The Challenges of Sciences. A Tribute to Carlos Chagas
Pontifical Academy of Sciences, *Scripta Varia* 103, Vatican City 2002
www.pas.va/content/dam/accademia/pdf/sv103/sv103-rich.pdf

FUNCTIONAL ASPECTS OF LEFT-HANDED NUCLEIC ACIDS

BERNARD A. BROWN II and ALEXANDER RICH

Introduction

Both DNA and RNA form stable double helices held together by Watson-Crick base pairs. However, the presence of an added oxygen atom in the backbone of RNA results in a considerable change in the geometry of the molecule. The B-DNA molecule has both major and minor grooves, with the major groove fully accessible. On the other hand, A-RNA, because of the altered pucker of the ribose ring, forms a double helix in which the minor groove is quite accessible, but the major groove is constricted so that it is almost inaccessible to neighboring molecules. Right-handed B-DNA and A-RNA are the most stable forms of these duplexes. Both of these conformations can be transformed with the input of energy into left-handed double helical conformations, which are still held together by Watson-Crick base pairs, but with the backbones in an unusual *zig-zag* shape, hence the name "Z"-conformation. In the Z-conformation, the puckers of the furanose sugar rings in the polynucleotide chain alternate between that found in B-DNA (C2' *endo*) and that found in A-RNA (C3' *endo*). It is this alternation that produces the unusual shape of the phosphate backbone, the most striking feature of the Z-conformation.

Here we discuss some of the research on the left-handed Z-forms of both DNA and RNA double helices. Much more is known about Z-DNA than Z-RNA at the present time.

DNA can assume many shapes (Rich 1993). A dramatic change in shape is found when the familiar right-handed B-DNA double helix changes to the slightly thinner and elongated left-handed Z-DNA conformation (Figure 1) (Wang *et al.* 1979). This conformational change occurs most readily in seg-

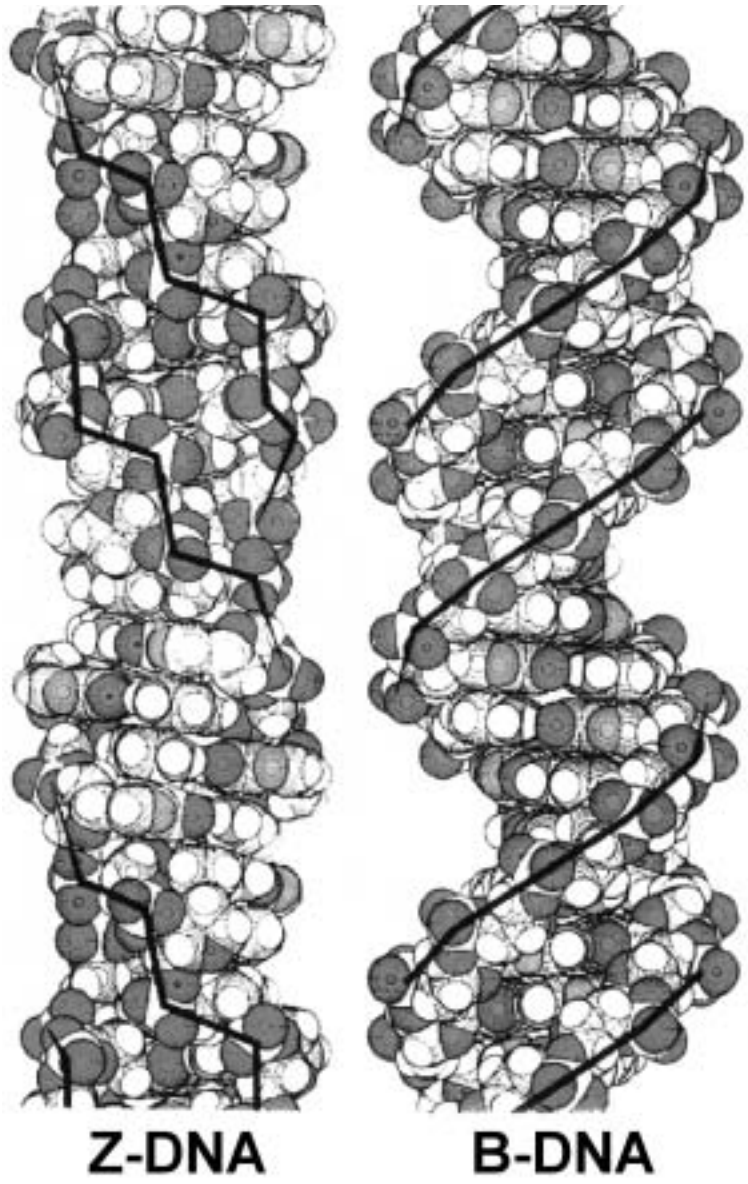

Figure 1. Overview of B- and Z-DNA helices (Wang *et al.* 1979; Wang *et al.* 1981). The "information-rich" residues that allow sequence-specific recognition of the major groove of B-DNA lie on the convex surface of left-handed Z-DNA helix. The two DNA strands of each duplex are highlighted by solid black lines. The "zig-zag" nature of the Z-DNA backbone is clearly seen. The sequence shown is $(dC\text{-}dG)_n$.

ments with specialized sequences, favored largely by alternations of purines and pyrimidines, especially alternating deoxycytidine and deoxyguanosine residues (Klysik *et al.* 1981; Haniford & Pulleyblank 1983; Peck *et al.* 1982). The alteration in ring pucker reflects differences in the stabilities of furanose sugar puckers for particular nucleosides.

