MUSCULAR DYSTROPHY

Backup Gene May Help Muscles Help Themselves

For a detailed look at progress in several areas of tissue regeneration, please see the Special Issue beginning on page 59.

Researchers are beginning to learn that, just as a single engine can keep a passenger jet aloft if the others fail, mammalian DNA contains redundant genes that might, in a pinch, be able to stand in for their counterparts. Now, scientists are trying to use one of those genes to treat a currently incurable human genetic disease: Duchenne type muscular dystrophy (DMD), which mainly affects boys.

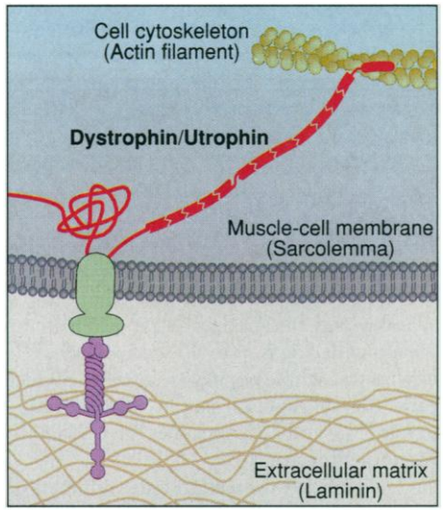
Every year, about 21,000 male babies worldwide are born with DMD, which is caused by a genetic defect that renders them incapable of making a key muscle-strengthening protein called dystrophin. As a result, their muscle cells slowly rupture and die, usually leading to heart or respiratory failure by age 20. But recent work in mice suggests that it may be possible to correct this defect by enticing muscle cells to make more of a very similar protein, called utrophin. These results, says Ron Schenkenberger, director of research for the Tuscon, Arizona–based Muscular Dystrophy Association, are "the most exciting development" in recent studies on DMD.

If researchers can find a way to duplicate the animal results in boys with DMD, it might be possible to prevent muscle-cell loss or even repair damaged muscles. For example, researchers might be able to find drugs that can turn up the activity of an existing utrophin gene—a strategy similar to one that has already proved effective in treating another genetic disease, sickle cell anemia.

Still, the history of efforts to come up with DMD therapies is tempering optimism. Discovery of the dystrophin gene in 1987 fueled hopes that dystrophin itself could be replenished in the muscles of those with DMD, either by injecting them with myoblasts—young, undifferentiated muscle cells from healthy donors that would then populate the muscles with normal cells—or by infecting diseased muscle cells with genetically engineered viruses carrying the dystrophin gene. But neither approach has yet panned out (*Science*, 24 July 1992, p. 472).

Aware of these problems, geneticist Kay Davies of Oxford University in the U.K. decided to see whether a healthy gene could pinch-hit for dystrophin. In a similar maneuver, researchers are already replacing hemoglobin—the protein impaired in sickle cell patients—using urea compounds that activate the gene for fetal hemoglobin, which is normally turned off after birth. Davies and her team had already identified the gene for a protein that might play such a role in DMD. In 1989, they had used dystrophin's own gene sequence as molecular bait to find similar genes—and thereby hooked the utrophin gene. The proteins encoded by the two genes turned out to be extremely similar: 80% of their amino acids are identical, although they have different functions.

Dystrophin is part of a complex of proteins that lash the fibers of muscle cells' internal skeletons, made of the protein actin, to an external support network, the extracellular matrix. These connections help buttress muscle fibers against the forces of stretching and contraction. In contrast, utrophin is found mainly at the synapses between muscles and their controlling nerves, where its role remains largely unknown.



Protein proxy. In transgenic mice, utrophin took over for its cousin, dystrophin (red), stemming muscle breakdown.

Davies had a tool to test her idea that utrophin could perhaps be made to stand in for dystrophin: the *mdx* mutant mouse, which produces little dystrophin. But first, she had to get the mice to produce utrophin in more of their muscle tissue than is usual. To do this, Davies, geneticist Jonathon Tinsley, and four other Oxford colleagues first genetically engineered a non-*mdx* strain to carry a condensed version of the utrophin gene attached to a promoter, or on-off switch, that would keep the gene turned on throughout an offspring's skeletal muscles. The mice carrying the utrophin transgene were then bred to *mdx* animals.

Before this experiment, other muscular dystrophy researchers doubted that utrophin could fill in for the missing dystrophin. If it could, they argued, muscular dystrophy patients would naturally up-regulate the utrophin gene to produce more of the protein. Because they do not, "most people didn't think the experiment would work," Davies recalls. But when the Oxford team examined the progeny of the two mouse strains, they found that male pups who had inherited both the dystrophin deficiency and the transgene had utrophin everywhere that dystrophin should be.

What's more, as the group reported in the 28 November 1996 issue of *Nature*, the utrophin appeared to decrease sharply the muscle damage in the animals. Dying muscles release an enzyme called creatine kinase into the bloodstream, and mice with the active utrophin gene had only about one-fourth as much of this enzyme in their blood as their nontransgenic littermates. Under the microscope, moreover, muscles such as the diaphragm showed no signs of cell breakdown. The Oxford findings "are certainly promising, certainly encouraging," says Eric Hoffman, a geneticist at the University of Pittsburgh School of Medicine who, in 1987, was a co-discoverer of the dystrophin gene.

In two papers in the 24 February issue of the *Journal of Cell Biology*, the Davies lab and that of neuroscientist Joshua Sanes at Washington University in St. Louis report what may be another sign that the two proteins are interchangeable. They found that mice with their utrophin genes knocked out have only mild motor impairment; this suggests either that utrophin is not essential at neuromuscular junctions, or that dystrophin, or some unknown third member of the dystrophin-utrophin family, can substitute for it.

Turning this apparent redundancy to the advantage of human DMD patients may take a long time, however. Utrophin will probably be no easier to sneak into muscle cells through gene therapy than dystrophin is, says Davies. Her original idea—boosting endogenous utrophin gene activity with a drug—may be more promising, but it hinges on whether researchers can find a compound that turns up utrophin production without producing intolerable side effects. Some 30 researchers discussed strategies for that search at a meeting in January at Long Island's Cold Spring Harbor Laboratory. And Oncogene Science Inc., of Uniondale, New York, is already screening for such a compound, with help from a $700,000 grant from the Association Française Contre Les Myopathies. "Within 2 years, we will know whether [up-regulating utrophin] will work," says Davies. "Right now, it's very difficult to tell. ... This is unknown territory."

–Wade Roush