosis transmembrane conductance regulator (CFTR; see *Lehninger Principles of Biochemistry, 3e*, Box 12-1, pp. 418-419). CFTR has turned out to be of even greater medical importance than originally thought. It is involved not only in the pathology of cystic fibrosis, but also in the pathology of the secretory diarrhea caused by bacterial endotoxins, which is the second largest cause of infant death in the developing world, killing several million young children each year. Intestinal water retention is defective in these children because chloride ion leaves the cell through CFTR chloride channels that are continuously open due to the effects of the bacterial toxin (see *Lehninger Principles of Biochemistry, 3e*, pp. 466-467). Water follows the chloride ion by osmosis, producing watery diarrhea and life-threatening dehydration in the patient.

The tissues most seriously affected in cystic fibrosis, the lungs, are relatively accessible; medications can be delivered directly to the affected cells by aerosol inhalation. One approach to treating cystic fibrosis is gene therapy—inserting an intact copy of the CFTR gene into a suitable vector (an attenuated respiratory virus, for example), then delivering the virus to the lungs in an aerosol. If enough epithelial cells are infected with the now-harmless virus carrying a good gene, enough CFTR function may be gained to overcome the worst of the pathology of cystic fibrosis. Clinical trials now underway to test this possibility are being carefully watched as one of the first tests of gene therapy in humans.

FURTHER READING

Akabas, M.H. (2000) Cystic fibrosis transmembrane conductance regulator: Structure and function of an epithelial chloride channel. *J. Biol. Chem.* 275, 3729-3732.

Pilewski, J.M. & Frizzell, R.A. (1999) Role of CFTR in airway disease. *Physiol. Rev.* 79 Suppl. 1, S215-S255. (Note: this supplement is a collection of 10 excellent reviews on various aspects of CFTR structure and function.)

Sheppard, D.N. & Welsh, M.J. (1999) Structure and function of the CFTR chloride channel. *Physiol. Rev.* 79 Suppl. 1, S23-S45.

QUESTIONS

1. The CFTR protein was named before its function was fully understood. What evidence clinches the argument that it is a Cl^- channel?
2. What are the roles of cAMP and protein kinases in the function of this protein?
3. The most common mutation leading to CF is the deletion of the trinucleotide sequence that normally encodes Phe_{508}. Why is Cl^- transport defective with these mutant proteins? What are the defects that result from mutations at other position?
4. The severity of the defects varies from patient to patient. Can you give a genetic or biochemical explanation for this?
5. Why is the adenovirus a particularly suitable vector for carrying a good CFTR gene into the lung epithelium? Do you see any dangers in using adenovirus as a vector? Why is gene therapy not likely to lead to a permanent cure of the defects in airways?

Cystic Fibrosis

The genetic defects underlying this lethal disease have now been shown to eliminate or hobble a critical channel through which a constituent of salt enters and leaves cells

by Michael J. Welsh and Alan E. Smith

Woe to that child which when kissed on the forehead tastes salty. He is bewitched and soon must die. This adage, from northern European folklore, is an early reference to the common genetic disease recognized today as cystic fibrosis. As the saying implies, the disorder once routinely killed children in infancy and is often identifiable by excessive salt in sweat. A salty brow is one of the more benign manifestations. The inherited genetic abnormality can also destroy the lungs and cause serious impairment of the pancreas, intestines and liver. Advances in therapy over the past few decades have brightened the outlook for afflicted children, enabling more than half of them to survive into their late twenties or beyond. But none of the approved treatments can yet correct the biochemical abnormality at the root of the condition, and none can remove the specter of an early death.

Hoping to do better, investigators began trying in the early 1980s to identify the specific genetic derangement that gives rise to cystic fibrosis. After almost a decade of struggle, they isolated the affected gene and pinpointed the mutation that most often leads to the disease. At the time, they could only guess at the gene's normal function—that is, at the role played by the protein produced from the healthy DNA. Since then, in an exciting series of discoveries, researchers have learned that the protein serves as a channel through which chloride, one component of salt, enters and leaves cells. They also have explained how damage to the gene blocks chloride transport, and they are exploring how the loss of chloride movement brings on the overt signs of cystic fibrosis. As was hoped, such findings are suggesting new ideas for therapy, some of which may one day cure the disorder.

The molecular advances that have led to this promising moment in medical history could not have been achieved without the pioneering efforts of physicians, many of whom gleaned their initial understanding of cystic fibrosis at the bedside. Indeed, for decades, clinical research yielded more information about the nature of the disease than did biochemical investigation.

One of the first major contributions came in 1938 from Dorothy H. Andersen of Columbia University. After performing autopsies on infants and children and reviewing the youngsters' case histories, Andersen provided the first comprehensive description of the symptoms of cystic fibrosis and of the changes produced in organs. Those changes, she noted, almost always included destruction of the pancreas (even in infants) and, often, infection of and damage to the lung airways. Andersen also gave the disease its name, calling it "cystic fibrosis of the pancreas," on the basis of microscopic features she observed in pancreatic tissue.

By the late 1940s physicians had further realized that ductal systems and other passageways in the organs affected by cystic fibrosis generally become clogged with unusually thick secretions. In the pancreas, for instance, ducts that deliver digestive enzymes to the intestines almost always become occluded, impairing the body's ability to break down food and extract nutrients from it.