Negative Supercoiling Stabilizes Z-DNA

An alternative conformation was first suggested by optical studies of Pohl and Jovin showing that polymers of alternating deoxyguanosine and deoxycytidine residues, $d(CG)_n$, produced a nearly inverted circular dichroism spectrum in *ca.* 4 M salt solutions (Pohl & Jovin 1972). The physical reason for this finding remained a mystery until an atomic resolution crystallographic study of $d(CG)_3$, surprisingly revealed a left-handed double helix, which maintained Watson-Crick base pairing (Figure 1) (Wang *et al.* 1979). The Z-DNA helix is formed by a d(CG) dinucleotide repeat with the deoxycytidines in the familiar *anti* conformation while the deoxyguanosines are in the unusual *syn* form. In Z-DNA, there is a single narrow groove that corresponds to the minor groove of B-DNA; there is no major groove. Instead, the information-rich residues that allow sequence-specific recog-

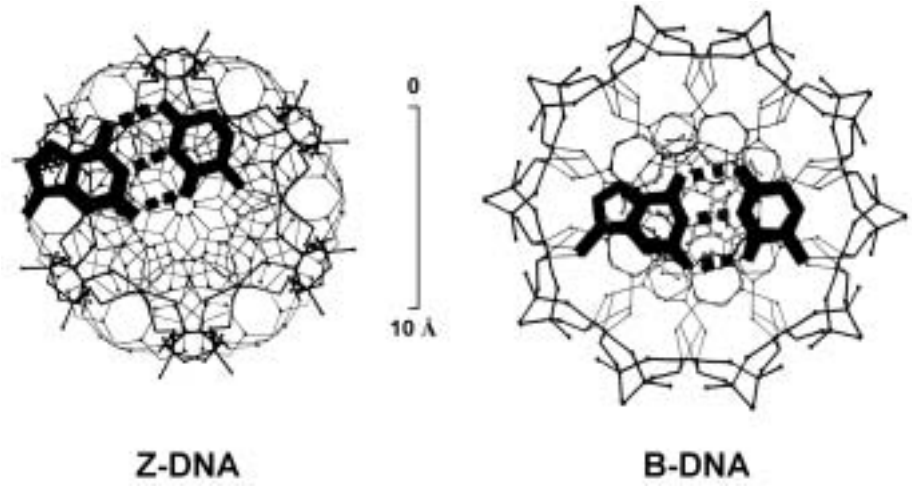

Figure 2. End views of Z-DNA and B-DNA.(Wang *et al.* 1979; Wang *et al.* 1981). End views of Z-DNA and B-DNA are shown in which a G·C base pair has been shaded. The guanine residues in B-DNA are located closer to the center of the molecule and the phosphates are on the outside, while in the thinner Z-DNA conformation, the base pair is displaced to one side, with the guanine C8 of the purine ring near the periphery of the helix.

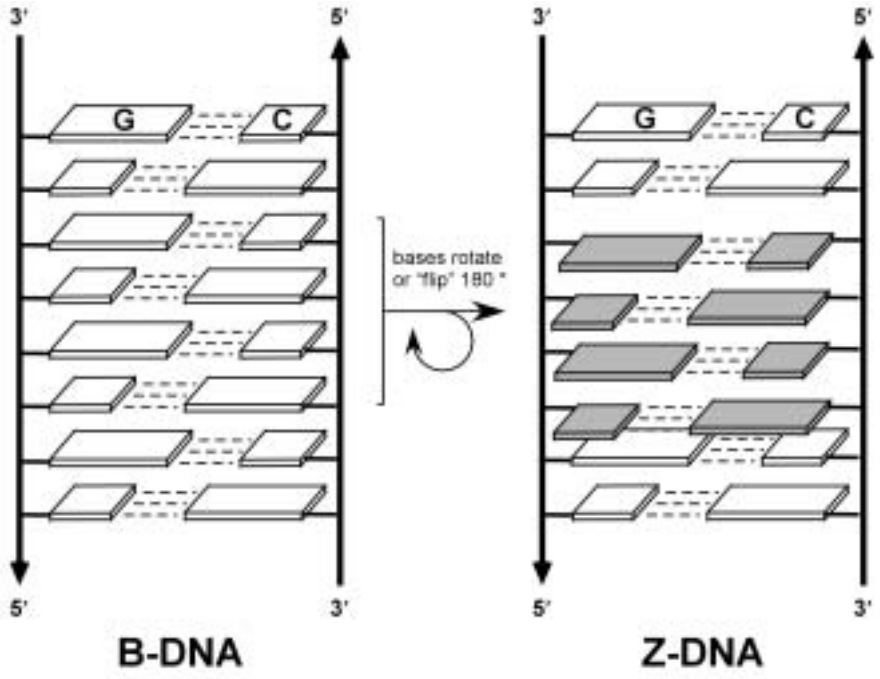

Figure 3. B-to-Z "Flipping" (Wang *et al.* 1979). This diagram illustrates the changes in topological relationship if a four-base pair segment of B-DNA were converted into Z-DNA. Base pairs are represented by flat boards; the base pairs in the Z-conformation are shaded. The conversion is associated with a rotation or "flipping" of the base pairs as indicated. Rotation of the guanine residues about the glycosidic bond yields deoxyguanosine in the *syn* conformation (with a C3' *endo* sugar pucker), as shown in Figure 4. In contrast, for deoxycytidine residues, both the cytidine base and deoxyribose are rotated, keeping cytosine in the *anti*-glycosidic orientation with a C2' *endo* sugar pucker.

nition of B-DNA lie exposed on the convex outer surface of Z-DNA. This is shown in an end view where in Z-DNA the base-pair is at the edge of the helix, especially the guanine base (Figure 2). The transition from B-DNA to Z-DNA involves "flipping" the base pairs upside down (Figure 3). During this process, deoxycytidine remains in the *anti* conformation because both the sugar and base flip over, while only the base of deoxyguanosine inverts, moving it into the *syn* conformation. In addition, the guanosine deoxyribose adopts the C3' *endo* sugar pucker, normally found in riboses of A-RNA, while the deoxycytidine deoxyribose remains in the normal C2' *endo* sugar

