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MEDICINE

A BREATH OF FRESH AIR

Fundamental understanding of basic biology has set the stage for new treatments for cystic fibrosis

By Steven M. Rowe, J. P. Clancy and Eric J. Sorscher

Illustration by John Hendrix

IN 1989 WHEN SCIENTISTS DISCOVERED THE DEFECTIVE GENE THAT CAUSES CYSTIC FIBROSIS, a serious hereditary disorder that primarily strikes children of European descent, it seemed as though a long-hoped-for cure might soon follow. After all, tests in many laboratories showed that providing normal copies of the gene should enable patients to make healthy copies of the protein specified by the gene. If successful, that feat would go a long way toward restoring health in the tens of thousands of people around the world who suffered from cystic fibrosis and typically died in their late 20s. (Half of all patients now live to their late 30s or beyond.) The question was whether researchers would be able to reliably insert the correct gene into the proper tissues in patients' bodies to rid them of the illness forever.

That task proved harder than anyone had believed. Although scientists successfully engineered viruses to ferry copies of the correct gene into patients' cells, the viruses did not do the job well. By the late 1990s additional unexpected complications made it increasingly obvious that another approach to addressing the fundamental problem in cystic fibrosis would need to be found.

Meanwhile cell biologists and their colleagues undertook the long, challenging task of determining exactly what the normal protein looked like, how it functioned and how defects led to the symptoms of cystic fibrosis. These efforts included understanding the protein's three-dimensional shape in increasingly fine detail as well as the various ways the abnormal protein failed in its cellular duties. Instead of creating normal proteins by replacing the broken gene with an effective one—as was gene therapy's goal—this group of researchers focused on a different objective: finding a drug that allowed the deficient protein to work better. A fruitful search might give people with cystic fibrosis many additional years of a healthier life.

Today it looks as though the gradual but steady approach is paying off. Several new compounds are in the final stages of being tested for use in the treatment of cystic fibrosis—and one of them looks particularly promising for certain patients. If successful, it would be the first medication that targets the underlying cause of the disease, as opposed to dealing with symptoms. But that is not all. Preliminary studies indicate that these potential new treatments may also work against other, more common conditions, such as bronchitis, chronic sinusitis and pancreatitis, among others.

A PROBLEM WITH SALT

THE STORY OF HOW these drugs were identified begins with a dogged search to understand the basic biology of cystic fibrosis. The disease has long been known to result generally from a failure in the ability of certain body tissues to transport salt (sodium chloride) across the membranes that envelop cells. The cells in these tissues extrude the chloride part of the salt to help maintain the right balance between their fluid-filled interior and the watery exterior environment. As the chloride ions accumulate on the outside of the cell, water molecules follow suit, diffusing across the membrane to the outside. When the cell is finished constructing these tiny chloride channels, it inserts them into the membrane, where each protein forms a passageway that spans the cell border.

The gene that in 1989 was found to cause cystic fibrosis codes for one of these proteins, known as the cystic fibrosis transmembrane conductance regulator, or CFTR. The normal version of this molecule is made of a precise sequence of ap-

IN BRIEF

Cystic fibrosis is a serious hereditary disorder that fills certain organs in the body with a sticky mucus that interferes with the ability to digest food or breathe.