In the lung it is the bronchial tubes and bronchioles that become obstructed. Those passages are usually bathed by a thin layer of mucus that traps inhaled particles and carries them to the throat for removal. But in patients with cystic fibrosis, the mucus is excessively thick and resistant to removal. This change by itself can narrow air passages and impair breathing. Moreover, when bacteria remain in the air passages, they can establish infections readily. These infections, which tend to recur, harm lung tissue by recruiting immune cells that secrete injurious chemicals and enzymes. As time goes by, chronic infection progressively destroys the bronchial passages and, together with the plugging of airways, ultimately leads to respiratory failure.

By 1946 studies of patients had also revealed something about the genetics of cystic fibrosis. After examining the pattern of disease inheritance in families, researchers deduced that cystic fibrosis was a recessive condition, probably caused by mutation of a single gene. If an infant inherited a damaged copy of the gene from both parents and therefore made no normal molecules of the protein specified by the gene, the child became ill; however, receipt of one good copy and one damaged copy did not produce disease.

Cystic fibrosis is now known to be among the most common genetic diseases and to strike mostly whites. About 5 percent of white Americans are asymptomatic carriers, harboring a single mutant version of the gene in their cells. One child in approximately 2,500 of European descent carries two defective copies and has the disease. In the U.S. such numbers translate into about 1,000 new cases a year and a total of some 30,000 people who live with the disorder today.

Help from a Heat Wave

Roughly seven years after the inheritance pattern was delineated, New York City baked in a heat wave. Hospitals saw a disproportionate number of children with cystic fibrosis, who apparently became dehydrated more readily than other youngsters. Paul di Sant'Agnese and his colleagues at Columbia University then found that boys and girls with cystic fibrosis lose an excessive amount of salt in sweat. The reason for the increased saltiness would not be discerned for many years, but the observation had great clinical value. It resulted in development of a test that remains the cornerstone of diagnosis: measurement of the chloride content in perspiration.

Over the years, such clinical work has led to earlier, more accurate diagnosis and better treatments. For example, pancreatic failure is rarely life-threaten-

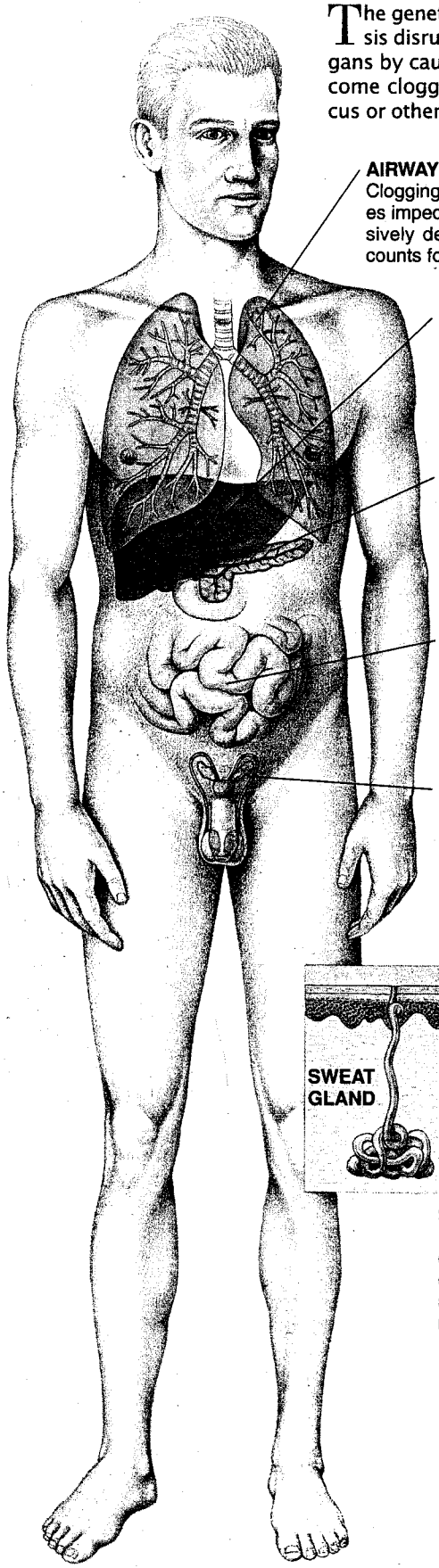
GENTLE POUNDING ON THE CHEST, or chest percussion, has long been a standard treatment for cystic fibrosis. The procedure aims to clear mucus from clogged airways in the lungs. Investigators hope that growing understanding of the molecular basis of the disease will lead to drug therapies that prevent airway obstruction in the first place. The child here is being tapped by her mother. The white unit on her arm delivers intravenous antibiotics to combat infection of the lung.

ing today because patients can replace their missing digestive enzymes with capsules taken when they eat. Now that the digestive problems can generally be controlled, the lung impairment accounts for more than 90 percent of the disability and death in patients with cystic fibrosis. Treatment options for the lung disease have expanded as well. Current therapy does include old standbys called postural drainage and chest percussion. Patients lie so that their head is tilted downward; someone then pounds gently and rapidly on their back or chest—as if hitting the bottom of a ketchup bottle—to try to clear mucus from the airways. But patients also benefit from a range of antibiotics that help to control the repeated infections (although usually without eliminating them). And about two years ago another treatment became available: inhalation of a drug called DNase. This compound aims to break up mucus by digesting long, sticky strands of DNA released from dying cells.

