Z-DNA: From the Crystal to the Fly

This unusual form of DNA now appears to have a biological function

From the very beginning, Z-DNA has been surprising. It was discovered quite by accident 2 years ago by Alexander Rich and his associates at the Massachusetts Institute of Technology and its structure was unlike any DNA molecule ever seen before. Some people thought it was just a chemical oddity but Rich, ever the believer that a structure so unusual must be exploited by cells, began searching for evidence that Z-DNA has a biological function. Now he has found evidence. To his own surprise, he discovered that it occurs in specific regions of chromosomes and, he proposes, it may be used to regulate gene expression.

Rich discovered Z-DNA in the course of trying to settle the structure of conventional B-DNA—the DNA whose structure was proposed by James Watson and Francis Crick. The problem with the B-DNA structure was that it was based on x-ray diffraction data from not very good crystals. Since it was impossible to see single atoms in the x-ray diffraction pattern, crystallographers had to guess at the DNA structure, and, as of a few years ago, several alternative models of B-DNA structure had been proposed.

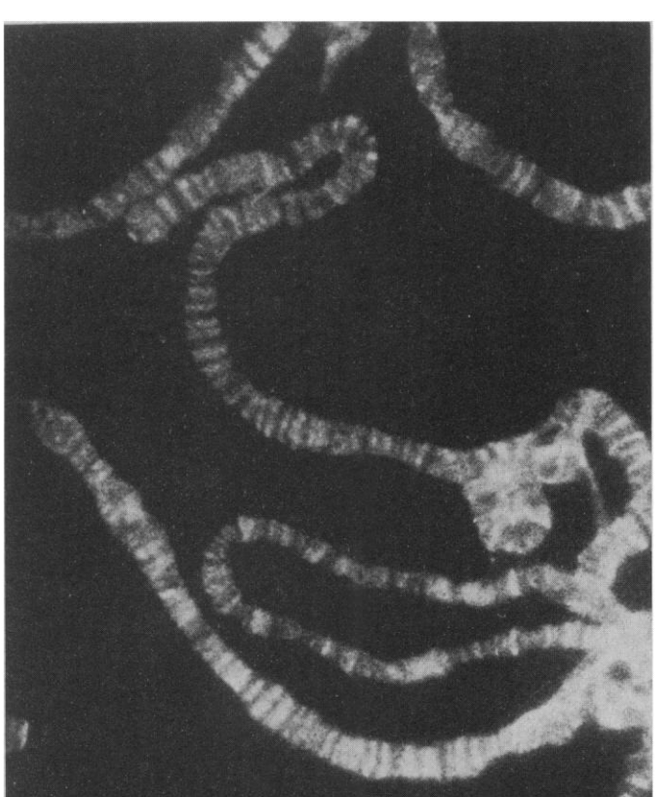
"I thought we could settle the matter with oligonucleotides," says Rich. "We could use a self-complementary DNA polymer, crystallize it, and solve the structure." Two organic chemists, Jacques van Boom and Gijs van der Marel of the University of Leiden, made tetramers consisting mostly of deoxyguanine and deoxycytosine. Rich and his associates, Andrew Wang, Gary Quigley, Francis Kolpak, and James Crawford, then crystallized the tetramers, hoping to get crystals that would give them atomic resolution in x-ray diffraction studies. (For atomic resolution, a crystal must diffract to a resolution of 1 angstrom or less.) "Out came a crystal that diffracted to 0.9 angstrom," says Rich. "We were very excited, but it was not an easy structure to solve. It took us 1½ years to solve it, and when the structure came out, it took us a long time to believe what we were seeing."

The polymer was a new form of DNA. The helix was left-handed rather than right-handed but, Rich remarks, "It was not simply a left-handed version of B-DNA. It's quite a different animal. You have to turn all the bases around and rotate them."

After considerable deliberation, Rich named the new DNA structure Z-DNA. He considered calling it D-DNA because already there were A, B, and C forms of the right-handed helix. But, since Z is so different, it seemed more appropriate to start at the other end of the alphabet. Besides, Z could stand for zigzag, because the backbone of Z-DNA, unlike that of B-DNA, zigzags down the molecule.

