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Left-handed Z-DNA: structure and function

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Abstract

Z-DNA is a high energy conformer of B-DNA that forms *in vivo* during transcription as a result of torsional strain generated by a moving polymerase. An understanding of the biological role of Z-DNA has advanced with the discovery that the RNA editing enzyme double-stranded RNA adenosine deaminase type I (ADAR1) has motifs specific for the Z-DNA conformation. Editing by ADAR1 requires a double-stranded RNA substrate. In the cases known, the substrate is formed by folding an intron back onto the exon that is targeted for modification. The use of introns to direct processing of exons requires that editing occurs before splicing. Recognition of Z-DNA by ADAR1 may allow editing of nascent transcripts to be initiated immediately after transcription, ensuring that editing and splicing are performed in the correct sequence. Structural characterization of the Z-DNA binding domain indicates that it belongs to the winged helix-turn-helix class of proteins and is similar to the globular domain of histone-H5.

Introduction

DNA is capable of assuming many different conformations other than the familiar right-handed B-DNA double helix [1]. One of the most dramatic examples is the Z-DNA conformer, which is left-handed [2]. Like B-DNA, the two strands of Z-DNA are antiparallel and joined by Watson–Crick base-pairing. In contrast to B-DNA, which has all its bases in the anti-conformation, the bases in the Z-DNA helix alternate between the anti-conformation and the unusual syn-conformation. This dinucleotide repeat causes the backbone to follow a zigzag path, giving rise to the name Z-DNA. In Z-DNA, there is only a single narrow groove that corresponds to the minor groove of B-DNA. No major groove exists. Instead, the 'information' rich residues that allow sequence-specific recognition of B-DNA lie exposed on the convex outer surface of Z-DNA (Figure 1). This transition from B- to Z-DNA occurs most readily in sequences, with alternations of purines and pyrimidines, especially alternating deoxycytosine and deoxyguanine residues [3–5].

The biological role of Z-DNA is an area of active study. The aim of these investigations is to determine whether this alternate DNA conformations exist *in vivo*, how its formation is regulated, and what information it conveys. Here we will review recent studies that bear on the role of Z-DNA in biological systems.

Formation of Z-DNA *in vitro*

The existence of Z-DNA was first suggested by optical studies demonstrating that a polymer of alternating guanine and cytosine residues ($d(CG)_n$) produced a nearly inverted circular dichroism spectrum in a high salt solution [6]. The physical reason for this finding remained a mystery until an atomic resolution crystallographic study of $d(CG)_3$, rather surprisingly revealed the existence of a left-handed double helix [2]. Further experiments using Raman spectroscopy confirmed that the crystal structure was the same as formed when poly($d(CG)_n$) was placed in a high salt solution [7]. Additional studies using circular dichroism to follow the transition from B- to Z-DNA demonstrated that Z-DNA can form from B-DNA under physiological salt conditions when deoxycytosine is 5-methylated [8]. The subsequent discovery that Z-DNA formed under conditions of negative superhelical stress raised considerable excitement as this brought the left-handed conformation within the realm of biology [3, 5, 9].

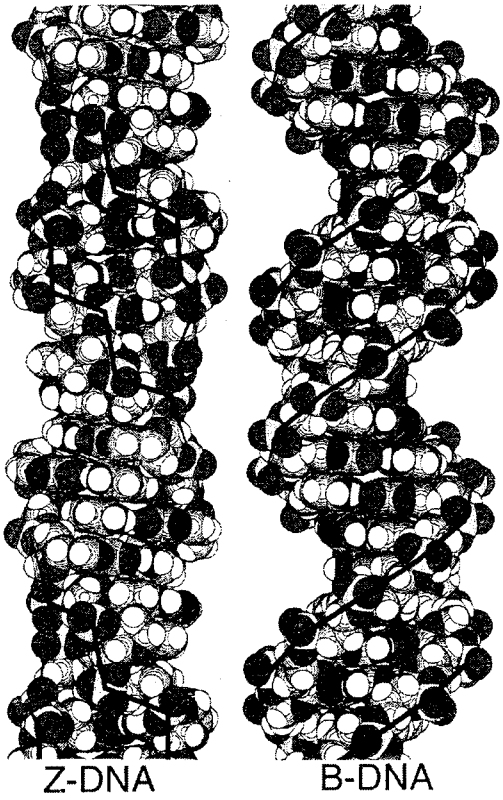

Figure 1. The 'information rich' residues that allow sequence-specific recognition of the major groove of B-DNA lie on the convex surface of left-handed Z-DNA helix. The two DNA strands of each duplex are highlighted by solid-black lines. The 'zigzag' nature of the Z-DNA backbone is clearly seen (adapted from [2]).

Stabilization of Z-DNA by negative supercoiling illustrates a number of features about this conformation. First, formation of Z-DNA requires energy. The amount necessary is proportional to the square of the number of negative supercoils lost from a covalently closed circular plasmid when a sequence fips into the Z-DNA conformation. For each turn of Z-DNA stabilized, approximately two supercoils are lost. The free energy required to effect the transition can be quantitated using two dimensional gel assays to follow the change in plasmid topology [10–14]. Second, sequences other than alternating purines and pyrimidines can form Z-DNA. The ease with which this occurs depends on the sequence – $d(CG)_n$ is best, $d(TG)_n$ is next, and a $d(GGGC)_n$ repeat is better than $d(TA)_n$ [12, 14, 15]. Third, formation of B–Z DNA junctions, each of which has a free energy ΔG near $+4$ kcal/mole, is a significant energetic barrier to Z-DNA formation [10].

Formation of Z-DNA *in vivo*

Due to the requirement for energy, formation of Z-DNA *in vivo* is an active process. One source of available energy is provided by transcription. As pointed out by Liu and Wang, negative supercoils arise behind a moving RNA polymerase as it ploughs through the DNA double helix [16], providing one mechanism for the initiation of Z-DNA formation *in vivo*. Computer models are consistent with this prediction. One analysis of 137 fully sequenced human genes demonstrated that sequences which could form Z-DNA easily were present in 98 genes. These sequences were distributed nonrandomly throughout a gene – sequences were ten times more frequent in 5′ than in 3′ regions [17]. They lie precisely in the regions of a gene where negative supercoiling is highest during transcription.

Experimental demonstration of Z-DNA formation *in vivo*

A number of experiments in prokaryotes have been used to demonstrate that Z-DNA forms *in vivo*, and that this occurs as a result of transcription. One approach is to detect Z-DNA using chemical modification of DNA. Through use of either osmium tetroxide or potassium permanganate, the formation within *E. coli* of Z-DNA in plasmids with a $d(CG)_n$ insert can be demonstrated [18, 19]. UV crosslinking of bacteria treated with psoralens have confirmed these results, and made possible a precise measurement of the amount of unrestrained supercoiling present within *E. coli* necessary to initiate formation of Z-DNA [20]. Another approach has used a construct in which an *EcoR1* site is embedded in a Z-DNA forming sequence [21–23]. In the bacterial cell, this fragment can be methylated when it is in the B-DNA conformation but it becomes resistant to methylation while in the Z-DNA conformation. Susceptibility to methylation of the *EcoR1* site thus provides an *in vivo* measure of Z-DNA formation. In *E. coli*, Z-DNA is formed in the absence of external perturbation and is increased by transcription, an effect that is enhanced by mutations inactivating topoisomerase I [22, 23]. In *Morganella*, *Klebsiella*, or *Enterobacter* formation of Z-DNA was not observed [23].