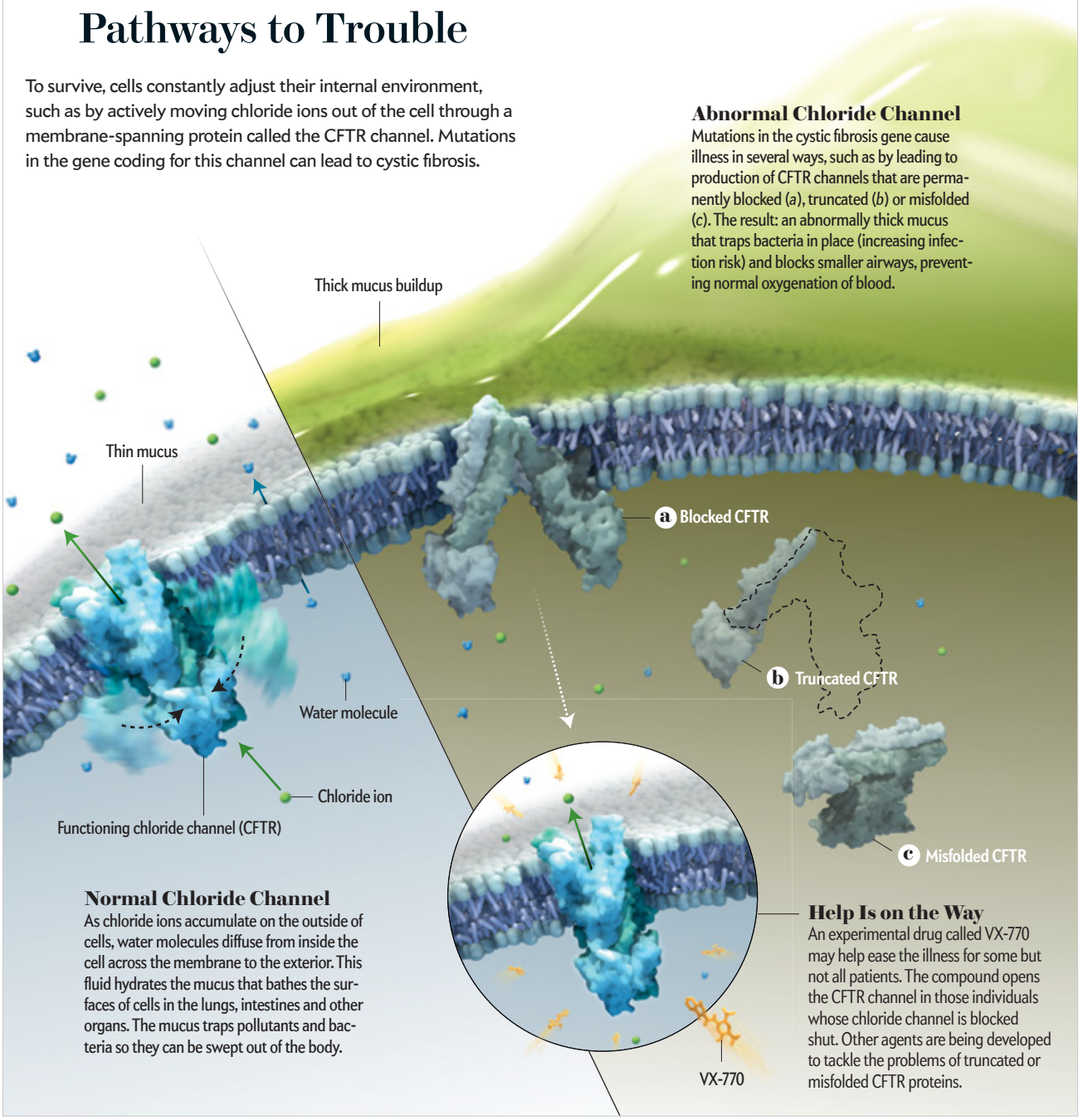
When researchers discovered the gene that causes cystic fibrosis in 1989, a long-hoped-for cure—in the form of gene therapy—seemed possible.

There were setbacks. But a new and different approach is now poised to deliver the first medications that address the fundamental causes of the disorder.

The Food and Drug Administration will probably begin to consider whether to approve a drug based on this latest research this year.

Pathways to Trouble

To survive, cells constantly adjust their internal environment, such as by actively moving chloride ions out of the cell through a membrane-spanning protein called the CFTR channel. Mutations in the gene coding for this channel can lead to cystic fibrosis.



proximately 1,500 amino acids folded intricately and gracefully into a series of three-dimensional loops and sheets that spiral or plunge to form a number of different subsections. The flow of water molecules elicited by the movement of chloride ions through the channel helps to move the mucus that coats the surfaces of the body's airways, as well as the many ducts found in the intestines, pancreas and liver. The CFTR channel can also transport certain other ions, such as bicarbonate.

Here is where the trouble starts. Mutation of the gene results in the body lacking a proper CFTR channel. As a result, people with cystic fibrosis produce a sticky mucus that is so thick it interferes with many physiological processes. In the lungs, the gel-like mucus hampers the diffusion of oxygen into the air sacs and makes the simple act of breathing, as one of our young patients described it, like "trying to breathe with someone's hands over your face." Furthermore, the viscous buildup becomes an ideal breeding ground for serious infections by harmful bacteria, often *Pseudomonas aeruginosa*. In the pancreas, the thick and immobile secretions prevent the passage of digestive enzymes through various ducts into the intestines, interfering with proper digestion and, as a result, frequently causing people with cystic fibrosis to be underweight

Illustration by AXS Biomedical Animation Studio

or undernourished. Meanwhile bile becomes trapped in the liver, so fats are not properly processed, and blockages in the intestines lead to constipation and sometimes even a life-threatening shutdown of the entire gastrointestinal tract.

Before the advent of antibiotics to treat recurrent lung infections and the discovery of better nutritional therapy, most children with cystic fibrosis died in infancy. Over the past few decades advances in medical and supportive care have substantially prolonged the lives of individuals with the disease. Some of the treatments can seem rough to the uninitiated: parents or others are taught how to vibrate or pound their children's chest to help move the thick secretions in the lungs and dislodge any mucus plugs. Several drugs have been developed that open the airways, suppress infection or help thin the airway secretions. Supplemental vitamins and enzymes aid the digestive process. It is as a result of these and other measures that half of all cystic fibrosis patients now live to 37 years of age or older. None of these treatments, however, addresses the underlying cause: the insufficient flow of chloride and other ions out of cells.

