

white blood cells, keratinocytes, fibroblasts, and liver cells. "Virtually no cell type used so far has delivered consistent high levels of gene product over a long period of time," says Blau. The others worked poorly for one of several reasons. Either the "on switch" of the therapeutic gene was inactivated, or the carrier cells were lost or destroyed in the host body, or the cells survived and produced protein, but became isolated from the blood supply, preventing the protein from getting into the blood.

Myoblasts do better than cells used in previous systems, both groups find, because they eventually differentiate and fuse to existing muscle tissue. "Because the cell is incorporated into an existing structure, it is not just tolerated, it is nurtured," says Blau. The result, she says, is that the implanted cells are likely to be longer lived than cells that have been injected into a foreign tissue. Theoretically, she adds, the implants could last the patient a lifetime.

In addition to these features, notes Leiden, the myoblast technique has some other qualities that could make it particularly useful for treating human patients. Myoblasts can easily be obtained by taking muscle tissue from an individual who needs gene therapy, and the genetically engineered cells can also be easily put back without causing damage to the patient's muscle.

But amidst the general enthusiasm for myoblasts, some gene delivery specialists are sounding cautionary notes. First, both the Stanford and Michigan groups used established myoblast cell lines, which have been perpetuated in culture for many years. The results could be different with primary cells—those freshly removed from an individual. Drawing from his own experience with fibroblasts, Inder Verma, a specialist in gene therapy research at the Salk Institute in La Jolla, California, notes, "There is a question as to whether you can get sustained expression in primary cells." And he adds, cell lines are not acceptable for use in humans because, "they make tremendous amounts of protein, but they always make tumors."

Furthermore, although myoblasts have the "potential to export any protein product into blood serum, what looks good in animals may not look good in humans," says noted muscular dystrophy researcher Louis Kunkel of Harvard Medical School. Indeed, attempts to treat muscular dystrophy with injections of normal myoblasts have so far not worked very well. Preliminary data obtained in one trial, conducted by George Karpati's team at the Montreal Neurological Institute of McGill University, show that "not enough donor myoblasts fused with host cells to make a func-

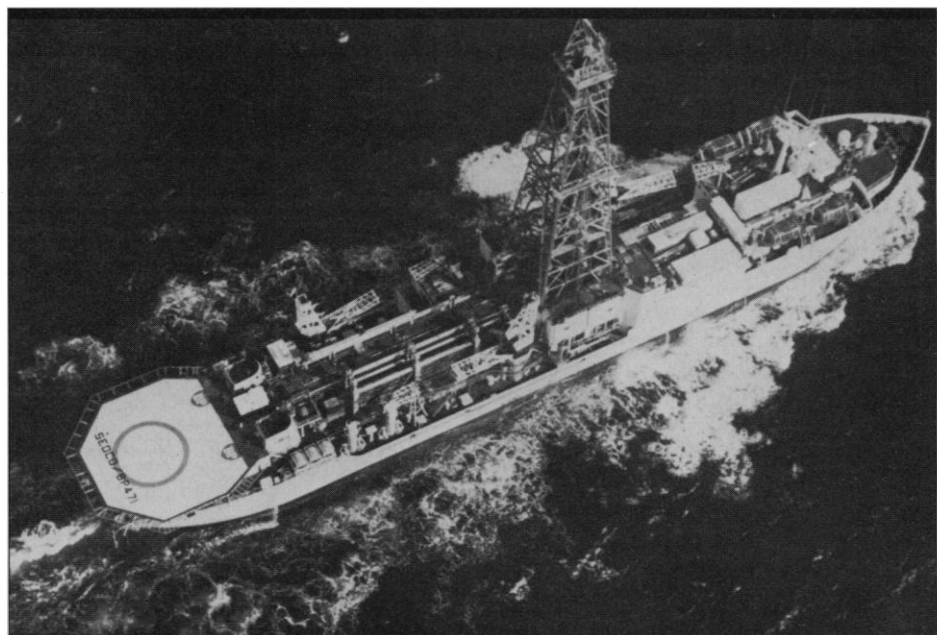
tional difference," says Karpati.