It has been difficult to directly demonstrate the existence of Z-DNA in eukaryotic systems due to their increased complexity. 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 recognize this conformation [24]. One natural source rich in anti-Z-DNA antibodies is the sera obtained from patients with auto-immune diseases, especially lupus erythematosus [25]. These antibodies are produced during the exacerbations of the disease, along with antibodies to many other nuclear components. The high specificity of these antibodies strongly suggest that Z-DNA is the cognate antigen and, by implication, that Z-DNA exists *in vivo*.

Antibodies raised in rabbits and sheep were used in staining experiments with both fixed [26] and unfixed polytene chromosomes of *Drosophila* [27]. These antibodies produced an unusual staining pattern of interband regions but did not stain bands. Staining was especially intense in the puffs which are associated with high levels of transcriptional activity (reviewed in [28]). Antibodies were also used in staining ciliated protozoa which have both a macronucleus and a micronucleus [29]. The micronucleus is used for genetic reproduction, but the macronucleus is the site of all transcriptional activity. Here, again, the macronucleus stained exclusively with no staining in the micronucleus. Both of these early experiments suggested, somewhat indirectly, a link between transcriptional activity and the presence of Z-DNA.

Analysis of intact mammalian systems has been more complicated. There are a number of limitations in these experiments. As yet, no phenotype has been associated with presence or absence of Z-DNA forming sequences, thus excluding the use of genetic approaches. In order to model Z-DNA formation *in vivo* a number of experiments have been carried out using metabolically active permeabilized mammalian nuclei which were formed by embedding intact cells in agarose microbeads using the method of Jackson and Cook [30]. Here, low concentrations of detergent are used to lyse the cytoplasmic membrane and permeabilize the nuclear membrane. These nuclei have been shown to replicate DNA at 85% of the rate observed in the intact cell, and they are transcriptionally competent [31]. In these experiments the amount of Z-DNA present in the gene is measured by diffusing biotin-labeled anti-Z-DNA monoclonal antibodies into the beads [32]. The amount of Z-DNA present can be measured by quantitating how much radioactive streptavidin binds within the nucleus. Such experiments show that, at low concentrations of antibody, the amount of Z-DNA measured was independent of the antibody added over a 100-fold change in antibody concentration, suggesting that the Z-DNA is present *de novo* in these preparations rather than being induced by antibody. Furthermore, the amount of Z-DNA present increased dramatically during active transcription, consistent with the model of Liu and Wang [16], but was largely unaffected by DNA replication [33].

In further experiments, it was found that individual genes could be assayed by cross linking the antibody to DNA using a 10-ns exposure of a laser at 266 nm [34]. Release of DNA fragments with attached antibody was accomplished by diffusing in restriction endonucleases and performing an *in situ* DNA digest. Following isolation of biotin-labeled antibody-DNA complexes with streptavidin magnetobeads, free DNA was obtained by proteolysis. These experiments made it possible to determine the site of Z-DNA formation in particular genes. Using hybridization or PCR techniques, the *c-myc* gene was studied in murine U937 cells [34]. Three transcription-dependent Z-DNA forming segments were identified in the 5′ region of the gene with two of them near promoters [35]. 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 by a rapid reduction in the amount of Z-DNA present in these three regions. In contrast, Z-DNA formation in the beta actin gene, which is not down regulated with differentiation, was detected under all the conditions tested.

In other studies with a primary liver cell line, induction of Z-DNA was measured in the corticotropin hormone-releasing gene [36]. 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.

Functional consequences of Z-DNA formation

The role of Z-DNA in biological processes is currently unknown. In principle, Z-DNA formation could have a functional role that need not involve its recognition by proteins. For example, *E. coli* RNA polymerase does not transcribe through Z-DNA [37] raising the possibility that the formation of Z-DNA

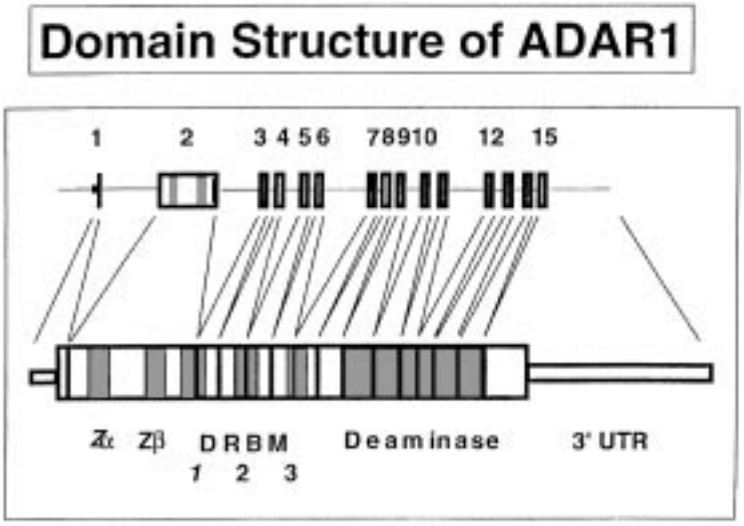

Figure 2. The domain structure of ADAR1. ADAR1 has two Z-DNA binding motifs, 3 double-stranded RNA binding motifs and a deaminase domain. The short form which starts at methionine 296, lacks the N-terminal Zα domain (adapted from [95]).

behind (5′) to a moving polymerase may block a trailing RNA polymerase from transcribing through that region of a gene until the torsional strain stabilizing the Z-DNA is relieved by topoisomerases. This mechanism might ensure spatial separation between successive polymerases. As a consequence, processing of an RNA would then be physically and temporally removed from that of subsequent transcripts, perhaps minimizing non-functional trans-splicing in eukaryotes.

Alternatively, formation of Z-DNA may relieve topological strain that arises when intact duplexes are intertwined as occurs during recombination events involving Holliday junction intermediates [38]. For example, the Z-forming $d(CA/GT)_n$ sequence has been shown to be recombinogenic in yeast [39], but is found to be less efficient than $d(CG)_n$ in human cells [40, 41]. Furthermore, several reports have correlated chromosomal breakpoints in human tumors to potential Z-DNA forming sequences, although no causal relationship has yet been established [42–46]. In addition, Z-DNA formation could affect the placement of nucleosomes as well as the organization of chromosomal domains by providing regions from which histones or other architectural proteins are excluded [47]. Lastly, Z-DNA may perform unexpected roles in organisms such as the primitive eukaryote dinoflagellate *Prorocentrum micans*, which lack histones and nucleosomes but forms immunologically detectable Z-DNA at the nuclear periphery and at the segregation fork of dividing chromosomes [48].