Research into the biochemical underpinnings of cystic fibrosis progressed more slowly than did the clinical work, but the pace intensified in the first half of the 1980s. During that time, scientists realized that malfunction of epithelial tissue was at fault in every organ impaired by cystic fibrosis. (An epithelium is a sheet of cells that forms a barrier between different compartments of the body; such sheets, which often secrete mucus, line the intestines and many ducts.) In particular, two avenues of investigation revealed that the epithelia of patients with cystic fibrosis were relatively impermeable to chloride. This discovery implied that some chloride-transporting channel in epithelial tissue was malfunctioning.

Organs Affected by Cystic Fibrosis

The genetic defect underlying cystic fibrosis disrupts the functioning of several organs by causing ducts or other tubes to become clogged, usually by thick, sticky mucus or other secretions.



AIRWAYS
Clogging and infection of bronchial passages impede breathing. The infections progressively destroy the lungs. Lung disease accounts for most deaths from cystic fibrosis.

LIVER
Plugging of small bile ducts impedes digestion and disrupts liver function in perhaps 5 percent of patients.

PANCREAS
Occlusion of ducts prevents the pancreas from delivering critical digestive enzymes to the bowel in 85 percent of patients. Diabetes can result as well.

SMALL INTESTINE
Obstruction of the gut by thick stool necessitates surgery in about 10 percent of newborns.

REPRODUCTIVE TRACT
Absence of fine ducts, such as the vas deferens, renders 95 percent of males infertile. Occasionally, women are made infertile by a dense plug of mucus that blocks sperm from entering the uterus.

SKIN
Malfunctioning of sweat glands causes perspiration to contain excessive salt (sodium chloride). Measurement of chloride in sweat is a mainstay of diagnosis.

ROBERTO OSTI

In one set of those investigations, Paul M. Quinton of the University of California at Riverside found that the epithelia lining the ducts of sweat glands failed to take up chloride efficiently from the cavity, or lumen, of the glands. This finding finally explained why people with cystic fibrosis have unusually salty sweat. Sweat is normally produced at the base of sweat glands; it then flows to the skin surface through a narrow duct. Initially the sweat is a solution rich in sodium and chloride ions—that is, the constituents of salt. But as the fluid traverses the duct, the ions escape into the epithelium, leaving the water behind. Thus, the sweat that emerges to cool the skin surface is only slightly salty. In patients with cystic fibrosis, in contrast, the inability of epithelial tissue to absorb chloride and the consequent impairment of sodium absorption from the duct lumen cause sweat to retain excess sodium and chloride and to become abnormally salty.

In the other line of study, Michael R. Knowles and Richard C. Boucher of the University of North Carolina at Chapel Hill examined the lungs. They found that chloride movement from epithelial tissue into the airway lumen was diminished and that sodium uptake by the epithelium was enhanced. Reduced chloride transport has now been demonstrated as well in the epithelia of the pancreatic ducts in mice and of the intestines in patients.

Finally, the Gene Is Found

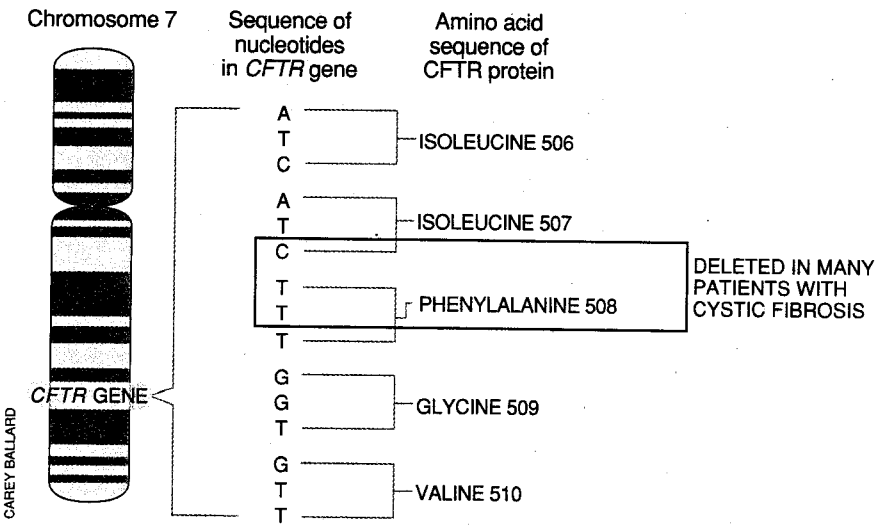
As these studies of chloride transport were progressing, many scientists were engaged in an intense race to find the gene responsible for cystic fibrosis. That effort culminated in 1989, when a large group of collaborators, led by Lap-Chee Tsui and John R. Riordan of the Hospital for Sick Children in Toronto and by Francis S. Collins, then at the University of Michigan, announced it had isolated the gene. Aware that the protein product of the gene probably influenced the movement of chloride directly or indirectly, they named the protein the cystic fibrosis transmembrane conductance regulator (CFTR). While searching for the gene, the team also identified an abnormality in the DNA that appeared to account for about 70 percent of cystic fibrosis cases. That aberration, often denoted as the ΔF508 mutation, consists of the deletion of three nucleotides (DNA building blocks) from the gene. That loss causes the protein product of the gene to lack a single amino acid: phenylalanine at position 508.