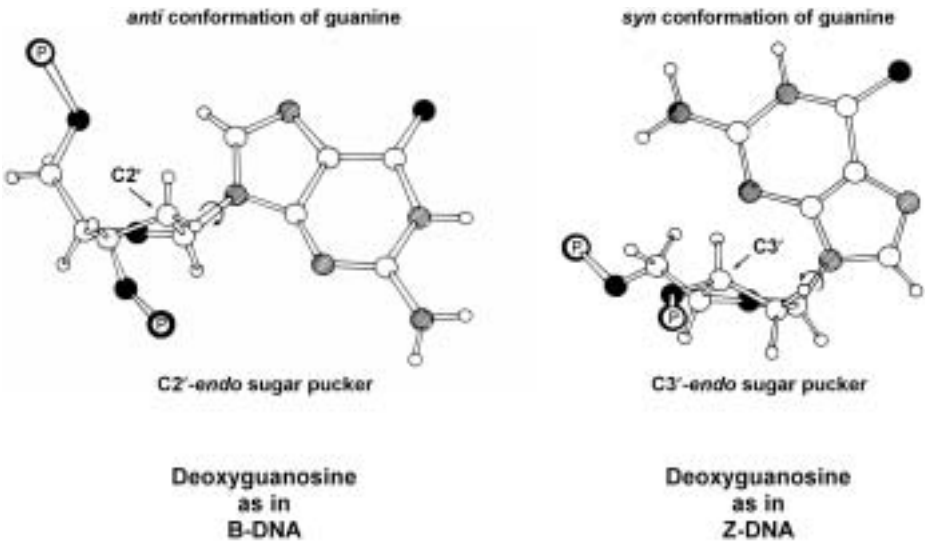

Figure 4. Conformation of deoxyguanosine in B- and Z-DNA (Wang *et al.* 1981). In both diagrams, the sugars are oriented so that the plane defined by C1'–O1'–C4' is horizontal. Atoms lying above this plane are in the *endo* conformation. In B-DNA all of the sugar puckers are C2' *endo*; in A-RNA all sugars are in the C3' *endo* conformation. In Z-DNA and Z-RNA the guanosine sugars adopt the C3' *endo* conformation. However, in the Z-conformation the guanine bases are in the *syn*-orientation with respect to the glycosidic-bond. In contrast, only the *anti*-position is found in B-DNA and A-RNA. A curved arrow around the glycosyl carbon-nitrogen bond indicates the site of rotation.

pucker (Figure 4). These features consequently mold the phosphate backbone into a zig-zag path (see Figure 1). B-DNA can form Z-DNA under physiological salt conditions when deoxycytidine is C5-methylated or brominated (Behe & Felsenfeld 1981). The demonstration that Z-DNA formed under conditions of negative superhelical stress was notable as this brought the left-handed conformation within the realm of biology (Klysik *et al.* 1981; Peck *et al.* 1982; Singleton *et al.* 1982).

Z-DNA is a higher-energy conformation than B-DNA and will only form in plasmids when they are torsionally stressed, thus Z-DNA is stabilized by negative supercoiling. The energy necessary to stabilize Z-DNA can be determined by measuring the plasmid superhelical density at which Z-DNA formation occurs, and it is proportional to the square of the number of negative supercoils (Peck & Wang 1983; Ellison *et al.* 1985). Sequences other

than alternating purines and pyrimidines can also form Z-DNA. The ease with which this occurs depends on the sequence; d(CG) is best, d(TG/AC) is next, and a d(GGGC) repeat is better than $d(TA)_2$ (McLean *et al.* 1986; Ellison *et al.* 1986). In addition, formation of a B-Z DNA junction, which has a ΔG of about 4 kcal/mol, is a significant energetic barrier to Z-DNA formation (Peck & Wang 1983). Based on many empirical findings, computer models have been developed to rank the Z-DNA-forming potential of naturally occurring sequences (Ho *et al.* 1986).

As pointed out by Liu and Wang (Liu & Wang 1987), negative supercoils arise behind a moving RNA polymerase as it ploughs through a DNA double helix. The torsional strain generated by passage of RNA polymerases then becomes a potent source of energy to stabilize Z-DNA. An analysis by Schroth et al. of 137 fully sequenced human genes demonstrated that sequences that could easily form Z-DNA were present in 98 and they were distributed non-randomly throughout the gene; sequences were 10 times more frequent in 5' than in 3' regions with the highest frequency near the transcription start site (Schroth *et al.* 1992). This finding supports the expectation that the energy necessary to form Z-DNA *in vivo* is generated by transcription.

Detection of Z-DNA

Z-DNA formation *in vivo* can be detected by chemical modification. Through use of either osmium tetroxide or potassium permanganate, plasmids containing a $d(CG)_n$ can be seen to form Z-DNA *in vivo* (Palecek *et al.* 1988). UV cross-linking of bacteria treated with psoralen dyes have confirmed these results and permitted precise quantitation of unrestrained supercoiling present within *Escherichia coli* (Zheng *et al.* 1991). A more indirect approach has used a construct in which an *Eco*RI restriction site is embedded within a Z-DNA-forming sequence (Jaworski *et al.* 1987). In the bacterial cell, this fragment can be methylated when it is in the B-DNA conformation, but is resistant to methylation while in the Z-DNA conformation. Susceptibility to methylation thus can be used as a measure of *in vivo* torsional strain. Results obtained with this system show that Z-DNA formation in *E. coli* occurs in the absence of external perturbation and is regulated by transcription, an effect that is enhanced by mutations inactivating topoisomerase I (Rahmouni & Wells 1989; Jaworski *et al.* 1991).

Krasilnikov and co-workers were able to quantitate the effects of negative supercoiling in *E. coli* by assessing the efficiency of cruciform forma-

tion at varying distances upstream of a promoter (Krasilnikov *et al.* 1999). Chemical probing assays showed cruciform formation decreased to one half by placing a promoter 800 bp upstream, and it could still be detected over 2 kb upstream. This was the first demonstration *in vivo* that supercoiling generated by transcription could change DNA structure at such great distances.

Detection of Z-DNA in eukaryotic systems is more complex, although a number of early observations clearly suggested its existence. Unlike B-DNA, Z-DNA is highly immunogenic, and polyclonal as well as monoclonal antibodies can be made that specifically recognize this conformation (Lafer *et al.* 1981). The first indication that Z-DNA exists in eukaryotic systems came from analyses of sera obtained from patients with autoimmune diseases such as systemic lupus erythematosis. These experiments showed that lupus patients produced antibodies which were highly specific for Z-DNA (Lafer *et al.* 1983). The blood concentrations increased during the exacerbations of the disease, together with antibodies to many other nuclear components.

Anti-Z-DNA antibodies raised in rabbits and goats have been used in staining fixed (Nordheim *et al.* 1981) and unfixed polytene chromosomes of *Drosophila melanogaster* (Lancillotti *et al.* 1987). These produced an unusual pattern with staining in the interband regions but not in the bands. Staining was especially intense in the puffs, which are associated with high levels of transcriptional activity (reviewed by Hill (Hill 1991)). Antibodies were also used in staining the ciliated protozoa *Stylonychia mytilus*, which has both a macronucleus and a micronucleus (Lipps *et al.* 1983). The micronucleus is used for genetic reproduction, but the macronucleus is the site of all transcriptional activity. In this case, the macronucleus stained exclusively, with no staining in the micronucleus, even though they both had the same DNA sequences. These findings suggested a link between transcriptional activity and the presence of Z-DNA.

