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."

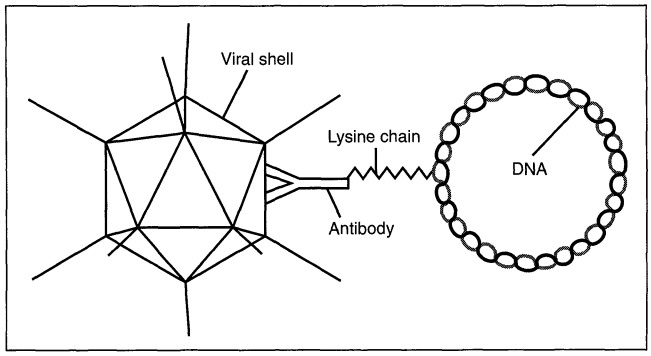
Riordan and others find the attention being paid to membrane flow and mucus proteins to be reminiscent of the "bad old days" in the CF field, before the chloride defect was found, when researchers wasted precious time and money chasing after mysterious "factors" in mucus, and other chimera that never 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