BIOCHEMISTRY

DNA Cuts Its Teeth—As an Enzyme

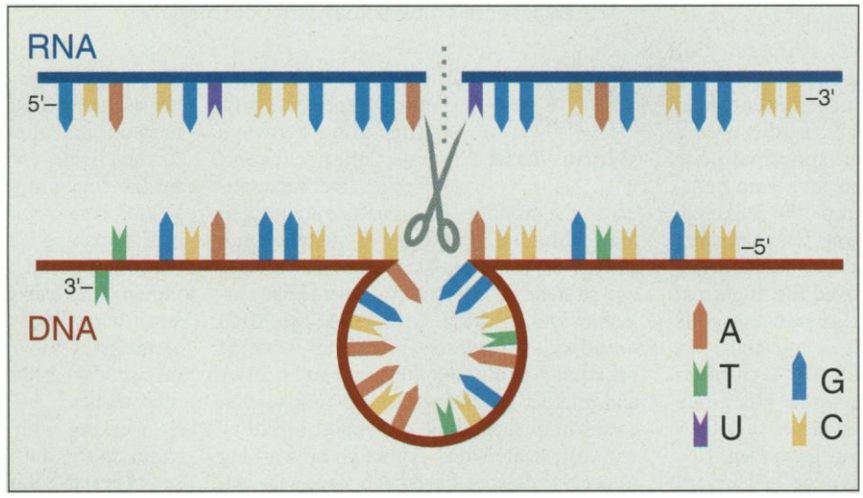
DNA enzymes can inactivate genes in laboratory tests by binding to and cleaving their RNA messages. These molecules may be moving toward the clinic

Each year some 1 million people worldwide with blocked coronary arteries opt for a procedure called balloon angioplasty. A surgeon inserts a catheter into the clogged artery and inflates a balloon that smears the atherosclerotic plaque against the vessel wall like peanut butter. It's simple and often effective. But 30% to 40% of these procedures fail because the cells of the artery wall react badly to the trauma the procedure inflicts: They furiously repair the damage, often totally clogging the artery in the process.

Molecular biologists have tried to stifle the key genes involved in this overzealous repair process by inactivating or destroying the RNA messages they produce. They have had varying degrees of success in animal models. Now, a team of researchers led by Levon Khachigian at the University of New South Wales in Sydney, Australia, has attacked the problem with a promising new gene-inactivating technology: an enzyme made from DNA.

The work, reported in the November issue of *Nature Medicine*, was an early test of the therapeutic potential of DNA enzymes, which until now have been mainly laboratory curiosities. Compared to other molecules that can inactivate genes, DNA enzymes are more stable and cheaper to make, and they may be more discriminating in choosing their targets, which could reduce side effects. And in Khachigian's test, they appeared to be effective. His DNA enzyme, designed to bind to and cleave the RNA made by a damage-sensing gene called *Egr-1*, seemed to keep ballooned arteries from closing up in rat models of heart disease. "This is an exciting new use of a powerful technology," says pathologist Tucker Collins of Harvard Medical School in Boston.

DNA enzymes are the able apprentices of RNA enzymes—RNA molecules that can catalyze chemical reactions, such as cleaving other RNA chains. Ribozymes, as they are called, were discovered in the early 1980s in living organisms; scientists soon made new variants in the laboratory, with different abilities, and then began to wonder whether DNA, RNA's chemical cousin, could follow the act.



Kind cut. A DNA enzyme snips the RNA message for a wound repair factor, Egr-1, that may play a role in narrowing coronary arteries that have been opened with balloon angioplasty.

No DNA enzymes are known in nature, but in 1994 Gerry Joyce of The Scripps Research Institute in La Jolla, California, and his then-postdoc, Ron Breaker, decided to try to create one using a laboratory version of natural selection. They hoped to produce a DNA molecule that could match the most elementary catalytic ability found in ribozymes: snipping the RNA phosphoester backbone. The starting ingredients for their primordial soup were trillions of random variants of a 50-base-long DNA chain, tethered to a matrix by a sequence that included an RNA nucleotide. When they washed the matrix with a lead solution, molecules capable of using the metal ion to help snip the tethering RNA nucleotide "selected" themselves into the column washings. The researchers then repeated the process several times, starting each round of selection with the population of molecules that had emerged from the previous round.

