Z-DNA: Still Searching for a Function

Six years after the discovery of Z-DNA questions remain about whether it exists naturally and what its functions might be

In the half-dozen years since Z-DNA was identified, this unusual structure has provoked both interest and controversy. According to some molecular biologists, notably including its discoverer, Alexander Rich of the Massachusetts Institute of Technology, Z-DNA is likely to play an important role in the life of the cell. He proposes, for example, that it might help to regulate gene activity.

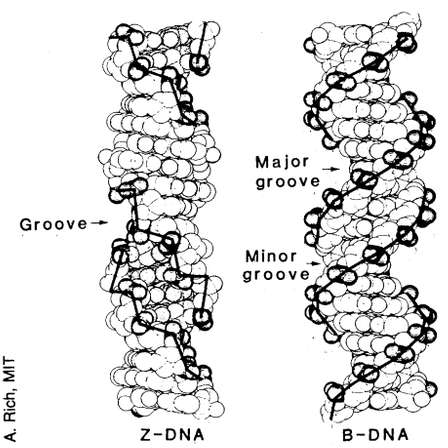
Not everyone agrees with this view, however, and over the past 2 to 3 years results from a variety of experiments have raised questions about the case for the physiological importance of Z-DNA, especially in gene regulation. Nevertheless, other recent evidence suggests that the structure might be a key intermediate in genetic recombination. Perhaps the clearest lesson to be learned from an examination of the current evidence regarding Z-DNA's proposed functions is that the issues are not easy to resolve.

Rich and his colleagues discovered Z-DNA in 1979 when they were attempting to confirm and refine the features of the three-dimensional structure of the classic B-DNA double helix, which was originally proposed by Francis Crick and James D. Watson. The MIT workers were startled when their analysis of crystals of a synthetic DNA molecule revealed a previously unknown structural configuration that was strikingly different from that of B-DNA.

Whereas the B-DNA double helix is right-handed and its surface is traced by two grooves, one major and one minor, the double helix formed by the synthetic DNA turned out to be left-handed and to have but a single groove. The backbones of the nucleic acid chains produce a zigzag pattern around the new helical form, which is one of the reasons why the "Z" designation was chosen for it. Moreover, the orientation of the bases in Z-DNA is quite different from that in B-DNA.

The questions surrounding Z-DNA today revolve around whether it is present in the genomes of living cells and, if it is, whether it has a function there. At least in the early studies, Z-DNA could only be induced to form in test-tube conditions, such as high salt concentrations, that would not be found in living cells. Investigators soon learned, however, that the Z conformation could be stabilized by modifications of the kind that occur naturally. These include addition of methyl groups to the cytosine bases and protein binding, both of which have been linked to gene regulation. Identification by the Rich group of cellular proteins that preferentially bind to Z-DNA buttressed the hypothesis that it might be present in cells, although as yet no function has been established for these proteins.

In addition, negative supercoiling of DNA stabilizes the Z conformation. The DNA in the circular chromosomes of bacteria is normally negatively supercoiled, a situation which facilitates gene expression by helping the double helix to unwind when the DNA is being transcribed into RNA. Whether the DNA of higher organisms is also negatively supercoiled is less clear.



A. Rich, MIT

Despite the evidence implying that Z-DNA might exist under physiological conditions, actually proving that it is present in living cells has been difficult. "What is important to realize is that we don't have direct evidence for the in vivo existence of Z-DNA or for its physiological function," says Alfred Nordheim, who previously worked with Rich at MIT and continues his investigations of Z-DNA at the University of Heidelberg.

Studies with antibodies to Z-DNA provided early evidence that the unusual conformation occurs naturally. Rich and Nordheim, with Mary Lou Pardue of MIT and B. David Stollar of Tufts University, found that the antibodies not only bound to the large chromosomes from the salivary gland of the fruit fly *Drosophila melanogaster*, but concentrated in specific regions, principally the light bands (*1*). The antibodies also stained some chromosome "puffs," which are areas of active gene expression.

