Novel Function Discovered For the Cystic Fibrosis Gene

In the 2 years since the cloning of the cystic fibrosis (CF) gene, evidence has mounted that the gene produces a protein that ushers chloride ions out of cells. Many researchers have come to believe that all of the symptoms of the deadly disease derive from the mutant protein's failure to perform this basic function. But a paper in this issue of *Science* (p. 530) by Neil Bradbury, Kevin Kirk, Robert Bridges, and their colleagues at the University of Alabama suggests that the CF protein controls other cellular processes besides chloride secretion. While some in the field say the finding may open fruitful new avenues of research, others see it as a potentially time-wasting distraction, with questionable relevance to the disease.

In the eyes of this second group, the finding runs counter to a great, unifying principle that has evolved in CF research: that all the symptoms of the disease are caused by faulty chloride secretion. Cells in the skin, airways, and digestive tract can't properly pass chloride through their membranes, and, as a result, they secrete less water than normal. That gives the skin a salty taste—indeed that taste was one of the first known signs of CF, long before the disease had a name. But in the airways the consequences are more serious. Without adequate water, the mucus thickens and refuses to flow properly, becoming a fertile breeding ground for often deadly infection by pneumonia-causing *Pseudomonas* bacteria.

That kind of reasoning is elegant and satisfying to those who remember the days when CF researchers were chasing multiple, unrelated, and inevitably fruitless leads. But some researchers worry that it won't answer all the questions posed by the disease. For example, the mucus proteins of CF patients have an abnormal composition that may be what attracts the *Pseudomonas* bacteria. Those differences in protein composition can't be explained "simply on the basis of water and chloride abnormalities," says University of Michigan CF researcher Francis Collins. They might be explained, however, if the CF protein played a role in the internal workings of the cell, where proteins are produced.

That's where the work of the Kirk and Bridges team comes in. Their group found that the CF protein (known to researchers as the CFTR, for cystic fibrosis transmembrane conductance regulator) regulates the insertion and removal of membrane on the surface of pancreatic cells—one of the cell types affected by CF. A defect in this process could potentially cause a host of changes in both the cells and their environment, since membrane insertion and removal is an important way for cells to control both secretion and placement of proteins on their surface. When membrane-bound vesicles inside the cell fuse with the plasma membrane that surrounds the cell, proteins inside the vesicles are released to the outside, and proteins in the vesicle membrane become part of the plasma membrane.

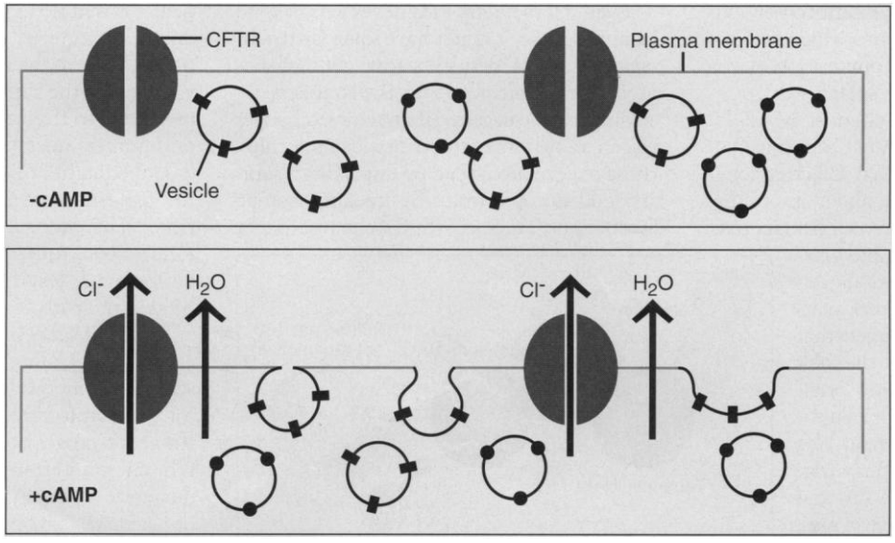
The inspiration for the group's experiments came from the finding of their Alabama colleagues Eric Sorscher and Ray Frizzell that a CFTR-containing cell line adds membrane to its surface in response to the intracellular messenger, cyclic AMP (cAMP). Since cAMP triggers CFTR-related chloride conductance, Kirk and Bridges wondered whether CFTR might also play a role in cAMP-stimulated membrane insertion, or exocytosis. To find out, the team studied a line of pancreatic cells derived from a tumor in a CF patient. The cells contain defective CFTR, and the researchers found that they don't have cAMP-stimulated exocytosis. But when normal CFTR was added to the cells, cAMP did trigger exocytosis, and also inhibited endocytosis, or membrane removal. This suggested CFTR was controlling both processes.