There are a number of limitations in the analysis of Z-DNA in intact mammalian systems. No phenotype has ever been associated with the presence or absence of Z-DNA-forming sequences, thus limiting the use of genetic approaches. Furthermore, regulation of Z-DNA is likely to be very complex. Moving RNA polymerases can generate negative torsional strain. RNA polymerase I is known to work on some favorable Z-DNA-forming sequences in ribosomal RNA genes, but it is not known how the torsional strain in regions 5' to RNA polymerase II promoters is regulated. The effect of potential Z-DNA-forming sequences upstream in a promoter must be

interpreted carefully. Deletion or mutation of such regions, as in the case of the SV40 enhancer which has regions of alternating purine/pyrimidine repeats, may have many different consequences (Gruskin & Rich 1993).

Several experiments have been carried out using metabolically active, permeabilized mammalian nuclei, which were formed by embedding living cells in agarose microbeads (Jackson & Cook 1985). A low concentration of detergent is used to lyse the cytoplasmic membrane and permeabilize the nuclear membrane. The treated nuclei are transcriptionally active and replicate DNA at 85% of the rate observed in the intact cell (Jackson *et al.* 1988). The amount of Z-DNA present under these conditions was detected by diffusing biotinylated anti-Z-DNA monoclonal antibodies into the permeabilized nucleus and measuring the amount of radioactive streptavidin that would bind (Wittig *et al.* 1989). The amount of Z-DNA measured was independent of the antibody infused, over a 100-fold range in antibody concentration. Furthermore, the amount of Z-DNA depended on DNA negative torsional strain. It increased dramatically as transcription increased, but was largely unaffected by DNA replication (Wittig *et al.* 1991).

Individual genes can be assayed by cross-linking the antibody to DNA using a 10-ns exposure of a laser at 266 nm (Wittig *et al.* 1992). The release of DNA fragments with cross-linked antibody was carried out by diffusing in restriction endonucleases, an *in situ* DNA digest. Following isolation of biotin-labeled antibody-DNA complexes with streptavidin magnetobeads, free DNA restriction fragments were obtained by proteolysis. Thus it was possible to determine which regions of a gene forms Z-DNA. Using hybridization or PCR techniques, the c-*myc* oncogene was studied in mouse U937 cells (Wittig *et al.* 1992). Three transcription-dependent Z-DNA-forming segments were identified in the 5' region of the gene with two of them near promoters (Wolfl *et al.* 1995b). Retinoic acid, which induces the cells to differentiate into macrophages, was then used to down-regulate expression of c-*myc*. Loss of c-*myc* expression was accompanied a loss of Z-DNA over 15–20 min. in these regions. As a control, Z-DNA was detectable by PCR amplification with probes for actin genes under all conditions tested; actin is not down-regulated during differentiation.

Induction of Z-DNA was also measured in the corticotropin hormone-releasing gene in a primary liver cell line (Wolfl *et al.* 1996). Z-DNA formation increased when the gene was up-regulated and decreased when it was down-regulated. This finding suggests that physiological events are being measured in these systems. A major conclusion from these studies is that Z-DNA forms largely, if not exclusively, behind a moving RNA polymerase

and is stabilized by the negative supercoiling generated by DNA transcription. After the polymerase stops transcribing, topoisomerase is able to catch up and release torsional strain caused by negative supercoiling, and the Z-DNA reverts to the lower energy B-conformation.

It is possible that Z-DNA formation has a functional role without recognition of its shape by proteins. For example, the *E. coli* RNA polymerase does not transcribe through Z-DNA (Peck & Wang 1985). Thus, the formation of Z-DNA behind a moving polymerase may block a following RNA polymerase from re-initiating transcription from that region of the gene. This might ensure spatial separation between successive polymerases. In a mammalian system, RNA transcripts would then be physically and temporally separated from other transcripts, perhaps minimizing non-functional eukaryotic trans-splicing (Rich 1994).

Alternatively, formation of Z-DNA could facilitate recombination of homologous chromosomal domains by relieving topological strain that arises when intact duplexes are intertwined (Pohl 1967). The Z-DNA-forming sequence $d(CA/GT)_n$ has been shown to be recombinogenic in yeast (Treco & Arnheim 1986) but is found to be less efficient than $d(CG)_n$ in human cells (Bullock *et al.* 1986; Wahls *et al.* 1990). Finally, Z-DNA formation could affect the placement of nucleosomes as well as the organization of chromosomal domains (Garner & Felsenfeld 1987).

An Adenosine Deaminase (ADAR1) Recognizes Z-DNA

A number of laboratories have searched for Z-DNA binding proteins. Early studies were unfruitful and caused widespread skepticism that Z-DNA would be associated with any biological function. Many of the positive results reported in these studies may have been due either to artifacts or misinterpretation of data (Wolfl *et al.* 1995a; Krishna *et al.* 1990; Rohner *et al.* 1990). However, absence of proof should not have been confused with absence of existence.

A protein which was found to specifically bind Z-DNA is the RNA editing enzyme double-stranded RNA adenosine deaminase. This enzyme deaminates adenine to create inosine. Inosine hydrogen bonds in a manner similar to guanine. In effect, the editing enzyme changes codons in mRNA by converting selected adenine residues to the functional equivalent of guanine. These enzymes are called adenosine deaminases acting on RNA (ADAR, formerly known as dsRAD or DRADA), and the enzyme that binds tightly to Z-DNA is ADAR1. The human ADAR1 protein is nearly 140 kDa

in size (1226 aa) and consists of three major domains. The C-terminal region contains the adenosine deaminase domain and the central region consists of three double-stranded RNA binding domains. These domains recognize double-stranded A-RNA and bind solely to that conformation in a sequence-independent manner. The N-terminal region consists of a bipartite Z-DNA binding domain, termed Zab, which has two homologous subdomains (Zα and Zβ) that are separated by a tandem-repeated linker (Herbert *et al.* 1995; Schwartz *et al.* 1999a).