The situation soon became more complicated, however. Other investigators found different patterns of antibody binding. According to Thomas Jovin and his colleagues at the Max-Planck-Institute for Biophysical Chemistry in Göttingen, antibodies to Z-DNA concentrated primarily in the dark bands (*2*). When the Max-Planck and MIT groups tried to resolve the discrepancy by exchanging antibodies, both obtained the same results as before. This suggested that the different binding patterns might have been caused by differences in the methods used by the two groups for preparing and fixing the chromosomes.

Stollar and Ronald Hill of the CSIRO Molecular and Cell Biology Unit in North Ryde, Australia, confirmed this suggestion in an experiment that is frequently cited by critics of the hypothesis that Z-DNA may be physiologically important. Ordinary methods for preparing polytene chromosomes require high concentrations of acetic acid, which had been used by the MIT and Max-Planck groups. Hill had devised a way of extracting the chromosomes from individual salivary gland cells with a fine needle. Chromosomes prepared by this much gentler procedure displayed little or no binding of the antibodies to Z-DNA (*3*).

Hill and Stollar went on to show that short treatment of these chromosomes with acetic acid results in an antibody-staining pattern like that observed by Rich and his colleagues, whereas longer treatments produce a pattern more like that seen by the Max-Planck group.

Stollar disagrees with the suggestion that this experiment proves that Z-DNA does not occur naturally but is only an artifact of the procedure used to prepare the polytene chromosomes. "I think the experiment has been overinterpreted as a negative," he maintains. Z-DNA might have been present in the original chromosomes, but masked by proteins that are removed by acetic acid treatments. Alternatively, the fixative treatment might have driven the conversion of B-DNA to Z-DNA. Nevertheless, Stollar says, the results indicate that potential Z-DNA-forming sequences are present in biologically interesting areas of the *Drosophila* genome.

Further support for this view comes from Hiroshi Hamada and his colleagues at the National Cancer Institute who found that the *Drosophila* genome contains some 2000 copies of a repetitive sequence in which purine bases alternate with pyrimidine bases. Such sequences more readily undergo the B- to Z-DNA transition than DNA segments without the alternating pattern. At one time purine-pyrimidine alternation was considered a prerequisite for Z-DNA formation, but Rich and his colleagues have more recently found that it is not (*4*, *5*). "We overestimated the extent to which we need alternating purines and pyrimidines," Rich explains, "but that means our ability to recognize Z-DNA-forming regions has gone down."

This problem and the difficulties encountered in the antibody-binding studies have increased the need for better methods of detecting Z-DNA. Rich's group and that of Winship Herr at Cold Spring Harbor Laboratory have been developing chemical reagents that are sensitive to the unusual conformation assumed by Z-DNA and may therefore permit more accurate identification of Z-DNA regions at the nucleotide level.

Attempting to define the physiological function of Z-DNA has proved even more problematical than trying to demonstrate its presence in living cells. Even Rich concedes that it is "by no means clear at the moment" what Z-DNA might be doing in the cell. The main difficulty, he suggests, is the dynamic nature of the system. Z-DNA is less stable than B-DNA and may form only transiently, depending on such factors as protein binding, degree of methylation, and the negative supercoiling status of the DNA.

By all accounts, the best evidence so far in support of a physiological role for Z-DNA comes from William Holloman of the Cornell University Medical College in New York City and Eric Kmiec of the University of Rochester. In work that was largely done while they were at the University of Florida College of Medicine in Gainesville, the investigators have implicated the structure as an important intermediate in genetic recombination in a simple eukaryote, the yeast-like fungus called Ustilago.

Chromosomes that are undergoing recombination first pair and then exchange segments. These reactions are promoted in Ustilago by an enzyme called rec1 that was identified a few years ago by Kmiec and Holloman. The investigators have now found in a test-tube model of recombination that Z-DNA is generated in double-stranded DNA at the site of pairing,

Dental Humans, Infant Apes

In examining the fossilized jaws of early human ancestors, paleoanthropologists have tended to apply modern human standards of sequence and timing of tooth eruption and wear in estimating the age of death of particular individuals. The assumption of a modern human pattern in early hominids might, however, be a mistake, suggest Timothy Bromage and Christopher Dean of University College, London. They conclude from a study of certain detailed growth patterns in tooth enamel of nine juvenile hominids that lived between 1 and 3.7 million years ago that the growth characteristics were more apelike than human-like (*1*). If correct, this conclusion has important behavioral implications.