"I think it's quite an important observation, that CFTR isn't a protein that just does its job in the plasma membrane but is also involved in intracellular processes," says Michigan's Collins. "It will open a new area of investigation."

Despite such enthusiasm on the part of some of his colleagues, Kirk is quick to warn against making too much of the finding. "The most I'd want to interpret from our work is that CFTR is a participant in the regulation of exocytosis and endocytosis," he says. By doing so, it could be influencing the placement of important proteins on the cell surface, but there is no evidence yet that that is so. And, he adds, several other big issues remain to be resolved. Does CFTR play a similar role in other cells known to be affected by cystic fibrosis? If it does, how does it control the membrane flow from the surface of the cell, or from the membrane-bound compartments inside the cell? And is it acting as a chloride channel, or in some completely novel way?

Columbia University researcher Qais Al-Awqati, a fan of the Kirk and Bridges work, proposes answers to some of those questions: The CFTR protein may act as a chloride channel in internal compartments of the cell, and its absence in CF may affect the way some proteins are processed. Among the affected proteins, says Al-Awqati, may be ones that play a role in exocytosis. Al-Awqati's hunch is based on work from his own lab, which last year showed that there is a chloride channel that is active in internal cell membranes, and acts to acidify the compartments where pH-sensitive enzymes modify proteins. In CF, the channel doesn't function. The result is a higher than normal pH, which changes the composition of mucus proteins.

Skeptics say spinning such scenarios from the Alabama team's data may be premature. "We can't answer whether [the finding] is important or not; it's just an observation," says CF researcher Michael Welsh, of the University of Iowa. "You can speculate, but the data to support the speculation are missing." And,



Two jobs. When it is triggered by cAMP, the cystic fibrosis protein lets chloride ions and water leave the cell, and also causes vesicles in the cell to fuse with the cell membrane. The relationship between the two functions is not known.

he adds, just because normal CFTR puts membrane on the cell surface doesn't mean that there are important proteins in that membrane whose absence contributes to the progress of the disease.

Jack Riordan of Toronto's Hospital for Sick Children joins the chorus of skeptics, taking issue with Collins' and Al-Awqati's view that chloride secretion can't explain all the pathology of CF. "In the end state of this very severe disease, you can measure differences of all kinds," says Riordan. But, he adds, those differences may not be significant. "I'm a proponent of the idea that all [the symptoms] follow directly from the chloride impermeability in the plasma membrane."

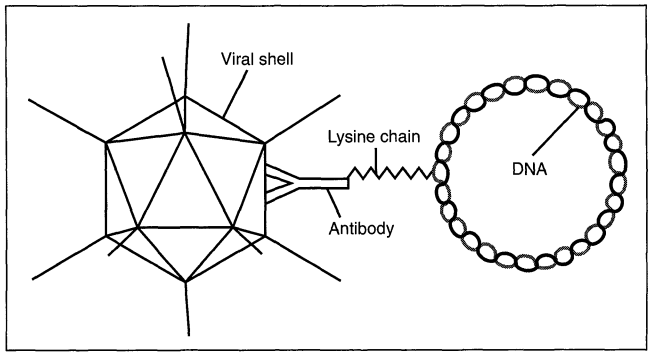
Riordan and others find the attention being paid to membrane flow and mucus proteins to be reminiscent of the "bad old days" in the CF field, before the chloride defect was found, when researchers wasted precious time and money chasing after mysterious "factors" in mucus, and other chimera that never materialized. "There is a real resistance in the field to moving back into the dark ages," says Alabama's Frizzell. "On the other hand, I think we should all be open-minded, and take the attitude that if we have a finding like this that clearly seems to be related to CFTR [function], that the mechanism of that is worth discovering." Until that mechanism and its significance are revealed, this debate, like others in the feisty field of CF research, is likely to rage on.