The Zα domain, containing approximately 80 amino acids, has been cloned and studied independently in great detail. Zα was found to bind to Z-DNA with a low nanomolar binding constant (Herbert *et al.* 1993; Herbert *et al.* 1995; Herbert *et al.* 1997). The interaction between Zα and DNA can be measured in several ways. Upon incubating poly(dGdC)n with increasing amounts of Zα, the circular dichroism changes from right-handed B-DNA to the left-handed Z form. In addition, the binding can be measured directly using surface plasmon resonance (BIACORE) or ultracentrifugation experiments (Herbert *et al.* 1998; Schade *et al.* 1999).

The ADAR enzymes exist as a small family. ADAR-2 contains an adenosine deaminase domain and a double-stranded RNA binding domain but does not have a Z-DNA binding domain (Melcher *et al.* 1996). These ADAR proteins are found in all metazoan tissues, suggesting that RNA editing is of great evolutionary significance (Bass 1993; Wagner & Nishikura 1988; Herbert 1996) . The activities of these enzymes may be an important source of phenotypic variation as they have the potential to significantly alter the linear flow of information from DNA to RNA (Herbert 1996; Maas & Rich 2000). A number of substrates have been identified including the glutamate and serotonin receptors in the central nervous system, as well as the α-2,6-sialyltransferase in the liver (Sommer *et al.* 1991; Lomeli *et al.* 1994; Burns *et al.* 1997; Ma *et al.* 1997; Maas & Rich 2000). In all examples the edited form of the protein, with changes in specific amino acids, results in the production of a modified function for the protein.

In the case of the glutamate receptor which is an ion channel, a glutamine (codon CAG) is edited to code for arginine (codon CGG) (Sommer *et al.* 1991). This is located in GluR-B, one of the subunits that make up the glutamate ion channel. The positively-charged arginine is found in the center of the ion channel, and its presence prevents the influx of calcium ions. This results in a rapid excitatory transmission, a change that is so beneficial to the organism that the GluR-B message is almost completely found in the edited form (Kask *et al.* 1998).

The serotonin receptor is a G-coupled protein; the edited form of the enzyme which interacts with the G protein is modified so that the coupling is somewhat weaker than the unedited form (Burns *et al.* 1997). This results in a modified serotonin receptor that produces a weaker signal Both of these receptors are used in the central nervous system, thereby permitting a finer-tuned level of serotonin regulation. In the liver α-2,6-sialyltransferase, the edited protein has a different secretory pathway, giving rise to a longer half life for the protein (Ma *et al.* 1997). Again, the unedited and the edited transcripts are both used in the organism, probably to gain greater control over the regulation of the sialyltransferase activity.

The crucial step in the editing process is the formation of a hairpin or fold-back structure in the pre-mRNA molecule, resulting in the formation of an RNA duplex (Higuchi *et al.* 1993). The RNA duplex is the binding site for the ADAR enzyme through its double-stranded RNA binding domains. This leads directly to deamination of an adenine residue somewhere in the duplex. The mechanism concerning the selection of the particular adenine are not well understood. An important point is that the

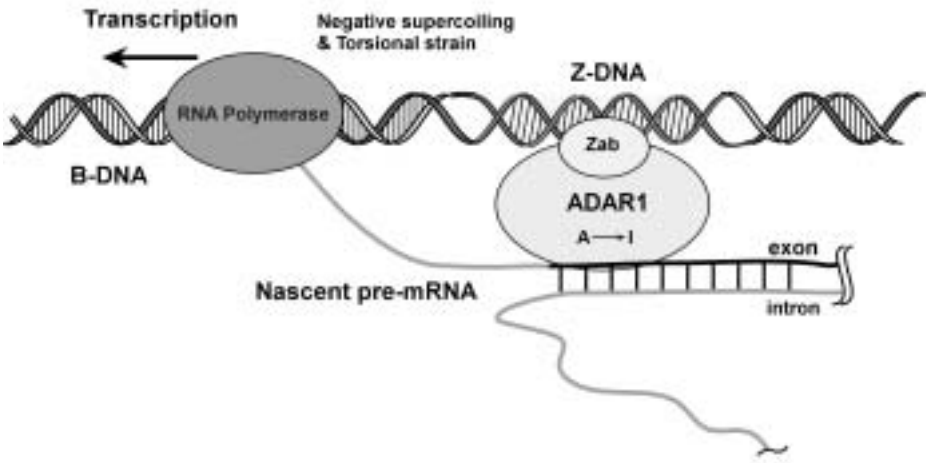

Figure 5. *In vivo*, Z-DNA is thought to be stabilized by the negative supercoiling generated by an RNA polymerase moving through a gene. Transcription also gives rise to regions of double-stranded RNA (dsRNA), formed when a nascent RNA transcript folds back on itself. The RNA editing enzyme, double-stranded RNA adenosine deaminase (ADAR1), has been shown to bind both Z-DNA and dsRNA with nanomolar affinity. Each nucleic acid is bound through a separate domain. This enzyme then catalyzes the hydrolytic deamination of an adenine within the dsRNA to form inosine, which is subsequently translated as guanine. Several editing sites may exist in a single pre-mRNA.

duplex RNA substrate is frequently formed by the pairing of an intron with an exon, and the exon is edited to change the amino acid codon. This has a number of interesting consequences. The control of the editing system rests in particular intronic sequences that are complementary to exonic sequences. In addition, it raises the question how does the enzyme manage to carry out all of its editing activity before the introns are removed by the splicing apparatus, which is known to be attached to the end of the nascent mRNA chain. This is where the postulated role of the Z-DNA binding domain becomes important.

The problem that the editing enzyme has is that of finding an actively transcribing gene in contrast to a gene that is not transcribing. Actively transcribing genes with their moving RNA polymerases generate the negative torsional strain upstream of the polymerase that transiently stabilizes Z-DNA while the polymerase is moving (Liu & Wang 1987). Hence, transcribing genes have Z-DNA in them, while non-transcribing genes do not. It is possible that the high-affinity Z-DNA binding domain at the N-terminus of ADAR1 localizes itself on the Z-DNA as a way of targeting a transcribing gene, as distinct from a non-transcribing one. In effect, it increases the local concentration of the ADAR1 editing enzyme in the vicinity of areas undergoing active transcription. The manner in which ADAR1 may bind to both Z-DNA and double-stranded RNA is shown in Figure 5.