The report was extraordinarily excit-

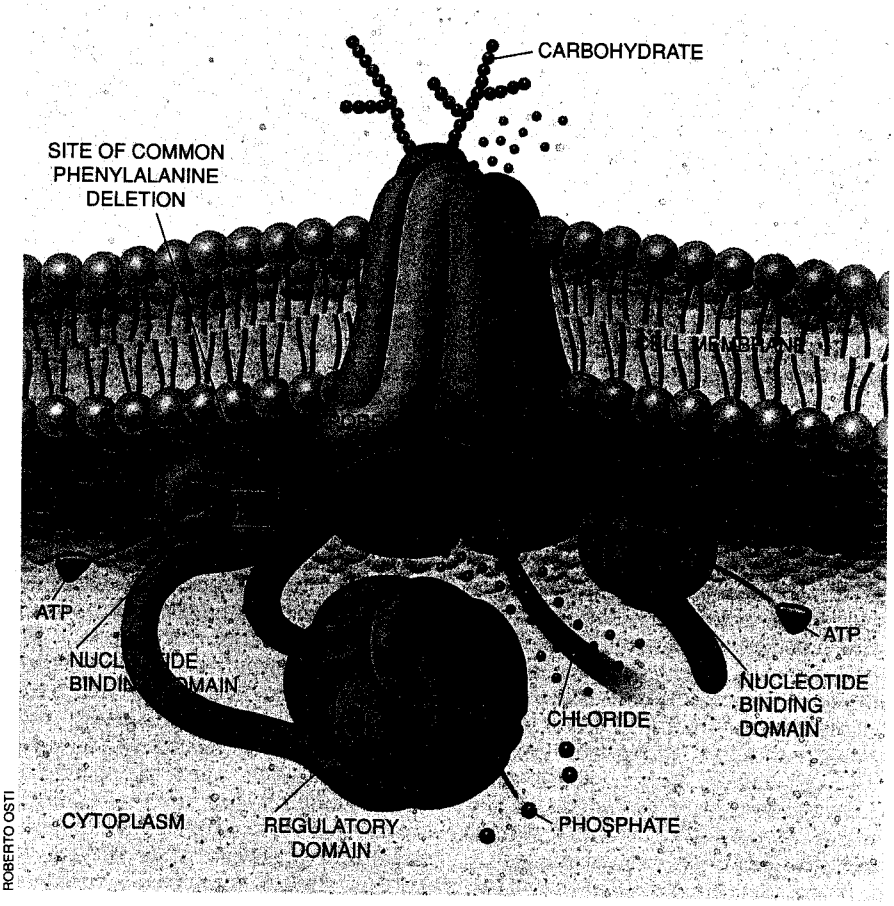
ing for everyone concerned with cystic fibrosis; it promised to open new vistas of understanding and new options for therapy. Nevertheless, investigators desired additional evidence that the correct gene had been isolated. Strong support could be obtained by inserting a healthy version into cells from a patient with cystic fibrosis and thereby correcting the chloride transport defect. Frustratingly, workers had difficulty constructing even a streamlined version of the gene. By the summer of 1990, however, our colleague Richard J. Gregory of Genzyme Corporation had solved the problem.

The two of us and our co-workers lost no time inserting the gene into epithelial cells isolated from the airways of patients with cystic fibrosis. Next we exposed the cells to cyclic AMP, a molecule that normally stimulates chloride transport in airway epithelium but has no effect on tissue from patients with cystic fibrosis. We were thrilled to see that cyclic AMP now caused chloride to stream out of the treated cells; the gene had apparently made the cells normal. We were not alone in our delight. Collins and a number of his colleagues had obtained similar findings using different methods in pancreatic epithelial cells.

The successes with cultured cells suggested that delivery of healthy *CFTR* genes to patients might correct their underlying biochemical abnormality—a tantalizing possibility. But we also knew, as will be seen, that there were many obstacles to attaining that goal. Meanwhile another obvious problem loomed over the field: resolving exactly how the CFTR protein influenced chloride movement.



CYSTIC FIBROSIS GENE resides on chromosome 7 (*left*) and normally gives rise to a protein called the cystic fibrosis transmembrane conductance regulator (CFTR). The defect that most often leads to the disease is the deletion of three nucleotides from the gene (*red letters in center column*); this alteration, known as the ΔF508 mutation, results in the loss of one amino acid—phenylalanine at position 508—in the CFTR protein (*right*). Phenylalanine is lost because the protein-making machinery of the cell now sees ATT (an alternative way to encode isoleucine) at the gene region coding for the protein's 507th amino acid, followed by the GGT sequence for the glycine that normally follows phenylalanine.