–Marcia Barinaga

GENE THERAPY

New Vector Puts Payload on the Outside

The quest for the perfect gene delivery system has at times seemed one of those impossible dreams. In the 8 years since scientists have been packaging healthy genes in viruses for delivery into cells, they have tested a dazzling and imaginative array of potential viral vectors in search of the one that combines maximum efficiency with optimum safety—but all approaches fell short of perfection, because those that were efficient weren't always safe. One reason for the problems was that in every one of those approaches, the DNA was packaged inside the virus, and this meant viral genes were transported into the cell along with the therapeutic genes. Now, though, a team of scientists from the University of North Carolina at Chapel Hill and the Research Institute of Molecular Pathology in Vienna, Austria, have announced a new vector that takes advantage of a virus's ability to get inside of cells, while inactivating the virus's genes. Instead of packaging the DNA inside the virus, they hook it onto the outside of the viral shell. "It's a totally different approach," says gene therapy expert Savio Woo of the Baylor College of Medicine in Houston. "Because there are no viral sequences, we don't have to contend with the safety issue."



Grabbing the ring. A new system for delivering genes to cells relies on an antibody molecule and a chain of amino acid units to hook DNA to the ouside of adenovirus.

Reporting in the April issue of *Human Gene Therapy*, David Curiel, Ed Hu, and colleagues at the University of North Carolina at Chapel Hill and collaborators Ernst Wagner, Matt Cotten, and Max Birnstiel at the Research Institute of Molecular Pathology in Vienna, Austria, describe the new vector, which in addition to its potential safety advantages can carry more DNA than traditional viral vectors.

As innovative as it is, however, this "new" vector is but a technical variation—though an intriguing one—on a well-established theme. Researchers in labs interested in curing respiratory illnesses like cystic fibrosis have used the same virus—adenovirus—because it is able to target the epithelial cells lining the respiratory tract. But until now, adenovirus had a large drawback. As Curiel puts it: "The good thing about adenovirus is that it enters cells, and that's what we want. The bad part is that it carries with it its own genes." So he and his co-workers decided to "capitalize on the virus in a selective manner" by using its "entry features, but dispensing completely with potentially harmful viral genes."

The capacity for entering a cell, Curiel notes, resides in the virus's outer protein shell; none of the viral genes are needed for entry or contribute to the system's efficacy. So Curiel and his colleagues have either deleted or otherwise inactivated the adenoviral genes. But the real innovation lies in their decision to tether the DNA to the outside of the viral coat using a chemical linker.

The transporter consisting of the virus and its linked DNA enters the cell via a surface receptor and gets taken into the nucleus via normal cellular uptake and transport processes. Once in the nucleus, the therapeutic gene can be expressed along with native host genes. In test tube studies, the imported genes were expressed at a high level, rivaling the best of the traditional gene delivery systems. Since developing the system, Curiel and his colleagues have been stunned by its potential versatility. "This system," says Curiel, "does it all; it is so plastic, so flexible."

Part of that versatility comes from the linker that attaches the DNA to the viral coat. The linker binds to the virus by means of an antibody molecule specific for adenovirus. At the other end of the antibody is a chain made up of lysine amino acid units. Lysine, notes Curiel, combines indiscriminately and spontaneously with nucleic acids, so just about any nucleic acid—not just DNA but also RNA—can be fastened to the virus. This, he says, may make the system useful for antiviral therapies that require antisense RNA as well as for gene therapy.

And there are other benefits to putting the DNA on the outside. Until now, the size of the viral shell, like a suitcase stuffed to bursting, dictated the size of the DNA molecule that could be transported, with most viruses capable of transporting molecules no larger than 7000 bases—slightly more than the cystic fibrosis gene without its regulatory regions. In contrast, Curiel and his colleagues have used the new vector to transport 48,000 bases of DNA successfully. And they are now gearing up to test their system with yeast artificial chromosomes that include several hundred thousand bases.

That's all well and good, but the real proving ground for any viral vector is in animal models—a hurdle Curiel's model hasn't yet leaped. Says gene therapy expert Inder Verma of the Salk Institute in LaJolla, California: "It is yet another interesting method, but the test is in whether you can get expression of the therapeutic gene in vivo and have it cure a disease." And the downside to adenovirus—in contrast to the retroviruses that many other gene therapy experts work with—is that foreign genes remain only transiently in their hosts, 6 weeks with adenovirus versus a lifetime with retroviruses. But, says Curiel, the system allows so much DNA to be transferred that sequences that make possible permanent integration into the genome could, in theory, be added to the therapeutic gene. And this new vector is so intriguing that many of its theoretical possibilities will no doubt fairly soon be put to the test.

–Michelle Hoffman