There have been many attempts to find proteins that bind to Z-DNA in the hope that they would indicate indirectly the presence of Z-DNA *in vivo*, and help establish a biological role for this conformation. Early studies were unfruitful and caused widespread skepticism that Z-DNA would be associated with any biological function. Many of the apparently positive results reported in these studies may have been due either to artefacts or misinterpretation of data [49–51]. However, absence of proof was confused with absence of existence.

Identification of a high affinity Z-DNA binding protein with enzymatic activity

Our work has recently shown that one type of double-stranded RNA adenosine deaminase (ADAR) [52] called ADAR1 binds Z-DNA *in vitro* with high affinity [53–55]. The dissociation constant of the Z-DNA binding domain is nanomolar, making it likely that this interaction is functional [56]. The binding of ADAR1–Z-DNA was identified initially in bandshift assays, using competition with high concentrations of unlabeled polynucleotides to indirectly confirm specificity of binding [53]. Mapping studies showed the presence in ADAR1 of two Z-DNA binding motifs, called Zα and Zβ [56] (Figure 2). Zα alone is able bind to Z-DNA with high affinity, but can interact with Zβ to form a domain with slightly different binding properties [57, 58]. The specificity of recombinant

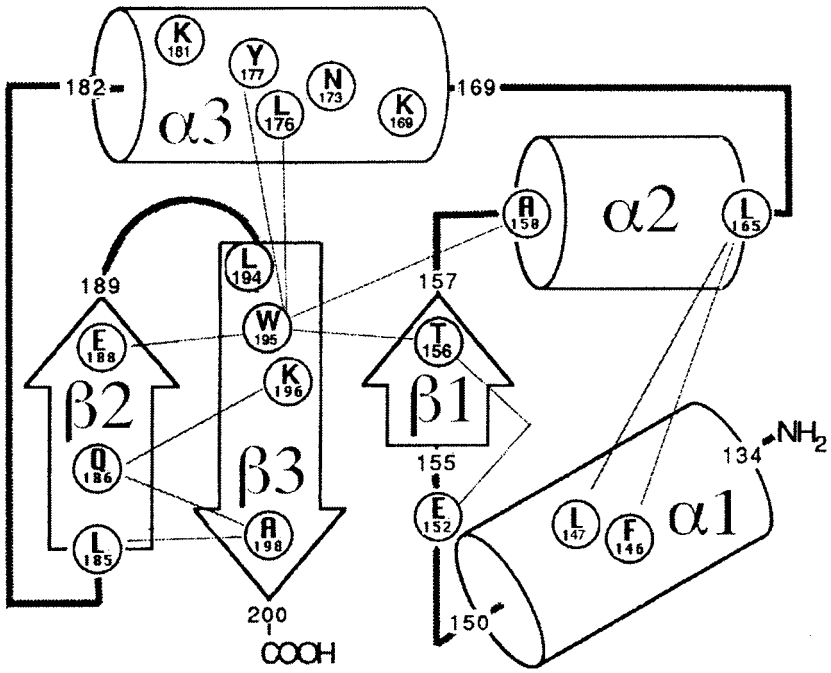

Figure 3. The topology and candidate Z-DNA contacts of Zα. The data show that the topology determined by NMR and the location of candidate contacts of Zα with Z-DNA determined by mutagenesis are in some respects similar to those of histone H5 [61] and HNF-3γ [62]. The position of α-helices and β-strands are indicated by cylinders and arrows connected with thick lines. Numbers correspond to amino acid residues. Long range NOEs are indicated with thin lines and show the interactions between the C-terminal β-sheet and the α-helices of Zα. W195 makes extensive contacts with other amino acids. Residues (K169, N173, Y177, K181) on the face of α3 that putatively contact Z-DNA are indicated (adapted from [63]).

Zα for Z-DNA has now been directly confirmed using biophysical techniques such as circular dichroism and Raman spectroscopy [59, 60]. NMR studies have confirmed structure predictions that Zα belongs to the winged-helix-turn-helix family of proteins (Figure 3). The fold is similar to that found in the globular domain of histone H5 [61] and the transcription factor HNF-γ3 [62]. The domain consists of a helix-turn-helix motif (incorporating α2 and α3 shown in Figure 3) and a C-terminal β-sheet that constrains the fold through contacts with residues lying between α1 and α2. Mutagenesis studies confirm that α3 has the properties of a recognition helix and also show that residues in the C-terminal β-sheet are also involved in binding to Z-DNA [63]. Further structural studies are necessary to resolve how the winged helix-turn-helix fold can be used to recognize both right- and left-handed DNA.

Domain structure of ADAR1

ADAR1 has a complex structure (Figure 2). It has two copies of a Z-DNA binding motif that are absent in ADAR2, the only other known member of this enzyme family [59]. In addition, ADAR1 has three copies of a double-stranded RNA binding motif (DRBM) as well as a catalytic domain related to that of *E. coli* cytidine deaminase [64–66].

A number of different variants of ADAR1 are produced in cells [67]. The DRBM for example show some variation arising from differential splicing that may affect substrate specificity [68]. A dramatic variation appears due to a splicing event that replaces the exon containing the methionine necessary to initiate translation of full length ADAR1. Instead a methionine at position 296 is used to produce a short form of ADAR1(compare sequence in Genbank accession number X79448 with that in X79449). The shorter form has only one copy of the Z-DNA binding motif and binds Z-DNA with a lower affinity when compared to the longer form [59, 69]. The splicing event that leads to the formation of the shorter form of ADAR1 is sensitive to the presence of interferon, which causes increased production of the long form of the enzyme [68]. The Z-DNA binding properties of ADAR1 can thereby be modulated, although the

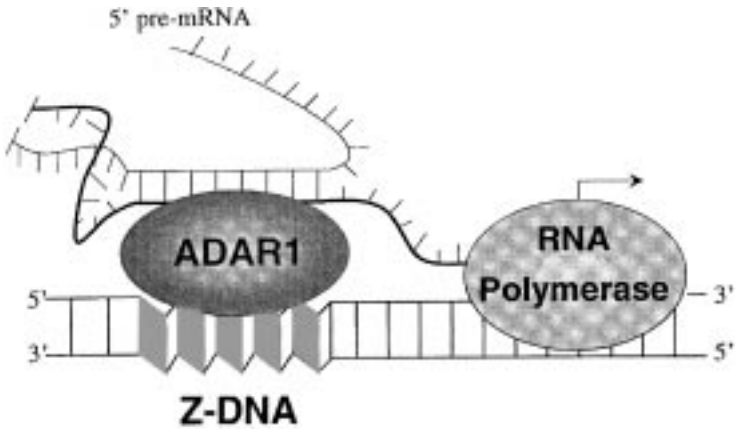

Figure 4. Model for regulation of ADAR1 activity by Z-DNA. *In vivo*, Z-DNA can be stabilized by the negative supercoiling generated by an RNA polymerase moving through a gene. Transcription also gives rise to regions of double-stranded RNA (dsRNA), formed when a nascent RNA transcript (pre-mRNA) folds back on itself. The RNA editing enzyme, dsRNA adenosine deaminase type 1 (ADAR1) has been shown to bind both Z-DNA and dsRNA with nanomolar affinity. It is proposed that binding to Z-DNA allosterically activates editing by ADAR1, initiating modification of a transcript as it forms and before splicing has occurred. This enzyme causes the hydrolytic deamination of adenine within the dsRNA to form inosine, which is subsequently translated as guanine. Editing thus changes the read-out of a gene. Several editing sites may exist in a particular pre-mRNA. ADAR1 thus utilizes the structural information encoded in Z-DNA and dsRNA to alter the linear flow of information from DNA to RNA.

physiological significance of this event remains to be determined.