Microscopic examination of tooth enamel reveals two types of incremental growth lines, which are presumed to be the result of rhythmic changes in the activity of the enamel-secreting ameloblasts. Although it has not yet been demonstrated beyond doubt, there is a good deal of agreement that the smaller of the two incremental lines represent 24-hour intervals. Bromage and Dean's interest, however, lies primarily with the larger increments, which are coarser lines that pass obliquely from the enamel-dentine junction to the surface of the enamel where they are sometimes visible as ridges known as perikymata. Although there is some variation in the number of smaller lines separating the coarser increments, the British researchers consider them to be the result of weekly fluctuations in enamel formation: there are, on average, seven to eight small striations between the larger ones. Count the number of ridges in a tooth crown and you know how many weeks it took to grow, which then allows an estimate of infancy.

This suggestion is met with some skepticism, not least because of an inability to explain the origin of biological fluctuations with a weekly period. Nevertheless, Bromage and Dean are able to cite several 7-day rhythms in physiological activity of soft and hard tissues, which, they say, adds credence to the idea of a 7- to 8-day periodicity in enamel formation. Lawrence Martin of the State University of New York at Stony Brook whose recent work on the evolution of human and ape enamel formation has had such an impact on the field (*2*), notes that critics can find many uncertainties to question in Bromage and Dean's scheme but guesses that it is unlikely to be far wrong.

For a number of technical reasons, incisor crowns offer the most appropriate target for counting perikymata and estimating age at death, which Bromage and Dean did for nine hominid specimens, including a 1.7+ million year *Homo*, from South and East Africa. In all cases the age at death was younger by at least one third compared to that inferred using modern human standards for tooth growth characteristics. If correct, this implies that infancy in these creatures was more like that in apes and had not yet become prolonged, as it is in modern humans. Dean reached similar conclusions based on a study of root growth patterns (*3*).

Prolonged infancy in humans is obviously important for protecting and nurturing the young, whose brain expands fourfold after birth as compared with a doubling in apes. Robert Martin also of University College, London, has calculated that special parental protection would be required for the hominid infant only when the adult brain size exceeded some 750 cubic centimeters (*4*). Had it lived, the *Homo* specimen in Bromage and Dean's survey would probably have developed an adult brain close to this size. Martin's conclusion about brain growth and infancy is therefore consistent with Bromage and Dean's estimate of infancy based on the teeth. Although the *Homo* species living at this time demonstrably made and used stone tools and, therefore, enjoyed a behavioral repertoire that in some ways at least was different from that of apes, these new data on tooth growth and infancy once again warn of the probable danger of thinking of early hominids simply as diminutive humans.—**ROGER LEWIN**

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which is brought about by rec1. Moreover, the enzyme binds to Z-DNA about 75 times more tightly than it binds to B-DNA (*6*). "This is the first example of a protein with a known function that binds preferentially to Z-DNA," Holloman says.

The investigators propose that the Z-DNA conformation represents the transition state for the pairing reaction catalyzed by rec1. Tight binding to the enzyme would be characteristic of this state. The proposed role for Z-DNA in genetic recombination is consistent with earlier findings in several laboratories that potential Z-DNA-forming sequences are "hotspots" for recombination.

Whether Z-DNA is important for controlling gene expression remains to be determined. Gene control is well understood only for certain bacterial genes—and these do not provide any evidence for Z-DNA involvement. Crystallographic studies of the structures of a few bacterial regulatory proteins and, in one case, of a cocrystal of a regulatory protein with its DNA recognition site, strongly suggest that the proteins recognize and bind to B-DNA without appreciably deforming this structure (*7*).

Moreover, Ann Hochschild, Nina Irwin, and Mark Ptashne of Harvard University have found that once a bacterial regulatory protein attaches to its control site, it apparently activates expression of the associated gene by binding RNA polymerase, the enzyme that begins protein synthesis by transcribing DNA into RNA (*8*). Z-DNA would appear not to be involved either in the recognition of the regulatory site by the protein or in the subsequent activation of the gene.