THREE PATHWAYS

THE FIRST STEP in finding a drug that might restore at least some function to a deficient chloride channel was to better understand on a microscopic level the precise details of what goes wrong. Geneticists have tested DNA samples from cystic fibrosis patients around the world and have so far discovered more than 1,600 different mutations in the CFTR gene that lead to serious illness. The deleterious effects on the resulting CFTR protein can be divided into several groups. In three of the best-studied categories, the channel never gets put in place in the cell membrane, or a truncated channel is synthesized (and the directions for making it are rapidly degraded), or a channel of normal length is made but is unable to open or transport chloride or other ions. A single drug developed to repair one of these problems might not be of much help for the other two. Therefore, it is likely that to help the entire population of cystic fibrosis patients, different drugs will need to be developed—each based on the genetic defect responsible for an individual's condition.

A completely missing chloride channel at the cell surface stems from the most common genetic mutation, which results in the deletion of just one of the channel's 1,500 amino acid building blocks. Because the missing amino acid is phenylalanine (designated "F" in protein parlance) and is the 508th amino acid in the chain, the mutation is referred to as F508del.

The F508del mutation causes disease in a fashion that was at first surprising. Despite the mutation, the cell is able to build a chloride channel, amino acid by amino acid. The final product is equipped to transport chloride ions to a limited extent. But the cell's own molecular quality-control apparatus prevents it from doing so. The cell has several hundred helper proteins and enzymes that ferry the nascent CFTR molecules around the cell, inspect the ways they are being folded and help to insert CFTRs in the cell membrane. Even seemingly minor defects in folding—such as the F508 omission—can be rapidly recognized, leading the cell to quickly destroy the mutant. As a result, the somewhat functional chloride channel never even makes it to the cell membrane.

Because F508del is the most common cause of cystic fibrosis, numerous facilities around the world (including our own) are trying to locate the precise cellular checkpoints at which the F508del CFTR molecule fails to "make the grade" and is routed to the recycling bin. The goal is to aid in the discovery of compounds that will ease cystic fibrosis by helping the protein fold correctly and avoiding its destruction without interfering with the ability of the cells to recognize and eliminate other aberrant amino acid chains.

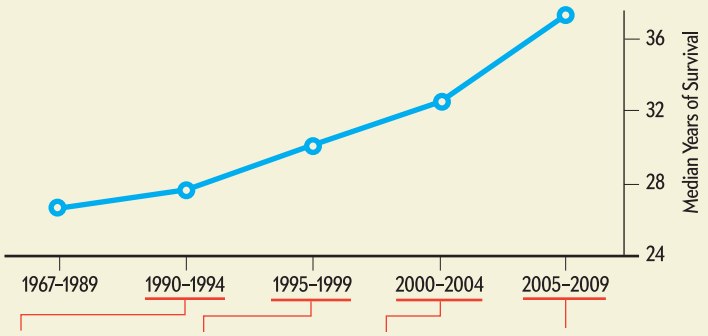
Figuring out how to adjust the cell's quality-control systems could offer benefits beyond treating cystic fibrosis. A number of chronic diseases—such as defects in cholesterol metabolism and some lung disorders (among them, alpha-1-antitrypsin deficiency)—occur because of protein misfolding. In at least certain diseases, evidence is growing that the true culprit is not the altered function of the protein per se but rather the propensity of the quality-control mechanism to degrade the abnormal molecule or deposit it in a tangled clump. It is conceivable that a number of slightly misfolded, mutant proteins would retain significant function if they were spared and allowed to do their intended jobs. Therapies that tackle the quality-control mechanism might therefore provide valuable insight into the biology and treatment of a wide range of diseases.

Mutations that result in abnormally foreshortened CFTR channels account for about 10 percent of cystic fibrosis cases worldwide. One

STEP BY STEP

Steady Progress

People with cystic fibrosis are already living longer thanks to new drugs and new approaches to delivering care. But agents that address the underlying biological cause would also help tremendously.