ADAR1 and dsRNA editing

What role does recognition of Z-DNA play in the biology of ADAR1? ADAR1 belongs to a family of deaminases that modify double-stranded mRNA by catalyzing the hydrolytic deamination of adenine to form inosine, which is subsequently translated as guanosine [70–73]. When this change occurs in the first two positions of a codon, an amino acid different from the one encoded by the gene may be placed at that site. Twenty-six codons specifying 12 amino acids can be substituted in this manner. An illustrative example is editing of the GluR-B receptor subtype RNA which specifies a component of the AMPA sensitive glutamic acid receptor. Glutamic acid is the major excitatory neurotransmitter in mammalian brains. As a result of editing, a glutamine (CAG) in the exon specifying the second transmembrane domain of the GluR-B subunit is replaced by arginine (CGG) [74]. This modification changes the electrophysiological properties of the assembled receptor, reducing the calcium conductance of the ion channel [75, 76]. Whether this change is brought about by ADAR1 or another member of the ADAR family, such as ADAR2, is currently controversial [77]. Other putative substrates for ADAR1 and ADAR2 are known. The list currently includes multiple sites in glutamic receptor sub-units GluR-A,B,C,D,5 and 6, the serotonin-2C receptor, a liver α 2,6 sialotransferase, and the squid voltage-gated potassium receptor [78–82]. Addition substrates are likely to exist as inosine is found in brain mRNA at a frequency of one in every 17,000 ribonucleotides [83]. A method has been recently described to allow detection of such edited messages [84]. Members of the ADAR family are also ubiquitous in metazoa [85]. These results suggest that A–I editing is of evolutionary significance [85, 86], producing phenotypic variation by altering the linear flow of information from DNA to RNA.

dsRNA editing and Z-DNA

In the cases so far examined, the double-stranded RNA editing substrate is formed by folding the 3′ intron back onto the exon to basepair with the site that is edited [79, 81, 87–89]. The involvement of introns requires that editing occurs soon after transcription of RNA, and before splicing. This sequence of events provides a rationale for the recognition of Z-DNA by ADAR1. As discussed above, Z-DNA *in vivo* is a transcription-dependent structure and will form when appropriate sequences are present behind (5′ to) a moving RNA polymerase. Transcription-induced Z-DNA may serve to localize the editing activity of ADAR1 to a particular region within a gene, preventing indiscriminate modification of other double-stranded RNAs (Figure 4). This mechanism allows

editing to initiate as the transcript is produced; allowing ADAR1 to act before the intron is removed by the splicing apparatus. Recognition of Z-DNA by ADAR1 may also block the gene from further transcription until editing of the RNA is complete. Furthermore, it is possible to imagine that the extent of adenosine to inosine modification at a particular site is related to the amount of Z-DNA formed, depending upon the ease with which surrounding sequences adopt the Z-DNA conformation as well as the local superhelical density.

Recent results have allowed this model to be refined further. We have shown that recognition of Z-DNA by Zα is conformation specific rather than sequence specific [57].

A rather dramatic example of the ability of Zα to bind different Z-DNA sequences is shown in Figure 5. In this circular dichroism experiment, short oligonucleotides with two 6 bp binding sites for Zα are used. The first site is $d(CG)_3$ and is used to initiate Z-DNA formation by the oligonucleotide. The second site can be varied to test for interaction of Zα with the Z-DNA conformation of that sequence. Two such substrates are shown in Figure 5. In Figure 5A both binding sites are $d(CG)_3$. Under low salt conditions, the oligonucleotide is in the B-DNA conformation (dotted line). However, in the presence of Zα (solid line), under these same low salt conditions, the spectrum inverts to give one characteristic of Z-DNA, as shown by comparison with the spectrum obtained when the oligonucleotide is placed in 4M NaCl (dashed line).

In Figure 5B the second site is $d(TA)_3$. The low salt (dotted line) and high salt spectrum of the oligonucleotide (dashed line) are shown. In 4M NaCl, the spectrum only undergoes partial inversion, indicating that even under these stringent conditions the $d(TA)_3$ site cannot fully adopt the Z-DNA conformation. However, in the presence of Zα under low salt conditions, inversion of the spectrum is complete showing that Zα binds to the $d(TA)_3$ as well as the $d(CG)_3$ site and stabilizes it in the Z-DNA conformation. This result is confirmed by bandshifting experiments that demonstrate that both sites on the oligonucleotide are occupied by Zα [57]. Similar results are obtained when other sequences are used in the second site, indicating that Zα is specific for the Z-DNA conformation rather than for a particular sequence [57]. Thus, Zα can recognize any sequence when conditions are such that it forms Z-DNA.

This result raises the question of how Zα targets a particular Z-DNA forming region within a gene, rather than all Z-DNA forming regions in the gen-