The myoblast fusion may have been poor because the cells may have difficulty penetrating the fibrous sheath of connective tissue that surrounds muscle fibers. Although previous work by Blau has shown that myoblasts can penetrate the connective tissue in mice, the number of myoblasts that can get across in humans "may be prohibitively low," says Karpati. Nevertheless, he notes that this may be more of a dilemma for replacing dystrophin, the protein lacking in muscular dystrophy, than for replacing secreted proteins. "You may not need so many myoblasts to take for a secreted protein like growth hormone," he says.

In spite of these difficulties, Leiden is very optimistic about the future of myoblasts for gene therapy. He envisions a day when scientists will be able to build a myoblast that

can do whatever they want them to—maybe even sense blood glucose levels and secrete insulin as needed in response. "That's far fetched, but I'm not sure it's out of the question," he says.

Other researchers are looking even further ahead. Noting that removing a patient's cells and reimplanting them is cumbersome, Nabel suggests that in the future gene therapy may involve direct injection of DNA into muscle cells, a technique pioneered last year by Jon Wolff and colleagues at the University of Wisconsin, Madison, and Vical Inc. in San Diego, California, who injected marker genes into muscle cells. While direct injection of DNA has not yet produced high protein expression levels, Nabel is hopeful that the method can be improved. If so, the gene therapy of the future may indeed be a shot in the arm. ■ MICHELLE HOFFMAN



Still pursuing the deepest crust. The 143-meter-long drill ship JOIDES Resolution may return to the deepest hole in the ocean floor for another try.

Coming up Short in a Crustal Quest

Last month, deep-sea drillers at a site in the eastern tropical Pacific were licking their lips as their drill bit slowly churned down toward a long-sought goal of the international Ocean Drilling Program: the lower-most region of the layer-cake-like ocean crust. Based on reflections from seismic waves sent down ahead of the drilling, the researchers expected that the bottom layer of the cake—the solidified remains of the magma chamber that gave birth to the crust millions of years ago at a midocean ridge—was only meters away. But once again in their long quest, the drillers came away without their goodies.

Even though they had bored through the ocean crust to a depth of 2 kilometers—deeper than ocean drilling had ever gone before—researchers aboard the drill ship *JOIDES Resolution* encountered no sign of the rock, called gabbro, that is typical of sub-sea magma chambers. "Once again, we discovered you should take seismic interpretations with a grain of salt," says Bruce Malfait, the National Science Foundation's program director for the Ocean Drilling Program. "It's not clear from shipboard data what they drilled through," he continues, "but it's definitely not" the top of the gabbro layer.

This deep-sea disappointment comes after 12 years of deepening the hole in an effort to sample a single section of ocean crust from top to bottom. The researchers had already pierced two of the three layers that are created as ocean crust is generated at a midocean ridge. First came a 600-meter bed of pillow lavas, formed as molten rock oozed onto the sea floor from the crest of the ridge, and then a kilometer of fossilized dikes—near-vertical channels that had fed magma into the ridge. Those findings had confirmed predictions about the structure of ocean crust that had been made from slivers of ocean floor pushed onto continents.

But the last layer—the remains of the underlying magma chamber—had eluded the researchers, as the hard rock and the deep hole taxed the technology of deep-ocean drilling to the limit. Over the years, it had taken six trips to the site, each lasting up to 2 months, to get this far. And all but the most recent leg of drilling had ended with the drill pipe broken off and lost in the hole, necessitating a “fishing” operation to recover it.

This time, at least, the drilling went off without a hitch—the researchers simply had no time to drill further when they didn’t reach the gabbro layer on schedule. The smooth drilling operation together with subtle signs in the rock that the top of the magma chamber is not far off will probably encourage the ocean drillers to return to the site yet again, says Malfait, perhaps as early as 1994.

Still, there is an easier way to get to the bottom of the crust: Go to one of the rare spots on the sea floor where rifting or faulting has exposed the deep crust. Ocean Drilling Program researchers in fact did just that in 1987, when they punched right into 500 meters of gabbro in the southern Indian Ocean without having to drill any other rock. By taking this shortcut, though, the drillers missed seeing the dikes and lavas that this magma chamber gave rise to, as well as the transitions between layers—among the details of ocean crust that they are eager to study.