1994 A drug called dornase alpha improves pulmonary function by breaking down thick mucus in the lungs

1997 Routine spraying of the antibiotic tobramycin into the lungs helps to control infections

2002 The Cystic Fibrosis Foundation launches a quality-improvement program that standardizes treatment of U.S. patients

2009 Mandatory screening of newborns across the U.S. for cystic fibrosis allows for earlier detection and treatment

SOURCE: CYSTIC FIBROSIS FOUNDATION PATIENT REGISTRY: ANNUAL DATA REPORT 2009

Graphic by Jen Christiansen

such genetic defect, dubbed W1282X, underlies about 40 percent of cystic fibrosis in Israelis. The protein ends up being truncated because the gene contains misguided instructions telling the protein-synthesizing machinery to stop attaching amino acids to the growing protein molecule at position 1282 in the chain, where the amino acid tryptophan (signified by "W") would normally reside. Such genetic instructions are called nonsense codons and are crucial to the proper manufacture of proteins—provided they occur in the right place. In this case, however, the protein-manufacturing process comes to a premature halt. In addition, the intermediate instructions (known as messenger RNA) that guide the production process are also recognized as abnormal and destroyed so that even if the stop signal could somehow be skipped over, not enough of the now functional protein would be made. Therefore, drug treatments for W1282X may need to attack two problems and not just one.

The last set of mutations we consider here disables the channel's ability to open and accounts for about 5 percent of cystic fibrosis cases worldwide. In effect, these mutations cause the doorway through the membrane to be stuck in the closed configuration, which leaves the channel less able to transport chloride ions to the extracellular environment. One of the mutations that act in this way (called G551D) causes particularly severe symptoms. Although it is generally true that each group of genetic defects may require its own specifically targeted treatment, researchers have shown that compounds designed to prop open a mutant CFTR gate might aid patients who do not have this mutation. For example, take the case of a drug that enabled a small amount of the F508del CFTR to travel to its proper location in the cell membrane. A second drug that braced the channel door open would allow this somewhat sluggish version of the channel to pass more chloride out of the cell.

PROMISING DRUG CANDIDATES

THE NEXT STEP in the long process of drug development was to search for compounds able to alleviate the effects of specific mutations in the CFTR gene. It made sense to start with F508del because of its high prevalence in patients with cystic fibrosis and because the resulting protein retains some residual functionality; if researchers could help F508del CFTR evade premature degradation, the protein could arguably provide partial activity and improve lung function without further prodding.

Knowledge of the ways the F508del CFTR protein misfolds and of how the cell's quality-control machinery detects that folding defect is far from complete. It is, however, reasonably straightforward to determine whether a particular compound can alleviate the effects of the folding error in human cells. By loading fluorescent molecules into a cell, investigators can measure small changes in the concentrations of chloride or other ions moving across the cell membrane. When ions traverse the membrane after exposure to a potentially therapeutic drug, researchers can infer that the impaired CFTR channel has regained some function. If the ions do not make it across, the search continues for a more active compound. By automating and computerizing the drug-screening process, millions of compounds can be analyzed in a relatively short time.

Biotechnology company Vertex Pharmaceuticals identified one compound, known as VX-809, that had encouraging preliminary results but that did not significantly improve lung function in test subjects. Another company, PTC Therapeutics, is directing clinical trials of a drug called ataluren that addresses the less common, CFTR-truncation mutations. This agent causes the protein-making machinery to read through some of the misplaced "stop" instructions, thereby allowing the chloride channel protein to avoid being foreshortened. This agent is also being tested for other hereditary disorders that involve aberrant stop codes, such as Hurler syndrome and Duchenne muscular dystrophy.

Adjusting the cell's quality-control systems could offer benefits beyond treating cystic fibrosis, such as fixing defects in cholesterol metabolism and other disorders.

Scientists have had the best results counteracting the G551D mutation. After testing 228,000 different potential compounds against cells harboring chloride channels that did not open easily, a group of researchers at Vertex discovered a compound that selectively activates the energy switch of the CFTR channel, boosting function to 50 percent of normal levels. The laboratory results were good, and the compound, named VX-770, has now undergone extensive testing. In these trials, the ability to breathe improved substantially in patients with the G551D mutation within weeks, and the benefits were sustained for the length of a one-year study. Just as important, treated individuals were hospitalized less often and gained an average of seven to eight pounds. One of us (Rowe) was the first clinician in the U.S. to administer VX-770 in a patient. Sometime later this year Vertex plans to petition the Food and Drug Administration for the right to bring the drug to market. Further testing to use VX-770 in combination with other agents is also showing some promise in clinical trials. (The University of Alabama at Birmingham is one of the sites for clinical trials of drugs developed by PTC Therapeutics and Vertex; Rowe and Clancy have advised both companies about study design.)

These clinical results are groundbreaking, and the discovery of several drug candidates that take aim at the root cause of cystic fibrosis validates decades of research into its basic biology and funding of such work by the National Institutes of Health and the Cystic Fibrosis Foundation. Although more clinical trials are under way to establish whether the available compounds will be safe and effective in the long term for a larger group of patients, optimism is growing that one day we will finally be able to treat the underlying causes of this difficult disease. SA

MORE TO EXPLORE

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SCIENTIFIC AMERICAN ONLINE

Young people with cystic fibrosis share their lives, philosophies and daily routines on video at ScientificAmerican.com/aug2011/cf-video