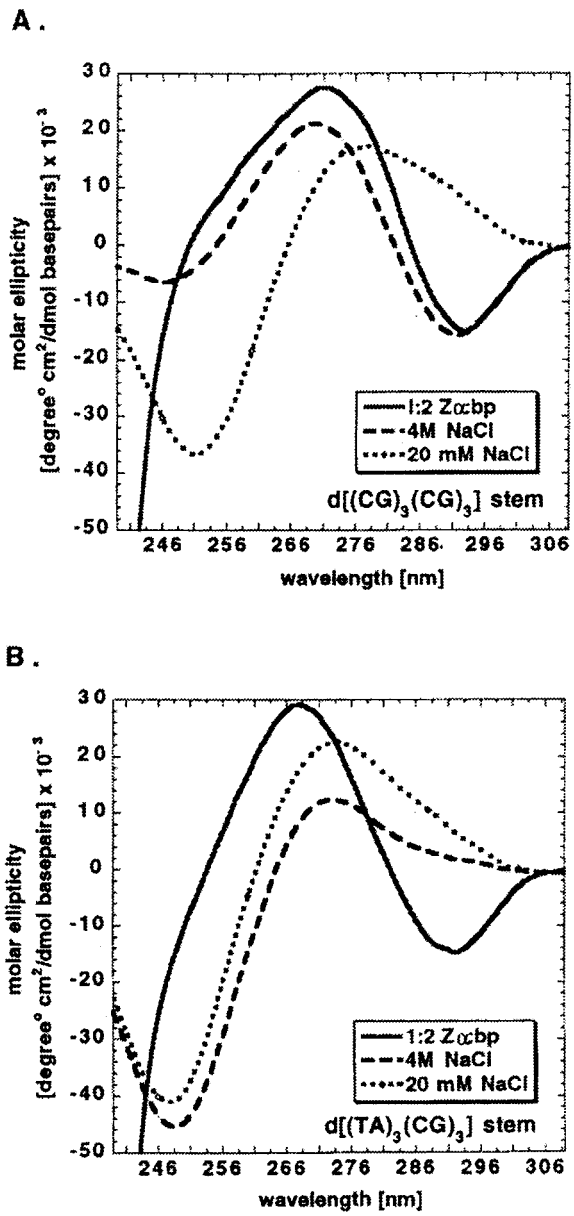

Figure 5. CD titration of Zα complexed to DNA hairpins with two binding sites. The DNA hairpins $d[(CG)_6T_3(CG)_6]$ (panel A.) and $d[(TA)_3(CG)_3T_3(CG)_3(TA)_3]$ (panel B) were titrated with Zα peptide in 50 mM Tris.HCl, 50 mM NaCl, 0.1 mM Na_2EDTA (pH 7.4) at 30°C in a Aviv 60DS spectrometer. Spectra obtained using a ratio of 1 mole of Zα to 2 moles of basepairs are shown (solid lines). Reference spectra obtained in the absence of protein (dotted line) and in 4M NaCl (dashed line) are also shown. In 4M salt hairpins $d[(CG)_6T_3d(CG)_6]$ forms Z-DNA, while $d[(TA)_3(CG)_3T_3(CG)_3(TA)_3]$ undergoes only a partial transition. However, Zα can stabilize $d(TA)_3$in the Z-DNA conformation as shown by the solid line in panel B. The CD signal produced by Zα alone is equivalent to the baseline in the region of 250–300 nm. The protein alone has a strong negative component below 240 nm (adapted from [57]).

ome. A number of models can be proposed. ADAR1 may, for example, travel with the polymerase as it elongates transcripts, dissociating when it sees a Z-DNA sequence. However, immunofluorescence studies fail to show a co-localization between Zα and RNA polymerase making this model unlikely (Herbert, unpublished data). We have shown that high affinity binding to Z-DNA requires two Zα molecules [57]. Other protein partners may confer on Zα sequence-specific recognition of Z-DNA. Zβ may be one such domain and there is some *in vitro* evidence to support this proposal [57, 58]. Alternatively ADAR1 may bind to a specific B-DNA sequence and wait until a passing polymerase induces the local formation of Z-DNA before it becomes active. In this case, the conformation specific recognition of Z-DNA may induce allosteric changes in ADAR1 that initiate binding of dsRNA and induce catalytic function. This mechanism allows Z-DNA to be captured as it forms and before it is dissipated by topoisomerases and other proteins that relieve superhelical stress. Current work, using UV cross-linking techniques, supports the idea that ADAR1 binds DNA *in vivo*, although the full complement of sequences bound are not yet known.

Other Z-DNA binding proteins

The winged-helix-turn-helix family of proteins to which Zα belongs is large and is involved in many aspects of growth and development. It is possible that other members of this family recognize Z-DNA in a manner similar to Zα, allowing their activities to be regulated by transcription. Many different roles in the spatial and temporal co-ordination of the cell's molecular machinery are possible.

One interesting element may be E3L protein from vaccinia virus, which shows some sequence similarity to Zα in its N-terminus. The carboxy terminus of E3L also contains a dsRNA binding domain that is essential to interferon resistance [59], making it similar in domain structure to ADAR1. It is also expressed in a long and a short form that lacks the N-terminal region. We have confirmed that the N-terminal domain binds to Z-DNA, but its function remains to be established.

Other proteins may exist that bind to Z-DNA with lower affinity than ADAR1. It has been demonstrated that peptides in which every second residue is lysine will stabilize Z-DNA *in vitro* at micromolar concentrations [90]. This provides a simple protein motif with which to recognize Z-DNA. This motif exists in a number of proteins, but it remains to be shown that such proteins interact with Z-DNA. In addition, evidence has been presented to show that topoisomerase II from *Drosophila*, humans and calf thymus recognize a number of different DNA conformations, including Z-DNA [91–93]. However, the domain interacting with Z-DNA has not yet been biochemically defined, nor has direct biophysical evidence been provided proving that this protein binds to Z-DNA rather than some other non-B-DNA conformation present in these polymers.

Future prospects

A role for Z-DNA *in vivo* has not yet been firmly established. The recognition of this conformation by ADAR1 provides a promising lead. Many questions remain unanswered. How does Z-DNA exactly affect dsRNA editing ADAR1? How many of the potential Z-DNA forming regions in a genome are used by ADAR1 to regulate editing? Are there other proteins that have a Z-DNA binding domain but a different enzymatic function? Are there other families of Z-DNA binding proteins that have so far escaped detection? These are difficult questions. As the quest for the biological role of Z-DNA has already shown, they are not for the faint-hearted. However, their solution will probably reveal many unexpected insights into how nature, the 'blind watchmaker' [94], utilizes subtle informational cues to co-ordinate its activities.

References

1. Rich A.: DNA comes in many forms, *Gene* **135** (1993): 99–109.
2. Wang A.H.-J., Quigley G.J., Kolpak F.J., Crawford J.I., van Boom J.H., van der Marel G. and Rich A.: Molecular structure of a left-handed double helical DNA fragment at atomic resolution, *Nature* **282** (1979) 680–686.
3. Klysik J., Stirdivant S.M., Larson J.E., Hart P.A. and Wells R.D.: Left-handed DNA in restriction fragments and a recombinant plasmid, *Nature* **290** (1981): 672–677.
4. Haniford D.B. and Pulleybank D.E.: Facile transition of poly[d(TG).d(CA)] into a left-handed helix in physiological conditions, *Nature* **302** (1983): 632–634.
5. Peck L.J., Nordheim A., Rich A. and Wang J.C.: Flipping of cloned $d(pCpG)_n.d(pCpG)_n$ DNA sequences from right- to left-handed helical structure by salt, Co(III), or negative supercoiling, *Proc. Natl. Acad. Sci. USA* **79** (1982): 4560–4564.
6. Pohl F.M. and Jovin T.M.: Salt induced co-operative conformational change of a synthetic DNA: Equilibrium and kinetic studies with poly(dG-dC), *J. Mol. Biol.* **67** (1972): 375–396.
7. Thamann T.J., Lord R.C., Wang A.H.-J and Rich A.: The high salt form of poly(dG-dC).ploy(dG-dC) is left-handed Z-DNA:

Raman spectra of crystals and solutions, *Nucleic Acids Res.* **9** (1981): 5443–5457.
8. Behe, M. and Felsenfeld G.: Effects of methylation on a synthetic polynucleotide: The B–Z transition in poly(dG-m^{5}dC)-poly(dG-m^{5}dC), *Proc. Natl. Acad. Sci. USA* **78** (1981): 1619–1623.
9. Singleton C.K., Klysik J., Stirdivant S.M. and Wells R.D.: Left-handed Z-DNA is induced by supercoiling in physiological ionic conditions, *Nature* **299** (1982): 312–316.
10. Peck L. and Wang J.C.: Energetics of B-to-Z transition in DNA, *Proc. Natl. Acad. Sci. USA* **80** (1983): 6206–6210.
11. Ellison M.J., Kelleher III R.J, Wang A.H.-J., Habener J.F. and Rich A.: Sequence-dependent energetics of the B–Z transition in supercoiled DNA containing nonalternating purine-pyrimidine sequences, *Proc. Natl. Acad. Sci. USA* **82** (1985): 8320–8324.
12. McLean M.J., Blaho J.A., Kilpatrick M.W. and Wells R.D.: Consecutive A–T pairs can adopt a left-handed DNA structure, *Proc. Natl. Acad. Sci. USA* **83** (1986): 5884–5888.
13. Zacharias W., O'Connor T.R. and Larson J.E.: Methylation of cytosine in the 5-position alters the structural and energetic properties of the supercoil-induced Z-helix and of B–Z junctions, *Biochemistry* **27** (1988): 2970–2978.
14. Ho P.S., Ellison M.J., Quigley G.J. and Rich A.: A computer aided thermodynamic approach for predicting the formation of Z-DNA in naturally occurring sequences, *EMBO J.* **5** (1986): 2737–2744.
15. Ellison M.J., Feigon J., Kelleher III R.J., Wang A.H.-J., Habener J.F. and Rich A.: An assessment of the Z-DNA forming potential of alternating dA–dT stretches in supercoiled plasmids, *Biochemistry* **25** (1986): 3648–3655.
16. Liu L.F. and Wang J.C.: Supercoiling of the DNA template during transcription, *Proc. Natl. Acad. Sci. USA* **84**(20) (1987): 7024–7027.
17. Schroth G.P., Chou P.J. and Ho P.S.: Mapping Z-DNA in the human genome. Computer-aided mapping reveals a non-random distribution of Z-DNA-forming sequences in human genes, *J. Biol. Chem.* **267** (1992): 11846–11855.
18. Jiang H., Zacharias W. and Amirhaeri S.: Potassium permanganate as an in situ probe for B–Z and Z–Z junctions, *Nucleic Acids Res.* **19** (1991): 6943–6948.
19. Palacek E., Rasvoka E. and Boublikova P.: Probing of DNA polymorphic structure in the cell with osmium tetraoxide, *Biochem. Biophys. Res. Commun.* **150** (1988): 731–738.
20. Zheng G., Kochel T., Hoepfner R.W., Timmons S.E. and Sinden R.R.: Torsionally tuned cruciform and Z-DNA probes for measuring unrestrained supercoiling at specific sites in DNA of living cells, *J. Mol. Biol.* **221** (1991): 107–129.
21. Jaworski A., Hsieh W.-T., Blaho J.A. and Larson J.E.: Left-handed DNA *in vivo*, *Science* **238** (1987): 773–777.
22. Rahmouni A.R. and Wells R.D.: Stabilization of Z-DNA *in vivo* by localized supercoiling, *Science* **246** (1989): 358–363.
23. Jaworski A., Higgins N.P., Wells R.D. and Zacharias W.: Topoisomerase mutants and physiological conditions control supercoiling and Z-DNA formation *in vivo*, *J. Biol. Chem.* **266** (1991): 2576–2581.
24. Lafer E.M., Valle R.P.C., Moller A., Nordheim A., Schur P.H., Rich A. and Stollar B.D.: Z-DNA-specific antibodies in Human systemic lupus erythematosis, *J. Clin. Invest.* **71** (1983): 314–321.
25. Lafer E.M., Sousa R., Ali R., Rich A. and Stollar B.D.: The effect of anti-Z-DNA antibodies on the B-DNA–Z-DNA equilibrium, *J. Biol. Chem.* **261** (1986): 6438–6443.
26. Nordheim A., Pardue M.L., Lafer E.M., Moller A., Stollar B.D. and Rich A.: Antibodies to left handed Z-DNA bind to interband regions of Drosophila polytene chromosomes, *Nature* **294** (1981): 417–422.
27. Lancillotti F., Lopez M.C., Arias P. and Alonco C.: Z-DNA in transcriptionally active chromosomes, *Proc. Natl. Acad. Sci. USA* **84** (1987): 1560–1564.
28. Hill R.J.: Z-DNA; A prodrome for the 1990, *J. Cell Sci.* **99** (1991): 675–680.
29. Lipps H.J., Nordheim A., Lafer E.M., Ammermann D., Stollar B.D. and Rich A.: Antibodies against Z-DNA react with the macronucleus but not the micronucleus of the hypotrichous ciliate *Stylonychia mytilus*, *Cell* **32** (1983): 435–441.
30. Jackson D.A. and Cook P.R.: A general method for preparing chromatin containing intact DNA, *EMBO J.* **4** (1985): 913–918.
31. Jackson D.A., Yuan J. and Cook P.R.: A gentle method for preparing cyto- and nucleo-skeletons and associated chromatin, *J. Cell Sci.* **90** (1988): 365–378.
32. Wittig B., Dorbic T. and Rich A.: The level of Z-DNA in the metabolically active, permeabilized mammalian cell nuclei is regulated by torsional strain, *J. Cell Biol.* **108** (1989): 755–764.
33. Wittig B., Dorbic T. and Rich A.: Transcription is associated with Z-DNA formation in metabolically active permeabilized mammalian cell nuclei, *Proc. Natl. Acad. Sci. USA* **88** (1991): 2259–2263.
34. Wittig B., Wolfl S., Dorbic T., Vahrson W. and Rich A.: Transcription of human c-myc in permeabilized nuclei is associated with formation of Z-DNA in three discrete regions of the gene, *EMBO J.* **11** (1992): 4653–4663.
35. Wolfl S., Wittig B. and Rich A.: Identification of transcriptionally induced Z-DNA segments in the human c-myc gene, *Biochim. Biophys. Acta* **1264** (1995): 294–302.
36. Wolfl S., Martinez C., Rich A. and Majzoub J.A.: Transcription of the human corticotropin-releasing hormone gene in NPLC cells is correlated with Z-DNA formation, *Proc. Natl. Acad. Sci. USA* **93** (1996).
37. Peck L.J. and Wang J.C.: Transcriptional block caused by a negative supercoiling induced structural change in an alternating CG sequence, *Cell* **40** (1985): 129–137.
38. Pohl F.M.: *Ein Modell der DNS-struktur*. Naturwissenschaften, 1967. **54**: p. 616.
39. Treco D. and Arnheim N.: The evolutionary conserved repetitive sequence d(TG.AC)$_n$ promotes reciprocal exchange and generates unusual recombinant tetrads during yeast meiosis, *Mol. Cell. Biol.* **6** (1986): 3934–3947.
40. Bullock P., Miller J. and Botchan M.: Effects of poly[d(pGpT).d(pApC)] and poly[d(pCpG).d(pCpG)] repeats on homologous recombination in somatic cells, *Mol. Cell. Biol.* **6**(11) (1986): 3948–3953.
41. Wahls W.P., Wallace L.J. and Moore P.D.: The Z-DNA motif d(TG)$_{30}$ promotes reception of information during gene conversion while stimulating homologous recombination in human cells in culture, *Mol. Cell. Biol.* **10**(2) (1990): 785–793.
42. Aplan P.D., Raimondi S.C. and Kirsch I.R.: Disruption of the SCL gene by a t(1;3) translocation in a patient with T cell acute lymphoblastic leukemia, *EMBO. J.* **8**(9) (1989): 2621–2631.
43. Boehm T., Mengle-Gaw L., Kees U.R., Spurr N., Lavenir I., Forster A. and Rabbitts T.H.: Alternating purine-pyrimidine tracts may promote chromosomal translocations seen in a variety of human lymphoid tumours, *EMBO J.* **8**(9) (1989): 2621–2631.

