Triplex DNA Finally Comes of Age

More than three decades after it was first synthesized, triple-stranded DNA is showing promise as a tool for cleaving genomes precisely and blocking gene transcription

BACK IN 1957, WHEN ALEXANDER RICH, David Davies, and Gary Felsenfeld, who were then all at the National Institutes of Health (NIH), produced a triple-stranded form of DNA while studying synthetic nucleic acids, few researchers paid the discovery much attention. The Watson-Crick double helix had been discovered just a few years before, and DNA was, after all, supposed to have two strands, not three. Triplex DNA, it seemed, was simply an interesting anomaly, a novel manmade structure with no physiological or practical importance. It rapidly sank in the flood of new information about nucleic acids unleashed by the discovery of the double helix. But triplex DNA was never entirely forgotten—and that's fortunate, because work in the past few years suggests that it may be put to good use after all.

Researchers in several labs are exploring the use of triplex DNA to produce a new type of "molecular scissors" for cutting DNA. Even the best of the restriction enzymes that are currently used for that purpose cut at many sites, mincing the genome into a multitude of small bits that are hard to separate and analyze. But triplex-based scissors may be able to cut at one or a few sites, giving a much more manageable collection of large DNA pieces. Such scissors would be a great help in mapping and sequencing the human genome and in isolating individual genes.

Triplex DNA is turning out to have therapeutic potential as well. It has recently been demonstrated that triplex DNA can be generated at specific sites on naturally occurring genes, and this can disrupt gene transcription—the copying of the genetic material into messenger RNA that is the first step in protein synthesis. Researchers are beginning to explore whether this might be a new strategy for treating viral diseases, such as AIDS, by blocking virus reproduction. Or it might be used to interfere with the expression of genes that contribute to cancer development. Indeed, several start-up companies, such as Triplex Pharmaceuticals of Houston, Texas, as well as established ones, including Ciba-Giegy and Rhône-Poulenc, are trying to exploit the new technology therapeutically.

"It's gratifying to learn that our old physical chemistry is getting to be biologically relevant," says triplex DNA codiscoverer Davies, who notes that it doesn't come as a complete surprise. It's a tenet of faith to x-ray crystallographers such as Davies, who is still at NIH, that structure determines function, so a novel structure might be expected to have a novel function.

The development that opened the door to these potential new applications came about 4 years ago. The original triplex DNA produced by Rich, Davies, and Felsenfeld was a totally synthetic polymer. But in 1987, Peter Dervan and his colleagues at the California Institute of Technology and a second group led by Claude Hélène of the Muséum National d'Histoire Naturelle in Paris independently obtained results indicating that triplex DNA could be formed by tacking a third strand of DNA onto a stretch of natural DNA containing actual genes.

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Dervan came upon this during a 14-year search for a chemical, rather than a biological, method for cutting double helical DNA at specific sites. Restriction enzymes cut promiscuously because they home in on DNA sequences containing four to eight base pairs, and there can be as many as tens of thousands of copies of such short sequences in large genomes like the human. Dervan was therefore looking for a chemical that would recognize sequences as long as 15 to 20 base pairs. Sequences that long might occur only once in the billion-base-pair human genome.

But he faced a tough problem: There were very few rules to guide researchers in designing compounds that would home in on whatever DNA sequences they wanted to cut. Dervan's first efforts focused on synthetic peptide analogs of the antibiotic distamycin. "It was not a blockbuster technique," he ruefully recalls. Attempts to use proteins to recognize specific DNA sequences worked no better.

Then in 1985, with two strikes already against them, Dervan and his colleagues decided to take another look at a third strategy that they had originally considered in 1978 but had rejected as being more high risk than the peptide analogue and protein approaches for recognizing DNA. The idea was to use a third strand of nucleic acid to recognize the DNA sites to be cut.