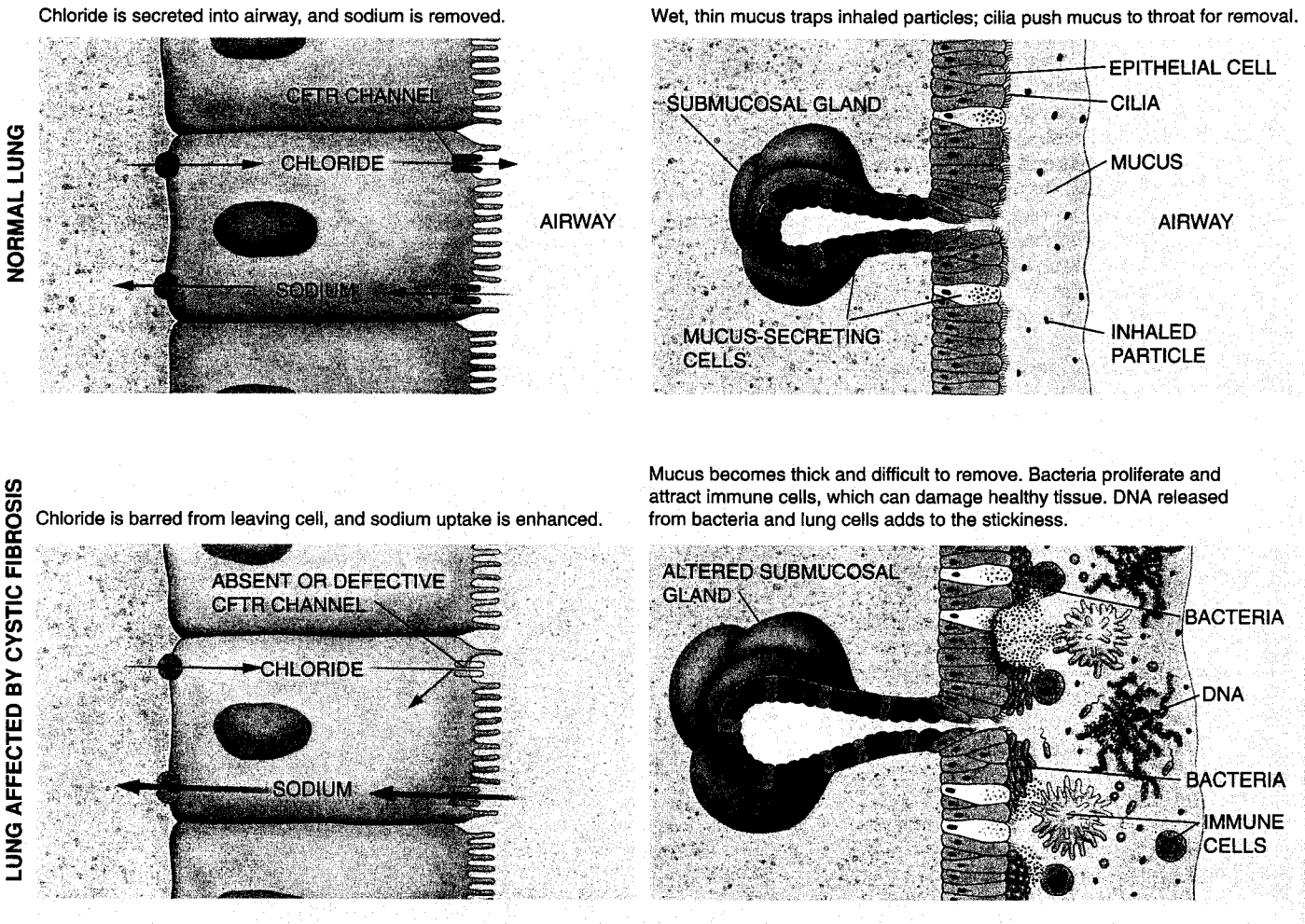


INTACT CFTR PROTEIN forms a chloride-permeable channel in the outer membrane of many cells. The precise structure has yet to be determined, but movement of chloride through the pore is known to be regulated by three cytoplasmic domains of the protein. Passage is allowed only when the two nucleotide binding domains dock with and cleave adenosine triphosphate (ATP) and when the regulatory domain becomes studded with phosphate groups.

What Does This Protein Do?

The linear sequence of amino acids in the protein, which was easily deduced once the gene was isolated, offered some immediate clues to the protein's normal behavior. Notably, the sequence was much like that found in a family of proteins called traffic ATPases or ABC transporters (because they carry what is known as an *ATP binding cassette*). The similarity implied that the CFTR protein might also resemble the family in its behavior and in its folded, three-dimensional structure.

The traffic ATPase family includes a number of proteins used by bacteria to pump nutrients across their cell membrane; it also includes the drug-resistance protein that unfortunately ejects chemotherapeutic drugs from cancer cells [see "Multidrug Resistance in Cancer," by Norbert Kartner and Victor Ling; SCIENTIFIC AMERICAN, March 1989].



MOLECULAR BASIS OF LUNG DISEASE in patients who have cystic fibrosis is complex. In healthy individuals (*top row*), the main epithelial cells lining the airways (*left panel*) display at least two types of channels at the surface facing the air passage. One—the CFTR channel (*red*)—releases chloride into the passage; the other (*blue*) takes up sodium. This arrangement somehow enables mucus made by other cells to remain wet, thin and easy to remove from the airways (*center panel*), and so the airways remain open (*right panel*). In patients with cystic fibrosis (*bottom row*), absence or malfunction of the CFTR channel prevents chloride movement (*left panel*) and indirectly causes cells to take up extra sodi-

When folded, these ATPases generally have four main structural parts, or domains: two that span the membrane (each of which contains several transmembrane segments) and two that dwell in the cytoplasm. The last two units, known as nucleotide binding domains, take up and cleave ATP (the nucleotide adenosine triphosphate) to obtain the energy required for pumping. The CFTR molecule was predicted to take essentially the same shape and, as will be seen, to have an added component residing in the cytoplasm.