44. Satyanarayana K. and Strominger J.L.: DNA sequences near a meiotic recombinational breakpoint within the human HLA-DQ region, *Immunogenetics* **35**(4) (1992): 235–240.
45. Steinmetz M., Stephan D. and Lindahl K.F.: Gene organization and recombinational hotspots in the murine major histocompatibility complex, *Cell* **44** (1986): 895–904.
46. Weinreb A., Katzenberg D.R., Gilmore G.L. and Birshtein B.K.: Site of unequal sister chromatid exchange contains a potential Z-DNA forming tract, *Proc. Natl. Acad. Sci. USA* **85**(2) (1991): 529–533.
47. Garner M.M. and Felsenfeld G.: Effect of Z-DNA on nucleosome placement, *J. Mol. Biol.* **196** (1987): 581–590.
48. Soyer-Gobillard M.O., Geraud M.L., Coulaud D., Barray M., Theveny B., Revet B. and Delain E.: Location of B- and Z-DNA in the chromosome of a primitive eukaryote dinoflagellate, *J. Cell. Biol.* **111**(2) (1990): 293–304.
49. Wolfl S., Vahrson W. and Herbert A.G.: Analysis of left handed Z-DNA in vivo. In: Saluz H.P. and Wiebauer K. (eds), *DNA and Nucleoprotein Structure in vivo*. Saluz H.P. and Wiebauer K. (ed.) Landes Co.: Austin, TX. 1995, p. 137–159.
50. Krishna P., Kennedy B.P., Waisman D.M., van de Sande J.H. and McGhee J.D.: Are many Z-DNA binding proteins actually phospholipid-binding proteins? *Proc. Natl. Acad. Sci. USA* **87** (1990): 1292–1295.
51. Rohner K.J., Hobi R. and Kuenzle C.C.: Z-DNA-binding proteins. Identification critically depends on the proper choice of ligands, *J. Biol. Chem.* **265** (1990): 19112–19115.
52. Bass B.L., Nihikura K., Keller W., Seeburg P.H., Emeson R.B., O'Connell M.A., Samuel C.E. and Herbert A.: A standardized nomenclature for adenosine deaminases that act on RNA, *RNA* **3** (1997): 947–949.
53. Herbert A.G. and Rich A.: A method to identify and characterize Z-DNA binding proteins using a linear oligodeoxynucleotide, *Nucleic Acids Res.* **21** (1993): 2669–2672.
54. Herbert A.G., Spitzner J.R., Lowenhaupt K. and Rich A.: Z-DNA binding protein from chicken blood nuclei, *Proc. Natl. Acad. Sci. USA* **90** (1993): 3339–3342.
55. Herbert A.G., Lowenhaupt K., Spitzner J.R. and Rich A.: Chicken double-stranded RNA adenosine deaminase has apparent specificity for Z-DNA, *Proc. Natl. Acad. Sci. USA* **92** (1995): 7550–7554.
56. Herbert A., Alfken J., Kim Y.-G., Mian S., Nishikura K. and Rich A.: A Z-DNA binding domain present in the human editing enzyme, double-stranded RNA adenosine deaminase, *Proc. Natl. Acad. Sci, USA* **94** (1997): 8421–8426.
57. Herbert A., Schade M., Lowenhaupt K., Alfken J., Schwartz T., Shlyakhtenko L.S., Lyubchenko Y.L. and Rich A.: The Za domain from human ADAR1 binds to the Z-DNA conformer of many different sequences, *Nucleic Acids Res.* **26** (1998): 3486–3493.
58. Schwartz T., Lowenhaupt K., Kim Y.-G., Li L., Brown II B.A., Herbert A. and Rich A.: Proteolytic dissection of Zab, the Z-DNA binding domain of human ADAR1, *J. Biol. Chem.* (1998): (in press).
59. Herbert A., Kim Y.-G., Alfken J., Nishikura K. and Rich A.: A Z-DNA binding domain from the human editing enzyme dsRNA adenosine deaminase, *Proc. Natl. Acad. Sci.* USA **94** (1997): 12875–12879.
60. Berger I., Manoharan W.W.R., Schwartz T., Alfken J., Kim Y.-G., Lowenhaupt K., Herbert A. and Rich A.: Spectroscopic characterization of Za, a novel DNA binding domain from human ADAR1, *Biochemistry* **37** (1998): 13313–13321.
61. Ramakrishnan V., Finch J.T., Graziano V., Lee P.L. and Sweet R.M.: Crystal structure of the globular domain of histone H5 and its implications for nucleosome binding, *Nature* **362** (1993): 219–223.
62. Clark K.L., Halay E.D., Lai E. and Burley S.K.: Co-crystal structure of the HNF-3/fork head DNA recognition motif resembles histone H5, *Nature* **364** (1993): 412–420.
63. Schade M., Turner C., Lowenhaupt K., Rich A. and Herbert A.: Structure/function analysis of the Z-DNA binding domain Za of ADAR1 reveals similarity to (a + b) family of helix-turn-helix proteins, *EMBO J.* (1998): (in press).
64. Kim U., Wang Y., Sanford T., Zeng Y. and Nishikura K.: Molecular cloning of a cDNA for double-stranded RNA adenosine deaminase, a candidate enzyme for nuclear RNA editing, *Proc. Natl. Acad. Sci. USA* **91** (1994): 11457–11461.
65. O'Connell M., Krause S., Higuchi M., Hsuan J.J., Totty N.F., Jenny A. and Keller W.: Cloning of cDNAs encoding mammalian double-stranded RNA-specific adenosine deaminase, *Mol. Cell. Biol.* **15**(3) (1995): 1389–1397.
66. Hough R.F. and Bass B.L.: Analysis of *Xenopus* dsRNA adenosine deaminase cDNAs reveals similarities to DNA methyltransferase, *RNA* **3** (1997): 356–370.
67. Liu Y., George C.X., Patterson J.R. and Samuel C.E.: Functionally distinct double-stranded RNA-binding domains associated with alternative splice variants of the interferon-inducible double-stranded RNA-specific adenosine deaminase, *J. Biol. Chem.* **14** (1997): 4419–4428.
68. Patterson J.B. and Samuel C.E.: Expression and regulation by interferon of a double-stranded-RNA-specific adenosine deaminase from human cells: Evidence for two forms of the deaminase, *Mol. Cell. Biol.* **15** (1995): 5376–5388.
69. Liu Y., Herbert A., Rich, A. and Samuel C.E.: Double-stranded RNA-specific adenosine deaminase nucleic acid binding properties, *Methods* **15** (1998): 199–205.
70. Bass B.L. and Weintraub H.: A developmentally regulated activity that unwinds RNA duplexes, *Cell* **48** (1987): 607–613.
71. Rebagliati M.R. and Melton D.A.: Antisense RNA injections in fertilized frog eggs reveal an RNA duplex unwinding activity, *Cell* **48** (1987): 599–605.
72. Bass B.L. and Weintraub H.: An unwinding activity that covalently modifies its double-stranded RNA substrate, *Cell* **55** (1988): 1089–1098.
73. Polson A.G., Crain P.F., Pomerantz S.C., McCloskey J.A. and Bass B.L.: The mechanism of adenosine to inosine conversion by the double-stranded RNA unwinding/modifying activity: A high performance liquid chromatography-mass spectrometry analysis, *Biochemistry* **30** (1991): 11507–11514.
74. Sommer B., Kohler M., Sprengel R. and Seeburg P.H.: RNA editing in brain controls a determinant of ion flow in glutamate-gated channels, *Cell* **67** (1991): 11–19.
75. Hume R.I., Dingledine R. and Heinemann S.F.: Identification of a site in the glutamate receptor subunits that controls calcium permeability, *Science* **253** (1991): 1028–1031.
76. Verdoorn T.A., Burnashev N., Monyer H., Seeburg P.H. and Sakmann B.: Structural determinants of ion flow through recombinant glutamate receptor channels, *Science* **252** (1991): 1715–1718.
77. Melcher T., Maas S., Sprengel R., Higuchi M. and Seeburg P.H.: RED2, a brain-specific member of the RNA-specific adenosine deaminase family, *J. Biol. Chem.* **271** (1996): 31795–31798.
78. Kohler M., Burnashev N., Sakmann B. and Seeburg P.H.: Determinants of Ca^{2+} permeability in both TM1 and TM2 of high affinity kainate receptor channels: Diversity by RNA editing, *Neuron* **10** (1993): 491–500.