This time the Caltech workers scored a hit. In 1987 they reported that synthetic DNA strands containing between 11 and 15 nucleotides would recognize and bind to specific sites on chromosomal DNA, forming a triple helix. And the binding followed chemical rules. The synthetic third strands, which contained only the two pyrimidine bases thymine and cytosine, bound so that the thymine recognized adenine-thymine base pairs in the target DNA and the cytosine recognized guanosine-cytosine base pairs. The thymines and cytosines in the third strand can therefore be arranged to recognize many gene sequences, Dervan says.

Moreover, by equipping the third strand of DNA with a chemical that oxidizes DNA (the chelating agent ethylenediaminetetraacetic acid complexed with iron), Dervan's group could turn it into a selective wrecking ball that would break the chromosomal DNA wherever it bound. Meanwhile in Paris, Hélène and his colleagues had showed that they could get specific DNA cutting by adding a light-activated cleaving compound to the third strand of the DNA triplex.

In the past year, Dervan's group has extended its earlier findings, showing that the triplex DNA technique could be used to cut a specific 20-base-pair sequence on chromosome III of yeast. The group's synthetic nucleic acid correctly targeted the desired sequence even though the yeast genome contains almost 14 million base pairs. And Dervan, with David Houseman of the Massachusetts Institute of Technology, may have achieved an even more impressive feat. The researchers have preliminary evidence

that the triplex method also works in the billion-base-pair human genome.

Those successes don't mean that all the bugs are out of the technique yet, however. "It is technically very exciting that triple helices can cut chromosomes," says Charles Cantor, currently principal scientist for the Department of Energy's human genome program, but he says he is not yet convinced that the technique can be used routinely.

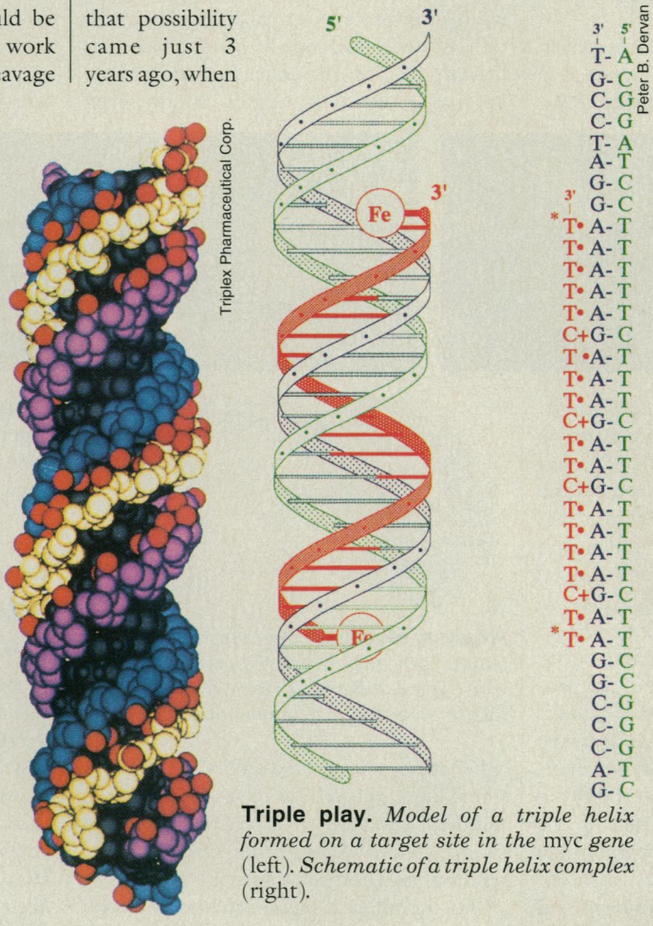
A major problem has been efficiency: The yeast genome work reported last year was successful only 6% of the time. But improvements are coming fast. Just 3 months ago, Dervan and his colleagues published results showing that this cleavage yield could be upped to 95%. The trick, he says, is to work a variation on the "Achilles heel" cleavage method pioneered by Waclaw Szybalski, Michael Koob, and their colleagues at the University of Wisconsin (*Science*, 15 July 1990, p. 127). In that method, the *lac* operator, a bacterial gene regulatory sequence containing 20 nucleotides, is modified to contain sites that can be clipped by restriction enzymes and inserted at the site in the genome that is to be cut. Then, when a protein called the *lac* repressor is added to the DNA, it seeks out and binds to the inserted *lac* operator.

