Hopping to a Better Protein

Clinical trials are under way to test an innovative use of antisense technology to stem paralysis in Duchenne muscular dystrophy

EXON SKIPPING. IT SOUNDS LIKE A GAME, like hopscotch. But it’s not child’s play. A half-dozen research teams around the world are scrambling to turn exon skipping—which involves tricking a cell’s protein-making machinery into skipping over defective parts of a gene—into a treatment for a devastating muscular disorder called Duchenne muscular dystrophy. “It’s the best shot” for stemming the progressive paralysis that puts teenagers in wheelchairs and, in many cases, leads to premature death, says Eric Hoffman, a geneticist at Children’s National Medical Center in Washington, D.C.

A concept first demonstrated in the mid-1990s, exon skipping uses short stretches of DNA-like molecules called antisense to shut down a faulty section of a gene. In the past 2 years, promising results in animals and in cell-culture tests, as well as from a safety evaluation in people, have energized the muscular dystrophy community. Clinical trials of antisense drugs that home in on defects in the gene in Duchenne muscular dystrophy are now under way to assess how much muscle function can be restored. “A lot of things are jelling,” says Hoffman.

Questions still remain. It’s not clear how to reach all the body’s affected tissue or how effective treatments will be over the long term. There is also much uncertainty about how these treatments would be regulated. “It’s got a long way to go to determine whether it’s going to be a drug or not,” says C. Frank Bennett of Isis Pharmaceuticals in Carlsbad, California, a company developing antisense drugs for other diseases. But a group of experts who met in Cold Spring Harbor, New York, in October to discuss the potential of exon skipping were optimistic that the obstacles are surmountable. Researchers understand the disease and the principles of exon skipping much better and have also improved antisense technologies, says Bennett. “It’s sort of a three-part stool that’s come together: All this progress is really beginning to pay off.”

Tough disease

Duchenne muscular dystrophy is the most common inherited childhood disorder. A sex-linked disease, it affects one in 3500 boys; without mechanical ventilation, death can occur before age 25. It is caused by mutations in the gene for dystrophin, a protein that helps stabilize the muscle cell membrane.

In severe cases, the mutations lead to an aberrant molecule of messenger RNA (mRNA), which carries the instructions for making the protein to the ribosomes, the cell’s protein-making factories. The result is a highly truncated dystrophin or no protein at all. Lacking functioning dystrophin, the muscle cell membranes leak, the muscles gradually waste away, and paralysis sets in. But some mutations result in a milder syndrome called Becker muscular dystrophy, in which the muscle cells produce dystrophin that isn’t perfect but has enough activity to keep the muscle up and running.

In the early 1990s, researchers in Japan and elsewhere began to explore exon skipping. One early convert, Steve Wilton, now a molecular biologist at the University of Western Australia in Perth, discovered that in a few muscle cells of patients with Duchenne muscular dystrophy or animal models of the disease, dystrophin was restored, suggesting that the faulty sections of the dystrophin gene were being bypassed. He wondered whether it was possible to trick all the muscle cells into skipping over the mutated regions to produce a functioning protein. “I knew what I wanted to do, but I wasn’t sure how,” he recalls—until he heard Ryszard Kole of the University of North Carolina, Chapel Hill, describe how he had restored normal function to a mutated beta-globin gene using antisense technology in 1996. “It hit me like a brick” that the approach might work with dystrophin, says Wilton.

In a normal cell, the gene’s protein-coding regions—the exons—produce stretches of RNA that are spliced together into a single mRNA molecule. But mutations in one exon can lead to faulty mRNA and make it impossible for the protein-making machinery to read the rest of the mRNA strand. The idea behind exon skipping is to target the disruptive exon, or a nearby one, with antisense drugs, so it is not transcribed into mRNA (see diagram, p. 1455). The resulting mRNA would be missing a section, and the protein produced would lack some amino acids. But it would still function, much like the dystrophin in Becker muscular dystrophy.

In a matter of months, Wilton and Kole had used antisense molecules to restore dystrophin gene function in cultured muscle cells, and a few years later, in live mice. His colleagues around the world were reporting similar successes, but Wilton had trouble getting support to follow up on the work. Antisense technology had been heavily touted in the early 1990s as a potential treatment for a variety of diseases, but it had not lived up to its promise (Science, 27 October 1995, p. 575). Wilton says one colleague even called exon skipping “a party trick.”
The skepticism was well-founded. The cell membrane doesn’t naturally let DNA molecules in because they may belong to a pathogen, yet antisense DNA needs to infiltrate the cell to do its work. That’s less of a problem in muscular dystrophy because the disease makes the cell membrane leaky, but once inside, the foreign DNA must avoid being degraded by enzymes. To make matters worse, the quantities of an antisense drug required can stimulate a strong immune response. And because mutations can occur all over the dystrophin gene, an individual patient could require an antisense molecule targeted to any one of the gene’s 79 exons.