Lawrence Peck and James Wang of Harvard University have evidence that indicates that Z-DNA does not form inside cells and also seems to militate against the participation of Z-DNA in gene activation (*9*). They have inserted a Z-forming sequence in a bacterial plasmid and found that in a test-tube system it stops transcription of the plasmid when in the Z, but not the B, configuration. If the plasmid is introduced into bacterial cells, however, it is transcribed normally. This probably means that the Z-forming insert does not assume the Z conformation in the bacterial cells. Alternatively, some component of the cellular machinery that permits Z-DNA transcription might have been missing from the artificial system, although this seems less likely in view of more recent results from Wang's laboratory that indicate that the plasmid insert forms Z-DNA inside the bacterial cells only under abnormal conditions.

According to Rich, what happens with the introduction of the artificial plasmid may not accurately reflect the situation of the DNA in the bacterial chromosome. In any event, he says, "I don't think that Z-DNA works by forming in the middle of a region that is being transcribed." He expects the sequences to be located in the regulatory regions before the start of the gene proper. In that position, they might cooperate with appropriate binding proteins either to turn genes on or off. The enhancer region of SV40 (simian virus 40) is one possible example.

"What is important to realize is that we don't have direct evidence for the in vivo existence of Z-DNA or for its physiological function."

A few years ago, Nordheim and Rich found that the SV40 enhancer contains two sequences of alternating purines and pyrimidines that apparently form Z-DNA, which they detected by antibody binding. The control regions of several other viruses carry comparable pairs of sequences in which purines and pyrimidines alternate.

The possibility that Z-DNA might participate in the control of SV40 gene expression received a boost about 2 years ago when Herr and Yakov Gluzman, also of Cold Spring Harbor Laboratory, found that mutations that destroy the purine-pyrimidine alternation of the Z-forming regions cause a decrease in ability of SV40 to reproduce itself. In contrast, they observed no such decrease in mutants in which the alternation was maintained.

Although the results implied that destroying the ability to form Z-DNA impairs viral gene expression, the result is not as clear-cut as it might seem at first glance. "It was a provocative result," Herr says, "but it doesn't address the question. No one has been able to show that we affected Z-DNA."

The situation of Z-DNA in the SV40 enhancer has been further clouded because Nordheim has now found that the antibodies used to identify the Z regions there also react with additional sites that do not have the alternating purine-pyrimidine structure. This finding raises questions about what the antibodies are recognizing, but might be explained if the additional regions can still assume the Z conformation. However, Herr has not been able to detect Z-DNA in the SV40 enhancer with the chemical reagent he is studying. The Rich group is currently probing the SV40 enhancer for Z-DNA with their reagents.

Other investigators have obtained different results with their enhancer mutants. Pierre Chambon and his colleagues at the Université de Strasbourg I-Université Louis Pasteur, did not detect any difference between mutants that destroyed or maintained the purine-pyrimidine alternation in the putative Z-DNA sequences. They observed equally large decreases in enhancer activity for both types of mutations.

"We conclude from our results that it is not just the alternating purine-pyrimidine sequence that is important," Chambon explains. "The absolute sequence is also important." Michael Botchan of the University of California at Berkeley came to a similar conclusion in studies of mutants in the alternating purine-pyrimidine sequences of bovine papilloma virus enhancer (*10*). However, these studies neither rule out nor support a role for Z-DNA in gene regulation.

Finally, the Z-DNA binding proteins identified by the Rich group include some that bind to the SV40 enhancer (*11*). "They are sitting at a site that is biologically important," Rich points out, although a regulatory function has yet to be demonstrated for any of the proteins.

Despite the current uncertainties over the role of Z-DNA, there are precedents for proteins altering the conformation of the DNA to which they bind. The DNA double helix can assume a range of conformations, of which the Z form is the most extreme to be identified so far. The questions about the existence and possible functions of Z-DNA in the cell may not have been definitively answered, but as Nordheim says, "What is important is that we have learned a lot while tackling the questions."—**JEAN L. MARX**

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