Based on the activities of the ATPases, some researchers favored the hypothesis that CFTR was an ATP-driven pump that actively transferred some substance into or out of epithelial cells; the transported substance then induced chloride transport across the cell membrane through a separate channel. They posited this complex scheme because no known ion channels (such as would be needed to move chloride more directly) resembled the predicted folded structure of CFTR.

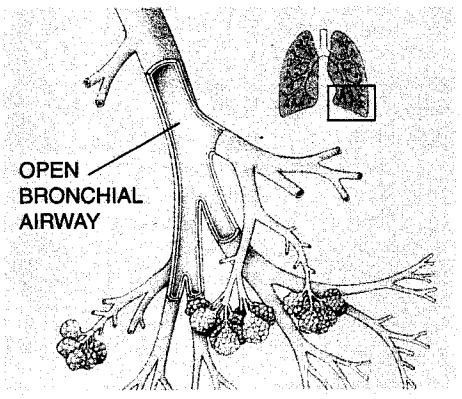
A second hypothesis proposed that CFTR itself attached to chloride channels and influenced their activity. And a third hypothesis held that CFTR might serve directly as a chloride channel even though its structure was unusual for any ion channel recognized at the time. In this scenario, the two membrane-spanning domains would form the pore through which chloride ions passed across the membrane.

As the work advanced, the data confirmed the third idea: CFTR formed a chloride channel on its own. We found that transfer of a gene for CFTR into chloride-impermeable cells conferred the ability to move that ion. If the gene was first altered in ways that affected parts of the CFTR protein thought to help chloride move through the channel, the channel's affinity for chloride decreased; this effect was shown by our colleague Matthew P. Anderson of the University of Iowa. Any last doubts were dispelled when Riordan and his colleagues inserted highly purified CFTR proteins into artificial cell membranes (lipid bilayers) containing no other channellike proteins. Addition of the protein allowed the ions to travel across the membrane.

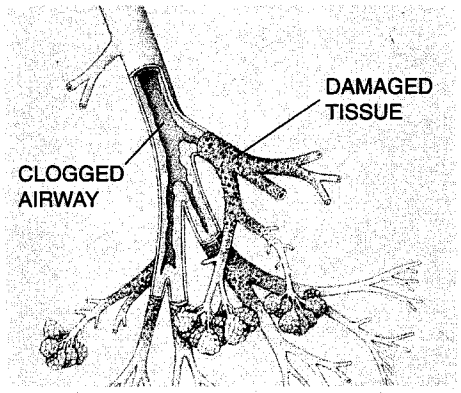
Subsequent investigations clarified the function of the "extra" CFTR component not found in traffic ATPases. On the basis of certain short sequences within that component, the mysterious

BRONCHIAL TUBES AND BRONCHIOLES

Airways stay clear for breathing.



Airways become plugged and begin to deteriorate.



ROBERTO OSTI

um (*thick blue arrow*). Then the mucus becomes thicker and more resistant to removal (*center panel*), and bacteria trapped there flourish. Together these changes plug the airways and lead to their destruction (*right panel*).

segment was deduced to be a regulatory domain—R—whose activity in the cytoplasm was controlled by the addition and removal of phosphate groups. Various experiments, including those by our colleagues Seng H. Cheng of Genzyme and Devra P. Rich of the University of Iowa, showed that when the R domain lacks phosphate groups, chloride ions cannot flow into the channel pore. But when chemical changes in a cell (specifically, rising levels of cyclic AMP) cause enzymes to dot the domain with phosphate, the addition promotes chloride movement through the pore.

It is helpful, though overly simplistic, to imagine that when the regulatory domain is not phosphorylated, it behaves like a gate blocking the cytoplasmic opening of the membrane pore. Addition of the phosphates somehow displaces the domain (opens the gate), allowing chloride ions to pass into the pore. Other analyses have demonstrated that the nucleotide binding domains influence the activity of the channel as well. For ions to go through the pore, those domains must bind to and probably cleave ATP.

How the Mutations Make Mischief

Knowing that the CFTR protein forms a chloride channel and having some idea of how the molecule functions leaves an important question still to be answered: Exactly how do mutations in the *CFTR* gene lead to loss of chloride transport? The effect of the most common DNA mutation—the deletion that leads to omission of phenylalanine 508 from the CFTR protein—has been the most extensively studied.

This deletion engenders what is known as an intracellular trafficking defect. Many proteins, among them the normal CFTR molecule, are processed after they are synthesized. They gain some sugar groups in a cellular compartment called the endoplasmic reticulum, after which they take up more sugar in the Golgi apparatus before being dispatched to the cell membrane. The mutant protein, in contrast, fails to leave the endoplasmic reticulum. Its travel is halted presumably because the quality-control system in the endoplasmic reticulum discerns that the protein is folded improperly. Proteins that are identified as defective are marked for degradation rather than being allowed to undergo further processing.

Although the phenylalanine 508 mutation is the most common one, hundreds of others have now been identified in people with cystic fibrosis. As is true of the 508 mutation, many of these changes block the protein from making its way to the cell membrane. Some prevent the CFTR protein from being made at all, and still others allow the protein to be produced and inserted into the cell membrane but bar the CFTR molecule from operating properly. In the last instance, the mutations may forestall chloride movement by disrupting the function of a nucleotide binding domain or by introducing a flaw into the lining of the ion-transporting pore.