Having solved the Z-DNA structure, Rich says, "the next thing I did was to familiarize myself with some work done in 1972 by F. M. Pohl and Thomas Jovin at the University of Göttingen." Pohl and Jovin had put a deoxycytosine-deoxyguanine polymer into a solution and studied how its spectral properties changed as they steadily increased the salt concentration of the solution. They measured the circular dichroism of the molecule, which tells of symmetry but not structure. What they found was that at high salt concentrations, the circular dichroism of this polymer changed dramatically. "The spectrum was almost inverted at high salt concentration," says Rich. Pohl and Jovin speculated that they were seeing the DNA flip from a right-handed helix to a left-handed one, but had no proof that this was so.

Pohl and Jovin had also observed that the Raman spectrum of their synthetic DNA molecules was different in high salt. Raman spectra are a measure of atomic vibrations and are largely insensitive to the physical form of a molecule—the spectra are the same whether a molecule is crystallized or tumbling in solution. Rich, Wang, and two MIT chemists, Thomas Thamann and Richard Lord, determined the Raman spectrum of their Z-DNA crystals. The spectrum was identical to that of the molecule that Pohl and Jovin found in high-salt solutions. This indicated that Pohl and Jovin were indeed looking at Z-DNA and that Z-DNA can exist in solution as well as in crystals. Thus it may be a naturally occurring structure.



Fluorescent antibodies to Z-DNA bound to* Drosophila *chromosomes.

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At this point, says Rich, "people were divided into two camps. Some thought that Z-DNA was just a chemical oddity. Others, including myself, reasoned that nature is inherently opportunistic. Here's a stable conformation of DNA. Nature is likely to use it. Our job is to find out how."

One objection to this theory that Z-DNA occurs in nature was that so far the molecules had only been found in high salt concentrations. (The Z-DNA crystals had been stabilized with magnesium ions.) These are hardly physiological conditions.

Then Gary Felsenfeld of the Natioal Institutes of Health discovered that Z-DNA occurs under normal salt concentrations if the deoxycytosine-deoxyguanine polymer is methylated. It was known that methylation of cytosine-guanine sequences of eukaryotic DNA is tied to gene expression. When these sequences are methylated, genes are inactive. When they are not methylated, genes are active. "This suggested to me that Z-DNA is involved in gene regulation," says Rich.

"It was not so obvious, however, how to find Z-DNA in biological systems," Rich recalls. One way would be to make antibodies to Z-DNA and then see if they bind specifically to naturally occurring DNA. Normally, animals do not make antibodies to B-DNA, so it was not clear whether Z-DNA would elicit antibodies. B-DNA is not immunogenic during embryo development because there is enough B-DNA around that animals become tolerant to it and do not recognize it as foreign.

Working with immunologists David Stollar and Eileen Lafer of Tufts University School of Medicine, Rich and his colleagues Alfred Nordheim and Achim Möller tried to get rabbits to make antibodies to Z-DNA. "To our surprise, we discovered that Z-DNA is a powerful immunogen," says Rich.

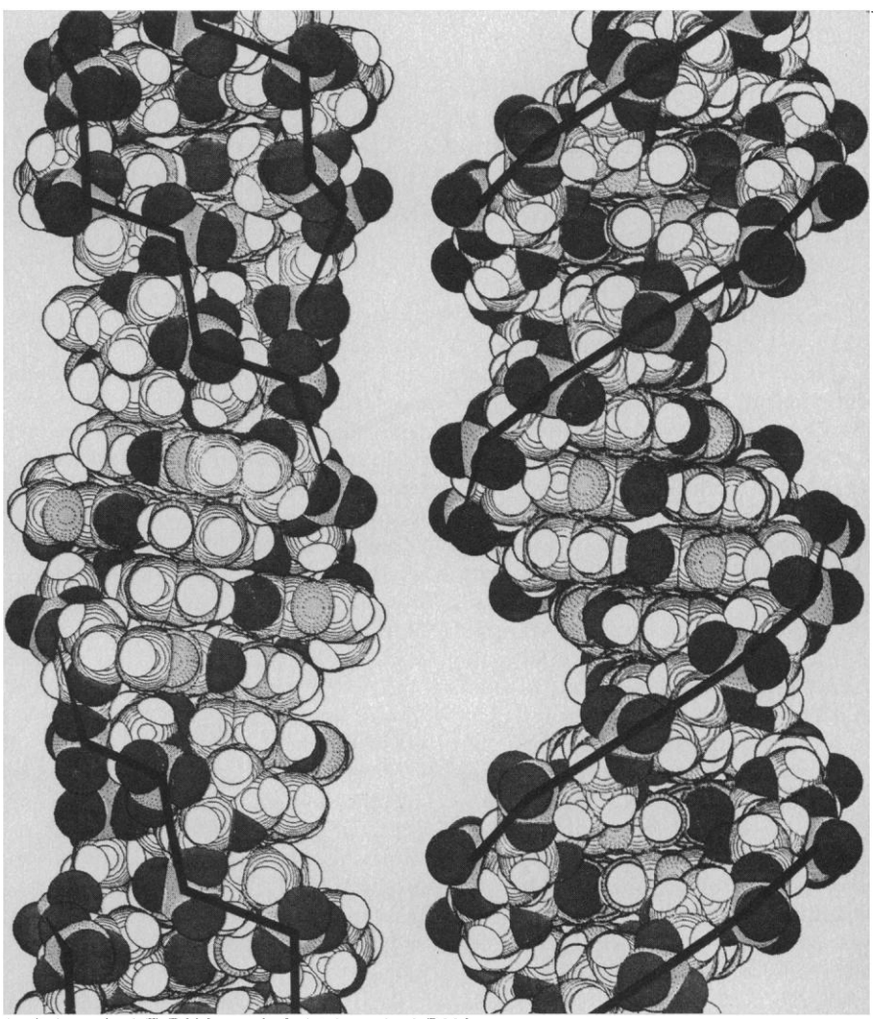
The next question the MIT and Tufts University group asked was whether Z-DNA antibodies might occur in nature, perhaps in autoimmune diseases. They looked at 12 mice with lupus, an autoimmune disease of unknown origin. All 12 had antibodies to Z-DNA, whereas normal mice did not. The implication is that Z-DNA must occur in mouse DNA. "It would be reasonable to look in humans with lupus to see if they too have antibodies to Z-DNA," says Rich.