After 3 days, Breaker had evolved an efficient RNA-cleaving DNA enzyme. And later work in Joyce's lab by graduate student Steve Santoro produced the prize specimen, known as 10-23, for the 10th generation and 23rd clone. 10-23 had a catalytic efficiency higher than that of any known ribozyme. "It's the best we've ever seen; it will target any RNA you want," boasts Joyce.

To target a specific RNA molecule, 10-23's RNA-cleaving catalytic domain can be fitted with stretches of DNA that bind to target sequences in the RNA. When the 10-23 catalytic domain is within range, it cleaves the RNA by speeding up the background reaction that makes RNA inherently unstable: the attack by an oxygen in one of RNA's ribose sugars on one of the bonds in the backbone. The so-called hammerhead ribozyme, originally found in plant viruses, works the same way, but 10-23 is less choosy about where it cleaves the sequence. Whereas the hammerhead prefers to bite where it finds a sequence of the nucleotides guanine, uracil, and cytosine—a target that may not be accessible on a tangled RNA molecule—10-23 can sink its teeth into any junction between the two kinds of nucleotides, purine and pyrimidine. Such a sequence is found at the start site of RNA messages, which usually dangles freely. Says Breaker, who is now at Yale University, "That's the beauty of 10-23; a target sequence is always accessible."

Because 10-23 is made of DNA, it has some other powerful advantages over ribozymes. DNA lacks the inherent instability of RNA. "We have seen our DNA enzymes last at least 48 hours in serum," compared to minutes for ribozymes, says Lun-Quan Sun, a biochemist at Johnson and Johnson Research in Sydney, which owns the patent for commercial applications of the 10-23 DNA enzyme. DNA is also easier and cheaper to make than RNA. And because it takes a more precise match for a DNA sequence to bind to RNA than for RNA to bind to another RNA molecule, DNA enzymes may be better than ribozymes at selecting their targets and ignoring similar sequences.

ILLUSTRATION: C. CAIN; SOURCE: L. M. KHACHIGIAN

One place DNA enzymes could not supplant ribozymes, however, is in gene therapy. A gene for a therapeutic ribozyme could be inserted into the body, which would then make the ribozyme continuously; a DNA enzyme, in contrast, has to be synthesized and given as a drug.

Santoro and Joyce published the 10-23 sequence in the April 1997 *Proceedings of the National Academy of Sciences*, putting RNA-cleaving DNA enzymes in the hands of any researchers who wanted to copy the catalytic sequence. Explains Breaker, "These would be the simplest enzymes anyone can make. Just send an e-mail to a company that makes DNA, and you can have it the next day."

In the last few months, researchers have reported promising test tube results with customized variants of the 10-23 DNA enzyme. Sun and colleagues, for example, designed a DNA enzyme to target the growth-stimulating gene *myc*, which effectively froze the growth of smooth muscle cells in a culture dish. And Kazunari Taira's group at the University of Tokyo and the National Institute of Advanced Interdisciplinary Research at Tsukuba Science City in Japan deployed a DNA enzyme against an abnormal gene message made in certain leukemic cells. The abnormal protein keeps the leukemic cells from self-destructing when normal cells do, leading to uncontrolled growth. The DNA enzyme destroyed the abnormal messenger RNA and triggered the self-destruct circuitry in the leukemic cells.

Khachigian's work with DNA enzymes is a first step toward the clinic. His group altered 10-23 so it would target the RNA from *Egr-1*, a wound-repair gene identified by Khachigian in Collins's lab at Harvard in 1996. *Egr-1* acts like a chief of disaster operations. Undetectable in healthy arteries, its protein appears on the scene within minutes of injury, recruits a crew of tissue repair factors, and disappears a few hours later. Because *Egr-1* acts early in wound repair, Khachigian thought it might be a strategic target. "It's a case of shooting the messenger," he says.