In general, people whose cells carry two copies of the gene bearing the phenylalanine 508 mutation tend to have severe disease, probably because little if any of the mutated protein escapes from the endoplasmic reticulum. In people whose genes permit at least some CFTR to reach the cell membrane and to transport chloride to an extent,

Testing Dilemmas

Now that many genetic mutations leading to cystic fibrosis have been pinpointed, prospective parents can easily find out whether they are likely to be carriers of the disease—that is, whether their cells silently harbor a defective copy of the *CFTR* gene. Couples can also learn whether an already developing fetus has inherited two altered copies of the gene (one from each parent) and will thus be afflicted with cystic fibrosis.

The difficulty for many people is deciding how to proceed once they receive their test results. The trouble arises in part because the laboratories that perform the genetic analyses do not detect every mutation in the *CFTR* gene. Consequently, a reassuring negative finding may not fully rule out the possibility that someone is a carrier or is affected with cystic fibrosis. (A favorable prenatal test result will be conclusive, however, if the fetus is shown to lack the specific *CFTR* mutants known to be carried by the parents.) Moreover, it is not yet possible to predict the extent of symptoms in a person who inherits two *CFTR* mutants; even if the inherited genes are usually associated with highly severe or less severe disease, such associations do not necessarily hold true in every individual.

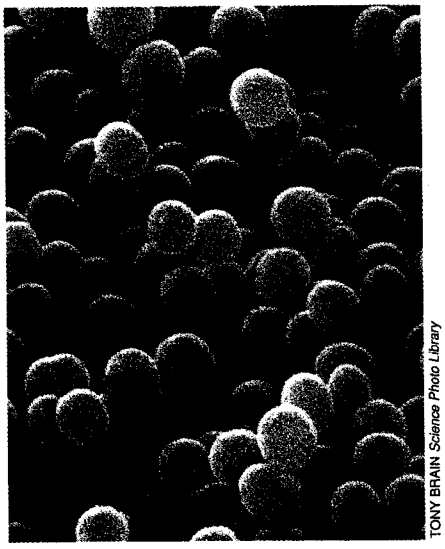
Some couples may be tempted to think that research will progress fast enough to protect children born today from the life-threatening lung damage characteristic of cystic fibrosis. Yet medical investigations often hit unexpected obstacles and suffer setbacks before they achieve their ultimate goals. Hence, although it is probable that treatment will become more effective—perhaps markedly so—in the coming years, no one can foretell exactly when cystic fibrosis will become significantly easier to manage. Prospective parents need to understand, therefore, that a child born with cystic fibrosis today will still have to cope with the disease and may not be spared a premature death.

Such uncertainties render decision making extremely challenging. This is an exciting time in cystic fibrosis research, but it is also a trying one for couples caught in the gap between current technology and anticipated advances that have not yet become a reality.—*M.J.W. and A.E.S.*

the residual activity can make for somewhat less severe symptoms. These patterns do not always hold, however, and so making predictions in individual cases remains problematic. Indeed, two patients with exactly the same mutations in both copies of their *CFTR* gene can differ significantly in the extent of organ damage they suffer. This divergence arises because other genetic and environmental factors that remain poorly understood can probably influence the course of the disease.

It is humbling to note that burgeoning understanding of the genetic defects has not yet fully explained how disordered chloride transport in the lung epithelium alters sodium transport and how those changes result in the accumulation of mucus in the bronchial passages. It has also been discovered that submucosal glands—mucus producers that lie below the surface epithelium—produce a large amount of the CFTR protein. What role do these glands play in the disease? Scientists are further puzzled by the fact that the airways of patients with cystic fibrosis are predisposed to infection by some bacteria more than by others. For instance, infections by *Pseudomonas aeruginosa* and *Staphylococcus aureus* are particularly common. An understanding of why certain organisms thrive is only now beginning to emerge.

Investigators wonder as well whether the CFTR protein has functions beyond its role as a chloride channel. Among the possibilities being considered is that CFTR may help regulate chloride channels distinct from CFTR. Researchers have also posited that the molecule may indirectly alter the mix of sugars on the epithelial surface in ways that favor colonization by certain bacteria.



TONY BRAIN *Science Photo Library*

DAVID M. PHILLIPS *Population Council, Science Source*

BACTERIA that often cause severe infections in the lungs of patients with cystic fibrosis include *Staphylococcus aureus* (*top*) and *Pseudomonas aeruginosa* (*bottom*). Once the infections are established, they almost invariably recur.

Strategies for Treatment

In spite of the unanswered questions, the knowledge gained since 1989 has already suggested several avenues for attacking cystic fibrosis. One is to compensate for the loss of the CFTR chloride channel by increasing the activity of a different class of chloride channel. For instance, channels controlled by calcium ions are known to exist in the lumen-facing surface of epithelial cells. Those molecules usually fail to counteract the loss of the CFTR channel, but perhaps their chloride conductance can be increased artificially. This possibility is being tested in patients.