79. Lomeli H., Mosbacher J., Melcher T., Hoger T., Geiger J.R., Kuner T., Monyer H., Higuchi M., Bach A. and Seeburg P.H.: Control of kinetic properties of AMPA receptor channels by nuclear RNA editing, *Science* **266** (1994): 1709–1713.
80. Ma J., Qian R., Rausa III F.M. and Colley K.J.: Two naturally occurring a2,6-Sialyltransferase forms with a single amino acid change in the catalytic domain differ in their catalytic activity and proteolytic processing, *J. Biol. Chem.* **272** (1997): 672–679.
81. Burns C.N., Chu H., Rueter S.M., Hutchinson L.K., Canton H., Sanders-Bush E. and Emeson R.: Regulation of serotonin-2C receptor G-protein coupling by RNA editing, *Nature* **387** (1997): 303–308.
82. Patton D.E., Silva T. and Bezanilla F.: RNA editing generates a diverse array of transcripts encoding squid Kv2 K^+ channels with altered functional properties, *Neuron* **19** (1997): 711–722.
83. Paul M.L. and Bass B.L.: Inosine exists in mRNA at tissue-specific levels and is most abundant in brain mRNA, *EMBO J.* **16** (1998): 1120–1127.
84. Morse D.P. and Bass B.L.: Detection of inosine in messenger RNA by inosine specific cleavage, *Biochemistry* **36** (1997): 8429–8434.
85. Bass B.L.: RNA editing: New uses for old players in the RNA world. In: Gesteland R.F. and Atkins J.F., (eds), *The RNA World*. Cold Spring Harbor Laboratory Press, Plainview, NY. 1993, 383–418.
86. Herbert A.G.: RNA editing, introns and evolution, *Trends in Genet.* **12**(1) (1996): 6–9.
87. Higuchi M., Single F.N., Kohler M., Sommer B., Sprengel R. and Seeburg P.H.: RNA editing of AMPA receptor subunit GluR-B: A base-paired intron-exon structure determines position and efficiency, *Cell* **75** (1993): 1361–1370.
88. Herb A., Higuchi M., Sprengel R. and Seeburg P.H.: Q/R site editing in kainate receptor GluR5 and GluR6 pre-mRNAs requires distant intronic sequences, *Proc. Natl. Acad. Sci. USA* **93** (1996): 1875–1880.
89. Egebjerg J., Kukekov V. and Heinemann S.F.: Intron sequence directs RNA editing of the glutamate receptor GluR2 coding sequence, *Proc. Natl. Acad. Sci. USA* **91** (1994): 10270–10274.
90. Takeuchi H., Hanamura N. and Harada I.: Structural specificity of peptides in Z-DNA formation and energetics of the peptide-induced B–Z transition of poly(dG-m^5C), *J. Mol. Biol.* **236**(2) (1994): 610–617.
91. Arndt-Jovin D.J., Udvardy A., Garner M.M., Ritter S. and Jovin T.: Z-DNA binding and inhibition by GTP of *Drosophila* Topoisomerase II, *Biochemistry* **32**(18) (1993): 4862–4872.
92. Bechert T., Diekmann S. and Arndt-Jovin D.J.: Human 170 kDa and 180 kDa topoisomerases II bind preferentially to curved and left-handed linear DNA, *J. Biomol. Struct. Dyn.* **12**(3) (1994): 605–623.
93. Glikin C.G., Jovin M.T. and Arndt-Jovin D.J.: Interactions of *Drosophila* DNA topoisomerase II with left-handed Z-DNA in supercoiled minicircles, *Nucleic Acids Res.* **19** (1991): 7139–7144.
94. Dawkins R.: The Blind Watchmaker, Harlow: Longman Scientific and Technical, 1986.
95. Wang J., Zeng Y., Murray J.M. and Nishikura K.: Genomic organization and chromosomal location of the human dsRNA adenosine deaminase gene: The enzyme for glutamate-activated ion channel RNA editing, *J. Mol. Biol.* **254** (1995): 184–195.