"Once you have an antibody with high specificity, it's like a hunting license," Rich remarks. "The first place we decided to hunt was in chick erythrocytes. We used a fluorescent label and we saw a greenish glow, but it was not enough to give us confidence that Z-DNA was there. You can't quite quantitate glows."

Rich and his associates then decided to look for Z-DNA in polytene chromosomes of *Drosophila*. These chromosomes, because of an accident of nature, are so suitable for studying chromosome structure that they have become standard material for geneticists. The polytene chromosomes are in the salivary glands of third instar larvae of *Drosophila*. There are about 100 cells in each gland that continue to replicate their DNA but that do not divide. The replicated chromosomes arrange themselves in perfect alignment so that there are 1000 to 2000 copies of each chromosome. Chromosome structure can be seen because each chromosome is amplified 1000 to 2000 times and thus appears 1000 to 2000 times thicker than normal. The chromosomes are banded; there are dark bands running down the chromosomes separated by light areas, called interbands. Seventy to 90 percent of the DNA is in the bands but no one knows whether genes are in the bands or the interbands, or both. "The nature of the bands and interbands is a mystery," says Rich.

When Rich and Nordheim, working with *Drosophila* specialist Mary Lou Pardue at MIT and with the Tufts University group, added Z-DNA antibodies to *Drosophila* polytene chromosomes, they got a dramatic result. "To our surprise and delight, we found that Z-DNA antibodies stain the interband regions and stain them in a reproducible way. This is not a random sort of stain," says Rich.

Asked whether the Z-DNA antibodies could be cross-reacting and therefore recognizing something other than Z-DNA, Rich said he did not think so because of the results of his control experiments. When he added the antibody to a polymer of deoxycytosine and deoxyguanine in the B form of DNA and then added the antibody to the polytene chromosomes, the antibody bound to the



Left-handed Z-DNA and right-handed DNA

A computer-generated diagram from a report by Alexander Rich and his associates in Science, *9 January 1981, pp. 171–176.*

interband regions. But when they added the antibody to a Z form of a deoxycytosine and deoxyguanine polymer, the antibody would no longer bind to the *Drosophila* chromosomes—presumably it had already combined with the Z-DNA of the synthetic polymer.

What is the biological function of Z-DNA? "We think Z-DNA is one of the elements that regulate gene transcription," says Rich. There are four ways to stabilize the Z-DNA form. It can be methylated, proteins can bind to it (when antibodies bind to Z-DNA, for example, they stabilize it), it can have negatively charged molecules like spermidine bound to it, or it can be supercoiled. All of these ways to stabilize Z-DNA could operate in gene control systems.

"Some people would call this wild-eyed speculation," Rich says as a preface to his theories of how Z-DNA might act as a regulatory structure. But, clearly, Rich himself believes his speculations are not out of line. He proposes two types of control involving Z-DNA, proximal and distal control.