Six hours before ballooning and during the procedure, the researchers applied the DNA to the outer surface of a rat's carotid artery, relying on chemical carriers to ferry it into the smooth muscle cells in the vessel wall. After 2 weeks, the new layer of cells lining the artery was twice as thick in untreated rats as it was in rats that had been treated with the DNA enzyme. Next, Khachigian plans to try this approach in pigs and then, if all goes well, in human patients.

But DNA enzymes modeled on the RNA-cleaving 10-23 molecule are just the beginning. In laboratory evolution experiments like the one that spawned 10-23, catalytic DNA molecules sporting bizarre new structures have emerged, some with four strands rather than the usual one or two, others co-opting amino acids—the building blocks of proteins—for extra catalytic power. Over the last year, these modified DNA variants have proved able to catalyze a whole new array of reactions in the test tube—for example, cutting, rejoining, and chemically modifying DNA strands. And Breaker thinks new DNA enzymes as potentially powerful as 10-23 could target proteins as well as RNA and DNA. "We must now consider the possibility that other enzymes like 10-23 are out there, with even greater catalytic power, just waiting to be discovered."

Says Breaker, "My goal is to develop the therapeutic warheads of the future."

–ELIZABETH FINKEL

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MEETING MATERIALS RESEARCH SOCIETY

Building the Small World Of the Future

BOSTON, MASSACHUSETTS—Nearly 4400 researchers gathered here from 29 November to 3 December to speed the way to future materials for everything from electronics to medicine. Highlights included new schemes for wiring molecular electronic devices and genetically engineered proteins that assemble materials.

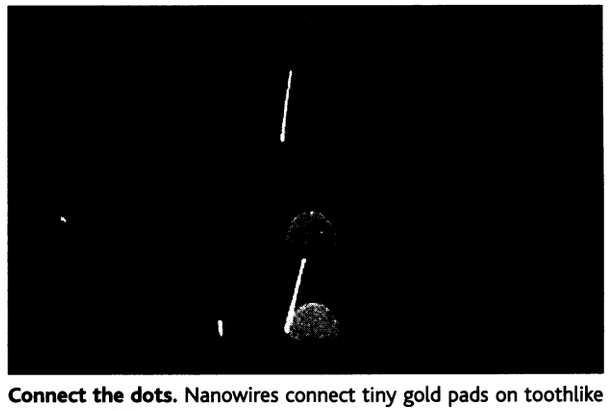
Wiring Up the Nanoworld

In computer technology, if not in space flight, smaller almost always means faster, cheaper, and better. But researchers at the frontiers of miniaturization, who are fashioning experimental switches and storage devices from single molecules, have outrun their ability to wire these devices together into working systems. At the MRS meeting, scientists described several schemes—including one that uses DNA and another involving electric fields—that could help them link molecular circuitry with nanoscale wires. Although no working molecular devices have been rigged up yet, experts say the approaches could help make dreams of molecular electronics a reality.

"This was really nice work," says Zhenan Bao, an advanced electronics specialist at Lucent Technologies' Bell Laboratories in Murray Hill, New Jersey. "It's the future direction the field [of molecular electronics] will take."

On today's computer chips, the smallest features are 250 billionths of a meter, or nanometers, across. Although that's vanishingly small, the devices can still be made and wired up with photolithography, the industry's workhorse patterning technology, which shines light through stencils to direct the etching of fine features on silicon chips. Molecular-scale devices, however, can measure just a few nanometers in some dimensions, and light simply can't be focused tightly enough to lay out the patterns for the fine wiring needed to connect them. Researchers have to take another tack, such as first making the minuscule wires, and then positioning and connecting them. But although they can already make sufficiently small wires—one technique condenses metal atoms in the pores of membranes to form tiny metal rods—achieving the connections is another matter.

Working with nanorods that have platinum shafts and gold tips, a team of researchers at Pennsylvania State University in University Park led by electrical engineers Theresa Mayer and Thomas Jackson, along with chemists Thomas Mallouk and Michael Natan, has now come up with two speedy ways to solve this problem. One as-



Connect the dots. Nanowires connect tiny gold pads on toothlike electrodes, 25 micrometers long from base to tip.

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