Zα·Z-DNA Crystal Structure

By carrying out controlled proteolysis of the Zab domain of ADAR1, it was possible to isolate a stable Zα domain which could be over-expressed and purified in sufficient quantities for crystallographic studies (Schwartz *et al.* 1999a; Schwartz *et al.* 1999c). Schwartz and coworkers were able to co-crystallize Zα with a segment of Z-DNA and the structure of this complex has been solved at 2.1 Å resolution (Schwartz *et al.* 1999b). The structure that emerged from the complex was quite surprising and revealed the mechanism that nature uses for detecting Z-DNA. One Zα molecule binds to each strand of a Z-DNA duplex, but the two proteins do not interact with one another. The protein domain is folded in the form of a helix-turn-helix (HTH), a motif that is widely used in transcription factors for the recognition of specific B-DNA sequences. In the case of B-DNA-binding HTH proteins, there usually is a recognition helix that fits into the broad, deep major groove of B-DNA and contacts specific base pairs. However, in Zα a recognition helix is used, but it does not bind in a groove, and rather it runs along

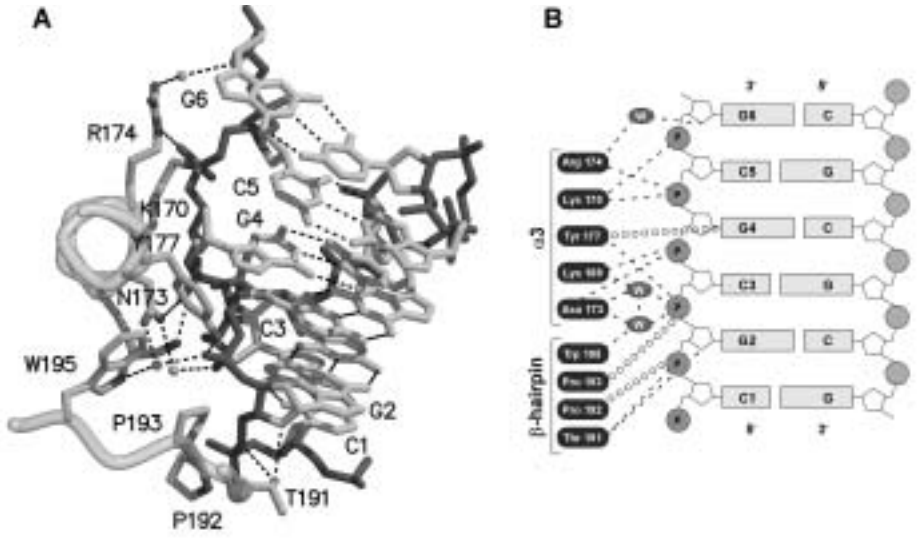

Figure 6. Recognition helix and specific interactions of the Zα·Z-DNA Complex (Schwartz *et al.* 1999b). (A) A view down the recognition helix (a3) axis shows the entire region of Z-DNA recognized by Zα. Five consecutive backbone phosphates of the Z-DNA segment are contacted by an extensive hydrogen bonding network. Protein side chains in direct or water-mediated contact with the DNA are labeled. Water molecules are represented as green spheres. Tyrosine 177 is involved in the only base-specific contact seen in the complex and is within van der Waals contact of the exposed carbon 8 of the guanine base G4; this feature is characteristic of Z-DNA. (B) Schematic of the protein-DNA contacts. Dashed lines represent H-bonds, and open circles show van der Waals contacts.

the side of the Z-DNA double helix where, together with an adjacent b-sheet, it recognizes five adjacent phosphate residues in the zig-zag backbone using a complex of 11 different hydrogen bonds. This recognition is shown in Figure 6, together with a schematic diagram illustrating the interaction modes. Another striking aspect of the interaction is the complementarity both in shape and in electrostatic interactions between the protein and the Z-DNA (Figure 7, see page VI). The only base-specific interaction is made by a tyrosine residue (Y177) which is in van der Waals contact with the C8 residue of a guanine in the *syn* conformation. By referring to Figure 2, it can be seen that the C8 residue is on the exterior of the Z-helix. This interaction is a stabilizing edge-to-face contact. On the other side of the tyrosine residue, a tryptophan is in van der Waals contact with the tyrosine

in a second stabilizing edge-to-face interaction. The tyrosine residue in this conformation can interact with guanine in the *syn* conformation, or any other base in the *syn* conformation. Thus, the Zα domain recognizes Z-DNA by its two most distinct features which differ from B-DNA, the zig-zag phosphate backbone and the *syn* conformation of a purine nucleotide.

The major difference between the interaction of Zα and Z-DNA and the structurally similar helix-turn-helix domain recognizing B-DNA is that the recognition helix has a different "angle of attack". In the B-DNA interaction, the recognition helix is almost perpendicular to the axis of B-DNA, while in the Z-DNA interaction, the recognition helix is rotated so that it is more in line with the helix axis.

The ADAR1 Zα domain also interacts with Z-RNA

When an RNA virus such as measles infects a cell, the anti-viral interferon response leads to increased activity of interferon-inducible genes. This includes the ADAR1 gene, which is strongly up-regulated and produces the full-length protein including the Zα domain (Patterson & Samuel 1995). In addition, the distribution of ADAR1 changes from primarily nuclear localization to both nuclear and cytoplasmic localization. The measles virus replicates in the cytoplasm (as do most RNA viruses), and late in infection it has been observed that the viral RNA has been subjected to hypermutation in which a significant fraction of adenines have been changed to guanines, and many uracil residues have been changed to cytosines (Cattaneo & Billeter 1992). Such mutations are the expected result of the action of ADAR1 on the viral RNA replication system, and may be an attempt on the part of the host cell to disable the virus. Hypermutation similar to that found in the measles virus has also been found in the RNA of vesicular stomatitis virus, respiratory syncytial virus and para-influenza virus 3 (Cattaneo 1994; Bass 1997).

RNA viruses generally utilize a double-stranded intermediate during some period of their life cycle (Jacobs & Langland 1996). Little is known about the conformation of this double-stranded RNA or the forces acting upon it during replication. However, the evidence of the interferon-inducible full-length variant of ADAR1 localized in the cytoplasm prompted us to investigate the possible interactions between Zα and double-stranded viral RNA in the Z-conformation.