But such shortcuts offer the only hope of reaching another long-sought goal of ocean drilling: the boundary between the crust and the mantle, called the Moho. A year from now *JOIDES Resolution* is scheduled to sink a hole near the Galapagos Islands starting in lower-crustal rock that was exposed by rifting. If the hole reaches the Moho, deep-sea drilling will have attained the goal sought 30 years ago in the first ocean drilling effort, Project Moho, which foundered in a financial, political, and technical morass. ■ RICHARD A. KERR

Excess Genetic Baggage Dumped

In recent years, putting new genes into plants has become routine, as plant scientists have genetically engineered a wide variety of plant strains having novel genes that help them resist pests and disease, improve their nutritional properties, and produce drugs and industrial enzymes. Not widely appreciated, however, is the fact that all those genes have been introduced into the plants in combination with so-called marker genes, usually encoding for antibiotic or herbicide resistance, that are used as laboratory tools to select the few plants that have acquired the novel genes from the many that have not.

The genetic engineers had previously been unable to remove the marker genes, but now two groups, one including David Ow and Emily Dale of the U.S. Department of Agriculture’s Plant Gene Expression Center in Albany, California, and the other lead by Brian Sauer of DuPont-Merck Pharmaceutical Company in Wilmington, Delaware, have independently come up with a new version of the gene transfer procedure that allows the marker genes to be expunged after plants have acquired the desired genes. “Doing any genetic manipulation in a directed way is significant, and the ability to pop out a sequence is particularly neat,” says Robert Goodman, a former executive vice president for research and development, at Calgene, a plant biotech firm in Davis, California, who is now at the University of Wisconsin, Madison.

Plant scientists have been eager for a method for popping out the marker genes. There’s currently no proof that the genes are harmful, but genetically engineered plants are beginning to move to market and there are concerns that trouble might arise as more and more acres are planted with them. There are fears, for example, that herbicide resistance genes might somehow be picked up by weeds or that pathogenic bacteria might acquire the antibiotic resistance genes, although the antibiotics involved, kanamycin and neomycin, are not widely used medically. Nevertheless, last spring Calgene asked the U.S. Food and Drug Administration (FDA) for an opinion on whether the kanamycin resistance gene can be used in genetically engineered tomato, cotton, and oilseed rape plants destined for commercial production. So far, the FDA has not issued an opinion, but should the agency rule against the markers, the

option for removing them is now available.

The markers have been so difficult to get rid of because they are introduced into plants on the same bit of DNA as the genes for the novel traits that researchers want to retain. As a result, the two genes are so tightly linked that the markers can’t be bred out of the plants by standard breeding procedures because if they go, the wanted genes go, too. What the Ow and Sauer groups did was develop a transgenic scissors that cuts the marker genes out. The scissors is an enzyme obtained from a bacterial virus. Known as “Cre,” for control of recombination, the enzyme neatly snips out any DNA located between a pair of identical 34-base pair sequences, called lox, for locus of crossing over.

The researchers construct the DNA used to make transgenic plants in such a way that the marker gene is flanked by lox sequences. After plants are successfully transformed with the DNA construct, they are crossed with another transgenic plant strain, this one carrying the gene for the Cre enzyme. In one-quarter of the progeny of this cross, the Cre enzyme produced in the plant cells cuts out the marker, leaving behind the desired gene. Those plants still have the Cre gene, but because it’s not on the same chromosome as the gene of interest, it’s easy to breed out of the plant strain. It takes a year or two, depending on the species, to

get plants that retain the desired recombinant gene, without any excess genetic baggage, Ow says.

Both Ow and Goodman agree that it’s too soon to know whether the technique will be picked up by the biotech industry, especially in view of the fact that

its use can delay the introduction of a new plant product by a year or two. Much depends on whether the FDA advises plant developers to remove them, and also on the U.S. public. If the public perceives the markers as dangerous, then the biotech industry will want to get rid of them whether or not they really are. As Ow points out, “This work could save the industry a lot of aggravation.” ■ ANNE SIMON MOFFAT

“This work could save the industry a lot of aggravation.”

—David Ow

ADDITIONAL READING

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