One day doctors also might deliver purified CFTR proteins to the cells that need them. Studies of cells in culture have shown that the protein molecules can correct chloride flow in cells carrying a mutant *CFTR* gene. In theory, another tactic would be to administer drugs able to escort mutant CFTR molecules from the endoplasmic reticulum through the Golgi apparatus and into the cell membrane. This idea seems worth pursuing because ΔF508 mutant CFTR proteins that become stuck in the endoplasmic reticulum usually function fairly well when experimentally inserted into the outer membrane of cells. At present, however, we know of no drugs that can correct the intracellular trafficking abnormality. A different approach, not yet tested, would be to use drugs to increase the activity of any mutant CFTR channels that do find their way into the cell membrane.

The treatment option attracting the most attention, however, is gene therapy, which aims to deliver a normal copy of the *CFTR* gene to the cells that need it. If all goes well, the DNA inserted into target cells should direct synthesis of the normal CFTR protein and reverse the primary biochemical abnormality at the root of cystic fibrosis. Introduction of the gene is a favored approach because it should replace all functions of the CFTR protein, including any that have not yet been recognized.

The best-studied method of gene therapy exploits the ability of viruses to enter cells, bringing their DNA with them. We and others have paid special attention to adenoviruses as gene carriers, or vectors, because those microbes are naturally able to infect human airways but will usually produce relatively innocuous disease, such as the common cold. The adenoviruses are altered in two ways: certain viral genes are removed to prevent the virus from reproducing in cells and causing symptoms. And the excised DNA is replaced with a normal *CFTR* gene. Our group, as well as those of Ronald G. Crystal, then at the National Heart, Lung and Blood Institute, and James M. Wilson, then at the University of Michigan, has demonstrated that such vectors can deliver the *CFTR* gene to cultured epithelial cells and to airway cells in animals. What is more, the cells use the DNA to synthesize CFTR molecules that function as healthy chloride channels.

On the basis of such experiments, several research groups have begun attempting to deliver the *CFTR* gene to patients via genetically engineered adenovirus vectors. The aim of these early experiments is primarily to assess safety. Even so, we and others have also tested the ability of a *CFTR*-bearing adenovirus to correct chloride transport in the nasal epithelium of patients. We chose the nasal epithelium because it is similar to that of the bronchial passages but is easier to reach.

Our first test was encouraging. For experimental purposes, we applied the altered virus directly to a small patch of epithelium in the nose. The treatment partially corrected chloride transport for a time. Since then, however, a similar study by us has been less successful, and one by another group showed no increase in chloride flow. These findings indicate that adenoviral vectors need to be improved substantially before they can serve as gene-delivery agents in therapy.

Even if ways are found to increase the efficiency of gene delivery by the viruses, another challenge would remain. Most cells in epithelial tissue are replaced every few months. Therefore, gene therapy would probably have to

Some Strategies for Treating Lung Abnormalities

The lung disease characteristic of cystic fibrosis can be attacked at many levels. Potential strategies range from reversing the genetic defect at the root of the pulmonary problems to replacing a failed lung with a healthy one.

ABNORMALITY	APPROACH	STATUS
Mutation in *CFTR* gene	Provide normal gene through gene therapy; provide normal CFTR protein to cells	Gene therapy is being tested in preliminary clinical trials; methods for protein delivery are inefficient
Defective delivery of CFTR protein to outer cell membrane	Supply drugs able to escort protein to cell membrane of epithelial cells	No candidate "escorts" have been identified
Defective movement of chloride ions through CFTR channels in cell membrane	Deliver drugs that increase activity of other classes of chloride channel in epithelial cells	Such drugs are being tested in preliminary clinical trials
Clogging of air passages by viscous mucus	Pound back and chest to help clear secretions; administer DNase and other drugs to liquefy secretions	Chest percussion is standard therapy; DNase is now in wide use, and similar drugs are being tested in animals
Development of recurrent infections that can damage lungs	Deliver antibiotics to destroy bacteria or provide antibodies (special molecules of immune system) to remove microbes	Antibiotics are in wide use; antibodies are being tested in preliminary clinical trials
Tissue damage caused by immune response to bacteria	Administer drugs that reduce harmful effects of immune response	Steroidal anti-inflammatory drugs are sometimes used; nonsteroidal anti-inflammatory agents (mainly ibuprofen) are being tested
Destruction of lung	Transplant healthy lung	Transplantation is sometimes an option

be administered a few times a year—at least until the rare, long-lived cells that give rise to the replacement cells can be induced to take up a normal *CFTR* gene permanently. Aside from inconvenience and expense, the need for multiple treatments is a concern because people respond to adenoviruses by mounting an immune response that ultimately eliminates the microbes and prevents repeated infection. For gene therapy to be successful, investigators will have to find ways to "hide" the adenoviruses from the immune system or to create viral or other vectors that do not elicit an immune response.

One appealing alternative to relying on viruses would be to coat the therapeutic gene with fatty molecules that are not recognized by the immune system but that nonetheless enable the DNA to enter cells. Recent studies conducted on human patients by Eric Alton and his co-workers at the Royal Brompton Hospital in London suggest this approach can restore chloride permeability to airway epithelium, although this group, like ours, has so far studied only nasal tissue. Moreover, delivery of genes by nonviral systems needs to be made more efficient.

Scientists have much to learn before they understand exactly how loss of the CFTR protein leads to the manifestations of cystic fibrosis. And a host of technical challenges must be eliminated before any therapy will routinely compensate for that loss. Nevertheless, progress is being made on many fronts. It is difficult not to be optimistic that the ongoing work will produce improved therapies within the next several years.

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Further Reading

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