Z-RNA was discovered a few years after Z-DNA (Hall *et al.* 1984). Although the low energy forms of right-handed duplexes of B-DNA and A-

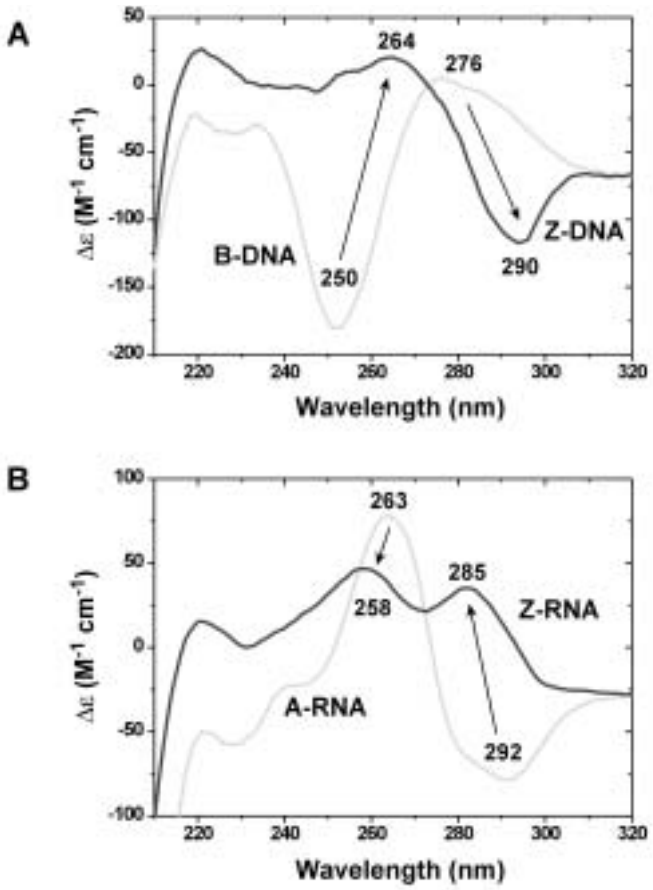

Figure 8. CD spectra of B, Z-DNA, and A, Z-RNA. Circular Dichroism is often used to observe the transition from the A-RNA, or B-DNA conformations to the Z-conformation. (A) CD spectra of the B- and Z- conformations of duplex $d(CG)_6$. B-DNA has a deep negative CD band at 250 nm and a broad positive ellipticity at 276 nm. Upon addition of NaCl to approximately 3.5 M, the spectrum nearly inverts to the Z-form with a positive ellipticity at 264 nm and a negative band at 290 nm. (B) CD Spectra of A- and Z-forms of duplex $r(CG)_6$. A-RNA has a distinctly different CD spectrum than B-DNA, characterized by a positive band at 263 nm and a broad negative ellipticity peak at 292 nm. Differences in base-stacking and the C3' *endo* sugar pucker both contribute to the differences compared to B-DNA. When $NaClO_4$ is added to *ca*. 6.5 M, the spectrum changes dramatically with the negative peak at 292 becoming a positive band at 285, and the band at 263 shifting to 258 nm, with reduced intensity. These spectral changes are due to the structural transitions in the phosphodiester backbone, alterations in base-stacking, and change of the cytosine sugar pucker. Note that much higher salt concentrations are required to shift A-RNA to the Z-conformation, than those necessary for the corresponding B-to-Z transition.

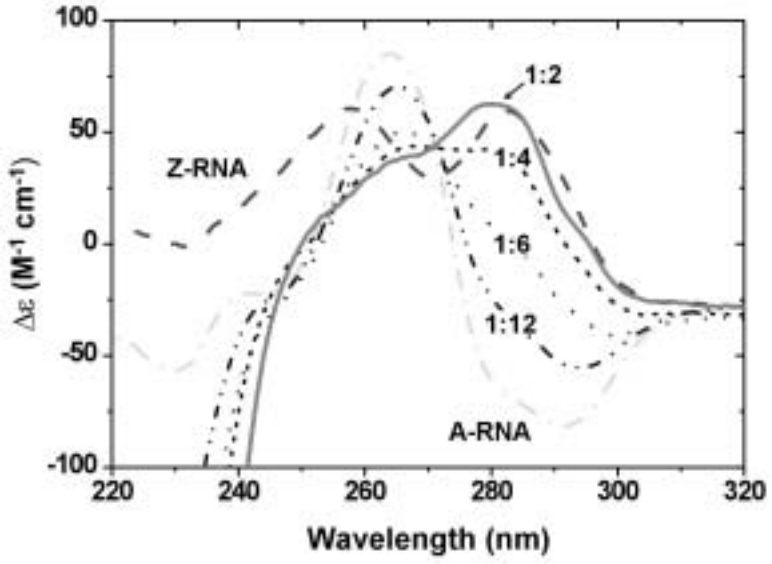

Figure 9. The Z-RNA conformation can be stabilized by Zα as shown by circular dichroism (CD) spectroscopy (Brown *et al.* 2000). Spectra are shown for 5 μM of duplex $r(CG)_6$ in the A-form (–·–·–). All samples contained 10 mM Na_2HPO_4 (pH 7), 20 mM NaCl, 0.5 mM EDTA. In 6.5 M $NaClO_4$, a typical Z-RNA spectrum is seen (– – –). The A-RNA spectrum changes as Zα is added (Zα has no CD signal above 250 nm, but a strong negative ellipticity below 250 nm). Spectra are shown for addition of 5 μM Zα (–··–··–), which is 1 Zα:12 bp; 10 mM Zα (· · · ·) 1:6; 15 mM Zα (- - - -) 1:4; and 30 μM Zα (——) 1:2. Inversion of the CD bands around 285 nm and the decrease in signal at 266 nm are characteristic of the A-to-Z-RNA transition.

