7. Frohlich, M. W. & Parker, D. S. The Mostly Male theory of flower evolutionary origins: from genes to fossils. *Syst. Bot.* **25**, 155–170 (2000).
8. Frohlich, M. W. in *Beyond Heterochrony: The Evolution of Development* (ed. Zelditch, M. L.) 59–106 (John Wiley, New York, 2001).
9. Frohlich, M. W. in *Developmental Genetics and Plant Evolution: Systematics Association Special Volume Series 65* (eds Cronk, Q. C. B. *et al.*) 85–108 (Taylor and Francis, London, 2002).
10. Doyle, J. A. Seed plant phylogeny and the relationships of Gnetales. *Int. J. Plant Sci.* **157**, 3–39 (1996).
11. Frohlich M. W. & Meyerowitz, E. M. The search for flower homeotic gene homologs in basal angiosperms and gnetales: a potential new source of data on the evolutionary origin of flowers. *Int. J. Plant Sci.* **158**, 131–142 (1997).
12. Baum, D. A. The evolution of plant development. *Curr. Opin. Plant Biol.* **1**, 79–86 (1998).
13. Lohmann, J. U. & Weigel, D. Building beauty: the genetic control of floral patterning. *Dev. Cell* **2**, 135–142 (2002).
14. Donoghue, M. J. & Doyle, J. A. Seed plant phylogeny: demise of the anthophyte hypothesis? *Curr. Biol.* **10**, 106–109 (2000).
15. Frohlich, M. W. & Estabrook, G. F. Wilkinson support calculated with exact probabilities: an example using Floricaula/LEAFY amino acid sequences that compares three hypotheses involving gene gain/loss in seed plants. *Mol. Biol. Evol.* **17**, 1914–1925 (2000).
16. Mouradov, A. *et al. NEEDLY*, a *Pinus radiata* ortholog of *FLORICAULA/LEAFY* genes, expressed in both reproductive and vegetative meristems. *Proc. Natl Acad. Sci. USA* **95**, 6537–6542 (1998).
17. Mellerowicz, E. J. *et al. PRFLL* a *Pinus radiata* homologue of *FLORICAULA* and *LEAFY* is expressed in buds containing vegetative shoot and undifferentiated male cone primordia. *Planta* **206**, 619–629 (1998).
18. Colombo, L. *et al.* The *Petunia* MADS box gene *FBP11* determines ovule identity. *Plant Cell* **7**, 1859–1868 (1995).
19. Decraene, L. P. R. & Smets, E. F. Notes on the evolution of androecial organisation in the Magnoliophytina (angiosperms). *Bot. Acta* **111**, 77–86 (1998).
20. Hufford, L. The morphology and evolution of male reproductive structures of Gnetales *Int. J. Plant Sci.* **157**, 95–112 (1996).
21. Endress, P. K. Structure and function of female and bisexual organ complexes in Gnetales. *Int. J. Plant Sci.* **157**, 113–125 (1996).
22. Yao, X. *et al.* The Corystosperm pollen organ *Pteruchus* from the Triassic of Antarctica. *Amer. J. Bot.* **82**, 535–546 (1995).
23. Klavins, S. D. *et al.* Anatomy of *Umkomasia* (Corystospermales) from the Triassic of Antarctica. *Amer. J. Bot.* **89**, 664–676 (2002).
24. Shindo, S. *et al.* Characterization of a *FLORICAULA/LEAFY* homologue of *Gnetum parvifolium* and its implications for the evolution of reproductive organs in seed plants. *Int. J. Plant Sci.* **162**, 1199–1209 (2001).
25. Long, J. A. *et al.* A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of *Arabidopsis*. *Nature* **379**, 66–69 (1996).
26. Brown, R. C. & Mogensen, H. L. Late ovule and early embryo development in *Quercus gambelii*. *Amer. J. Bot.* **59**, 311–316 (1972).
27. Bowman, J. L. *et al.* Establishment of polarity in angiosperm lateral organs. *Trends Genet.* **18**, 134–141 (2002).
28. Svoma, E. Seed development and function in *Artabotrys hexapetalus* (Annonaceae). *Plant Syst. Evol.* **207**, 205–223 (1997).
29. Taylor, T. N. *et al.* Permineralized seed fern cupules from the Triassic of Antarctica — implications for cupule and carpel evolution. *Amer. J. Bot.* **81**, 666–677 (1994).
30. Griffith, M. E. *et al. PETAL LOSS* gene regulates initiation and orientation of second whorl organs in the *Arabidopsis* flower. *Development* **126**, 5635–5644 (1999).
31. Nelson, J. M. *et al.* Expression of a mutant maize gene in the ventral leaf epidermis is sufficient to signal a switch of the leaf's dorsoventral axis. *Development* **129**, 4581–4589 (2002).
32. Groß-Hardt, R. *et al. WUSCHEL* signaling functions in interregional communication during *Arabidopsis* ovule development. *Genes Dev.* **16**, 1129–1138 (2002).
33. Winter, K. U. *et al.* Evolution of class B floral homeotic proteins: obligate heterodimerization originated from homodimerization. *Mol. Biol. Evol.* **19**, 587–596 (2002).
34. Becker, A. *et al.* A novel MADS-box gene subfamily with a sister-group relationship to class B floral homeotic genes. *Mol. Genet. Genomics* **266**, 942–950 (2002).
35. Nesi, N. *et al.* The *TRANSPARENT TESTA16* locus encodes the *ARABIDOPSIS* BSISTER MADS domain protein and is required for proper development and pigmentation of the seed coat. *Plant Cell* **14**, 2463–2479 (2002).
36. Bowe, L. M. *et al.* Phylogeny of seed plants based on all three genomic compartments: extant gymnosperms are monophyletic and Gnetales' closest relatives are conifers. *Proc. Natl Acad. Sci. USA* **97**, 4092–4097 (2000).
37. Chaw, S. M. *et al.* Seed plant phylogeny inferred from all three plant genomes: monophyly of extant gymnosperms and origin of Gnetales from conifers. *Proc. Natl Acad. Sci. USA* **97**, 4086–4091 (2000).
38. Qiu, Y.-L. *et al.* Phylogeny of basal angiosperms: analyses of five genes from three genomes. *Int. J. Plant Sci.* **161**, 3–27 (2000).
39. Mathews, S. & Donoghue, M. J. Basal angiosperm phylogeny inferred from duplicate phytochromes A and C. *Int. J. Plant Sci.* **161**, 41–55 (2000).
40. Soltis, D. E. *et al.* Missing links: the genetic architecture of flower and floral diversification. *Trends Plant Sci.* **7**, 22–31 (2002).
41. Lanfranchi, G. *et al.* Identification of 4370 expressed sequence tags from a 3′-end-specific cDNA library of human skeletal muscle by DNA sequencing and filter hybridization. *Genome Res.* **6**, 35–42 (1996).
42. Albert, V. A. *et al.* Pleiotropy, redundancy and the evolution of flowers. *Trends Plant Sci.* **7**, 297–301 (2002).
43. Theissen, G. *et al.* in *Developmental Genetics and Plant Evolution: Systematics Association Special Volume Series 65* (eds Cronk, Q. C. B. *et al.*) 173–206 (Taylor and Francis, London, 2002).
44. Lamark, J. B. *Zoological Philosophy (1809)* 122 (Univ. Chicago Press, Chicago, 1984) (Translated by H. Elliot).
45. Mayr, E. *The Growth of Biological Thought* (Belknap, Cambridge, Massachusetts, 1982).
46. Frohlich, M. W. MADS about Gnetales. *Proc. Natl Acad. Sci. USA* **96**, 8811–8813 (1999).
47. Gould, S. J. Sociobiology: the art of storytelling. *New Sci.* **80**, 530–533 (1978).
48. Gould, S. J. & Lewontin, R. C. The Spandrels of San-Marco and the Panglossian paradigm — a critique of the adaptationist program. *Proc. Royal Soc. London B* **205**, 581–598 (1979).
49. Iltis, H. H. From teosinte to maize: the catastrophic sexual transmutation. *Science* **222**, 886–894 (1983).
50. Iltis, H. H. Homeotic sexual translocations and the origin of maize (*Zea mays*, Poaceae): a new look at an old problem. *Econ. Bot.* **54**, 7–42 (2000).
51. Lauter, N. & Doebley, J. Genetic variation for phenotypically invariant traits detected in teosinte: implications for the evolution of novel forms. *Genetics* **160**, 333–342 (2002).
52. Gibson, G. Developmental evolution: going beyond the 'just so'. *Curr. Biol.* **9**, 942–945 (1999).