In distal control, the supercoiling of Z-DNA comes into play. "We know that DNA in chromosomes is normally supercoiled and that it is packed with minimal torsional stress," Rich explains. "When a region of Z-DNA is made to convert back to B-DNA, the DNA would have to turn more and so there would be more torsional stress and the DNA would open up, the double helix would unwind." He takes out a rubber band to demonstrate, twisting it tightly. "This is supercoiling," he says. Then he continues to twist and the two strands of the rubber band open up.

Rich proposes that when genes are turned on, regions of Z-DNA which previously were methylated lose their methyl groups. This would destabilize the Z form and the DNA would revert to B-DNA. As a consequence, DNA at some distance from the Z-DNA area would unwind and a so-called hypersensitive region would appear. These hypersensitive regions of DNA are extremely susceptible to degradation by enzymes that break down DNA, including S1 nuclease, which only attacks single-stranded DNA. They are known to appear upstream from genes that are active and to be necessary for gene activity (*Science*, 13 November, p. 775).

Proximal control, Rich speculates, is a bit simpler. He proposes that control regions of DNA adjacent to genes can be in B or Z form. When they are in Z form, certain proteins used in gene transcription cannot bind. When, through demethylation or through one of the other ways of converting Z-DNA to B-DNA, the control regions are converted to the B form, genes adjacent to them can be transcribed.

Now Rich is ready to start looking for evidence that Z-DNA is involved in gene regulation and is ready to look for evidence that gene transcription can be prevented if the Z-to-B switch cannot operate. Why is he so sure this is the way to proceed? "Well," he says, ever the optimist, "we've done some experiments." But, he cautions, he is not ready to discuss his results because "we haven't yet done the controls."—GINA KOLATA

Mauna Kea (II): Coming of Age

The Institute for Astronomy has grown enormously in the last 15 years —but its passage has not been easy

For nearly 18 years it has been John T. Jefferies' ambition to build the University of Hawaii into a world-class center of astronomical research—and, not incidentally, to create on the summit of Mauna Kea one of the great observatories of the world.

In 1965, when the National Aeronautics and Space Administration gave Hawaii the contract to build an 88-inch telescope on Mauna Kea, the university's entire astronomy program consisted of three solar astronomers working under Jefferies within the Institute of Geophysics. But it wasn't long before Jefferies was lobbying for a separate institute. "I felt we were going to grow to such an extent that we would have been disproportionately large within geophysics," he explains now, "and I felt that the specific needs of astronomy should be uppermost in management's mind." He was persuasive, and the Institute for Astronomy was formally established on 1 July 1967.

At first it was hard to hire anyone, Jefferies recalls. For one thing, there were many more jobs than applicants in those days. Worse, too many astronomers seemed to think of Hawaii as some kind of never-never land full of palm trees, pineapples, and tourists. They certainly did not see it as a place to live and to do serious astronomy. "We were engaged in a brand new enterprise in a location not noted for other cultural or academic advantages," Jefferies notes wryly. He was thousands of kilometers from any other graduate university, and in no position to attract academic superstars. So he began to staff his institute with people who were young and adventurous, some just a year or two out of graduate school.

Meanwhile, Jefferies was quietly urging other institutions to consider building telescopes on Mauna Kea. As an observatory site, he pointed out, it is unsurpassed for the clarity, dryness, and stability of its air, the darkness of its night sky, and its relative freedom from clouds.

Among the first to be convinced, in the early 1970's, were the Canadians and the French, who were planning a joint national observatory with a 4-meter class optical telescope. In 1973 representatives of the partnership came out to Hawaii to strike a deal with Jefferies: Canada and France agreed to split the cost of building the telescope; the University of Hawaii, which held the lease on the summit area, donated the land and agreed to build a permanent midlevel facility to house visiting astronomers and staff. Canadian and French astronomers would split 85 percent of the observing time while University of Hawaii faculty got the other 15 percent. Operating costs would be allocated in a like ratio. The Canada-France-Hawaii Telescope (CFHT) Corporation was born. Completed in 1979, the $30-million, 3.6-meter CFHT is the most lavishly appointed telescope on Mauna Kea. The most obvious detail is an aerodynamically designed dome that looks disconcertingly like a stocky white mushroom—but which allows the CFHT to operate in 100-kilometer-per-hour winds. (The others have to shut down at 60 kilometers per hour.)

About the same time that Jefferies was negotiating with CFHT, he was also concluding a similar arrangement with the

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