Better chemistry

None of those problems deterred Wilton and others. They began designing DNA sequences that would bind very specifically to individual exons. Wilton now has 40 that he says are ready for clinical trials. At the same time, private companies began to harness ways to disguise the antisense molecules to avoid destruction by the body. The point is to have it “look less and less like a nucleic acid,” says Hoffman.

The four bases that make up DNA hang off a molecular backbone that consists of alternating sugar and phosphate molecules. Taking one approach, AVI BioPharma in Corvallis, Oregon, has replaced the pentagon-shaped sugar with a hexagon-shaped “morpholino” and substituted a linker called phosphorodiamidate for the phosphates. A Dutch biotech company, Prosensa, based in Leiden, has masked the sugar backbone by adding a methyl group.

Gert-Jan B. van Ommen of Leiden University Medical Center in the Netherlands, another pioneer in exon skipping for muscular dystrophy, has been working with Prosensa for the past 5 years testing its antisense drug candidates. “We successfully get skipping in human and mouse cells and in vivo in mouse,” says Van Ommen. In 2006, he and his colleagues took the next step: They injected an antisense molecule targeted against exon 51 into the leg muscles of four patients and looked for the appearance of dystrophin at the injection sites. In each, “we got expression,” says molecular biologist Gerard Platenga, Prosensa’s CEO.

The results, published in the 27 December 2007 issue of The New England Journal of Medicine, caused quite a stir. “After the paper came out with the beautiful pictures of dystrophin expression, the community as a whole has been excited,” says Jane Larkindale, research program coordinator for the Muscular Dystrophy Association in Tucson, Arizona. “[Duchenne muscular dystrophy] patients and their families are very eager to try any therapy that has potential to work.” Now Prosensa is midway through a larger study to determine the dosage requirements for effective treatment.

In the United Kingdom, another research group is testing a morpholino antisense drug. Francesco Muntoni of the University College London Institute of Child Health and colleagues have injected the feet of five of seven patients and are planning a larger study that will involve periodic injections of 16 individuals.

Qi Long Lu, a pathologist at Carolinas Medical Center in Charlotte, North Carolina, has added a peptide rich in the amino acid arginine to the morpholino antisense. It readily slips into the heart muscle in a mouse model of muscular dystrophy, he and his colleagues reported in the 30 September issue of the Proceedings of the National Academy of Sciences. That could be a big advance because previous studies have suggested it’s almost impossible to get antisense molecules into heart muscle, which would be a major potential limitation to the utility of this therapy, Lu points out.

Another approach is in the works. Luis Garcia, a molecular biologist at the Institut de Myologie in Paris, is pursuing exon skipping with a twist. He has come up with a minigene that codes for an antisense molecule and is developing ways to transfer the gene into the patient’s own muscle stem cells to create a more permanent source of error correction with just a single injection. The approach so far has restored dystrophin in a mouse model of muscular dystrophy using patients’ cells, and he’s trying it out in a dog model of muscular dystrophy, he says.

Regulatory hurdles

But even if antisense drugs are successful in mice, in dogs, and even in people, the nature of these small molecules is raising regulatory challenges. Because antisense drugs are a hybrid between a “biological” and a “small molecule drug,” it’s not completely clear which regulatory rules apply. For example, the U.S. Food and Drug Administration guidelines for determining the correct drug dosages for humans based on animal studies might not work for these molecules, says Hoffman.

Another concern is that an antisense drug might turn off an exon in another gene that has a very similar sequence to the target. Assessing that sort of toxicity will be hard to do in animals, in which such similar exons might not exist.

Furthermore, these drugs will truly be “personalized,” as the antisense sequence must be tailored to the particular exon mutated in each patient. “For the rare mutations, there will not be sufficient patients to do a clinical trial,” says Larkindale. And some patients may require a cocktail of antisense molecules that will coordinate the skipping of more than one exon, complicating approval procedures even further.

“There are lots of issues,” says Hoffman. Nonetheless, “you have to give Wilton and others a lot of credit for doggedly pursuing this and having it emerge as [the] winning horse.” And Wilton thinks more than Duchenne muscular dystrophy is riding on their work. Others are exploring using antisense technology to treat the blood disorder thalassemia, an aging syndrome called progeria, and another degenerative disease, spinal muscular atrophy. “If exon skipping does not work for Duchenne muscular dystrophy,” says Wilton, “I find it difficult to believe it could work for any other conditions.”

—ELIZABETH PENNISI