The next step is to treat the DNA with an enzyme that adds methyl groups to the bases, an addition that prevents restriction enzymes from cutting the DNA. But the *lac* repressor protein protects the *lac* operator from being methylated, so appropriate restriction enzymes can still cleave there—and only there—with their normal high efficiency once the repressor is removed.

In the Dervan group's variation, a third DNA strand is used to protect the site to be cut from methylation. The cleavage efficiency is just as good as when the *lac* repressor is used. Indeed, Dervan and his Caltech colleague Scott Strobel got essentially complete cleavage of a yeast chromosome with their approach. And besides that, Dervan says, use of triplex DNA may be more generally applicable because it doesn't require the insertion of the *lac* operator into the DNA or the availability of rare DNA-binding proteins. It does require, however, that the target site contain a sequence recognized by a restriction enzyme.

Another recent development may help improve DNA cleavage by triplex DNA by broadening the number of sequences that can be targeted. The synthetic strands used in the early work did not contain the full complement of bases found in natural DNA—they contained the pyrimidines cytosine and thymine but not the purines adenine and guanosine. In 1988, however, Michael Hogan's group at Baylor College of Medicine got triplex DNA formation with synthetic strands containing purine bases in addition to the pyrimidines. Dervan and Hélène have recently done so too.

Work is moving apace on the therapeutic front as well. Though it was earlier expected that triplex DNA might be used to inhibit gene transcription, the first experimental verification of that possibility came just 3 years ago, when Hogan and his Baylor colleagues showed that, at least in a test-tube assay, triplex formation could selectively inhibit a target gene. Further, this was correlated to repression of the activity of the human *myc* gene.



Triple play. *Model of a triple helix formed on a target site in the* myc *gene* (left). *Schematic of a triple helix complex* (right).

About a year later, the Caltech group and the Hélène researchers showed that synthetic nucleic acids, functioning as third strands, could block the activity of the transcription factors that regulate the genes of higher organisms. Hélène says that a key challenge now is to prove that these oligonucleotides have their effect through the formation of triple helixes rather than some other way. The uptake of such synthetic third strands into the nucleus must also be improved.

Hogan was so impressed with the therapeutic potential of a technique that could, in theory, block the expression of any gene that he formed a company, Triplex Pharmaceuticals, dedicated to devising new therapies based on the triple helix concept. The first application, he says, is likely to be in AIDS therapy. He and his colleagues have already shown that a synthetic DNA strand can slow down replication of the virus in infected cells growing in lab cultures.

Another possible application, currently under investigation in Bert O'Malley's lab, also at Baylor, is in a new type of abortifacient. The hormone progesterone stimulates the growth of the uterine lining needed for normal implantation of the embryo. It may be possible to prevent implantation, O'Malley says, by using triplex DNA formation to block the activity of the genes through which progesterone exerts its effects. He has preliminary evidence that a synthetic DNA strand that binds to the regulatory regions of those genes inhibits their expression in cells in the test tube.

But will triplex DNA work in animals as well as it works in these *in vitro* systems? Some researchers are skeptical. "I think it's great there is renewed interest in triplex DNA," says transcription expert Harold Weintraub of the Fred Hutchinson Cancer Research Center in Seattle. "But I will wait and see whether it can turn off transcription *in vivo*."

Hogan thinks Weintraub's wait will be rewarded—and sooner rather than later. "We are gathering solid evidence that triple helix DNA can form in the nucleus, remain stable, and elicit an outcome, such as selective gene inhibition, that can be predicted," says Hogan, noting that the supporting data are in press in *Nucleic Acid Research*. This work is still limited to cultured cells, but Hogan also predicts that reports showing that gene transcription can also be altered in living animals will appear in the refereed literature within the year.

While much of the research on triplex DNA is admittedly high risk and speculative, the researchers take heart in knowing that such was the case for basic studies of nucleic acids themselves way back in 1957.

■ ANNE SIMON MOFFAT