RNA are structurally very different, both adopt similar left-handed Z-conformations (Teng *et al.* 1989; Davis *et al.* 1990). The Z-DNA conformation is stabilized *in vitro* by high concentrations of salt and other agents that screen repulsion between electronegative phosphate residues, which are closer together in the Z-conformation. The transition from the right-handed A-form of duplex RNA to the left-handed Z-form is much less favorable than the B-to-Z-DNA transition; consequently, higher concentrations of chaotrophic salts or low dielectric solvents combined with elevated temperatures are required to induce the transition *in vitro* (Tinoco *et al.* 1986; Klump & Jovin 1987). In order to shift from the right-handed A-RNA duplex, every other residue must change pucker from the C3'-*endo* into the C2'-*endo* conformation as both Z-DNA and Z-RNA alternate sugar puckers

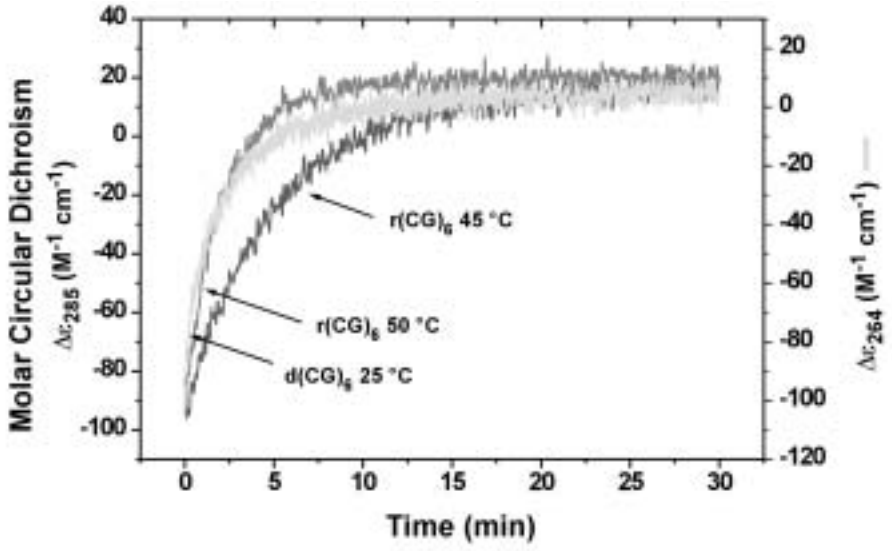

Figure 10. Temperature dependence of the B-to-Z-DNA and A-to-Z-RNA transitions (Brown *et al.* 2000). The rates of the A-to-Z-DNA transitions for the r$(CG)_6$ duplex at 45 and 50 °C (monitored at 285 nm) are comparable to the B-to-Z-RNA transition of d$(CG)_6$ (monitored at 264 nm) at 25 °C, demonstrating the higher energy requirements of the A-to-Z-RNA transition.

in the Z-conformation. The energy for changing the sugar pucker of a ribonucleotide is considerably greater than for a deoxyribonucleotide (Olson & Sussman 1982; Sanger 1984). This difference in energy accounts for the high concentrations of salts or increased temperatures which are necessary to stabilize poly r$(CG)_n$ in the Z-conformation.

It is easiest to observe the change from right-handed RNA duplex A-conformation to the Z-conformation by observing changes in the circular dichroism. Figure 8 illustrates the CD spectroscopic changes of a 12 base pair $(CG)_6$ duplex of DNA or RNA when they change from the right-handed duplex to the left-handed Z-conformation resulting from the addition of 3.5 M NaCl for the DNA and 6.5 M $NaClO_4$ for the RNA. It can be seen that there are near inversions of the CD spectrum although the actual direction of the changes is different, depending if one starts with right-handed B-DNA or right-handed A-RNA. When similar experiments were carried out using r$(CG)_6$ and gradually increasing amounts of Zα, the spectroscopic changes shown in Figure 9 were observed. This clearly indicated that the A-

RNA changed into the Z-conformation in the presence of Zα in a manner analogous to that which had been previously observed for DNA (Herbert *et al.* 1998; Schwartz *et al.* 1999a). The presence of Z-RNA in this complex was corroborated by Raman spectroscopic studies (Brown *et al.* 2000).

Analysis of the change of $r(CG)_6$ in the presence of Zα revealed that it took place at a slower rate than the conversion of $d(CG)_6$ to the Z-conformation in the presence of Zα. Figure 10 shows a scan of the change in the circular dichroism signal at fixed wavelength as a function of the rate of change from the A- and B-forms of RNA or DNA to the Z-conformation (Brown *et al.* 2000). The rate of conversion of the duplex $r(CG)_6$ at 50 ºC is approximately equal to that of the analogous DNA duplex, $d(CG)_6$, at 25 ºC. This is a reflection of the activation energy required for the transition to the Z-conformation which is 24 kilocalories per mole for $d(CG)_6$, compared to 38 kcal mole-1 for $r(CG)_6$. The increased activation energy for RNA is largely due to the energy required to change the sugar pucker of ribonucleotides compared to that required for deoxyribonucleotides.

It is not surprising that proteins which bind to B-DNA do not bind to A-RNA, and *vice versa*, since these right-handed duplexes differ significantly in shape; however, the left-handed Z-form duplexes are very similar. Zα may be the first nucleic acid binding domain that binds specifically to *both duplex DNA and RNA*. The role of this domain in the hypermutation of RNA viruses has yet to be explored. A great deal is known about the negative superhelicity generated by transcription of dsDNA, but little is known about negative torsional strain in replicating RNA molecules. This subject needs to be more fully explored in order to understand the possible participation of the Zα binding domain in the hypermutational activities of ADAR1 during infections by RNA viruses.

Conclusions

Since the discovery of Z-DNA in 1979 and Z-RNA in 1984, many groups have worked to understand whether specific biological functions are associated with these unusual nucleic acid conformations. The discovery that the N-terminus of ADAR1 bound Z-DNA with high affinity and the subsequent efforts, including a detailed structural view of the interaction, have shed much light into this interesting system, but this is only a first step. Less is known about Z-RNA. Although a few studies attempted to identify this structure in cells (Zarling *et al.* 1987), research on Z-RNA has been dor-

mant for almost a decade. The recent finding which demonstrated that the Zα domain of ADAR1 could also bind Z-RNA has reinitiated interest, and raised more questions than it has answered.

The future for the Z-conformations of nucleic acid has many difficult questions which need to be addressed. Work is ongoing to identify biological activities that may be associated with these structures and the current abundance of genomic information has fueled efforts to seek additional proteins which may specifically interact with the Z-conformations and to identify sequences which have the propensity to form Z-DNA or Z-RNA. New developments from biochemical and structural studies of other organisms may provide insight into the problems of biological functions There is little doubt, however, that some of the answers will be unexpected.

Acknowledgements

This work was supported by grants to A.R. from the NIH and NSF. B.A.B. was supported by an Aid for Cancer Research postdoctoral fellowship.

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