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TIMELINE

# Z-DNA: the long road to biological function

*Alexander Rich and Shuguang Zhang*


Biologists were puzzled by the discovery of left-handed Z-DNA because it seemed unnecessary. Z-DNA was stabilized by the negative supercoiling generated by transcription, which indicated a transient localized conformational change. Few laboratories worked on the biology of Z-DNA. However, the discovery that certain classes of proteins bound to Z-DNA with high affinity and great specificity indicated a biological role. The most recent data show that some of these proteins participate in the pathology of poxviruses.


When Watson and Crick proposed their model for the right-handed double helical structure of DNA (B-DNA) in 1953, it was compatible with the only experimental data on the structure at that time: DNA FIBRE X-RAY DIFFRACTION analysis. However, fibre diffraction yielded too little data to 'prove' the structure. It was not until the late 1970s, when DNA synthesis was developed, that it became possible to carry out SINGLE-CRYSTAL X-RAY DIFFRACTION on specific molecules to define the structure. Amazingly, the familiar right-handed B-DNA double helix that had been the focus of molecular biology for the preceding 25 years did not appear in this first atomic-resolution view of the double helix. Instead, the first single-crystal X-ray structure of a DNA fragment — a self-complementary DNA hexamer $d(CG)_3$ — showed a left-handed double helix with two anti-parallel chains that were held together by Watson–Crick base pairs[1] (see TIMELINE). The alternative structure pointed to an unusual function for this form of DNA.

The organization of the molecule was completely different from that which had

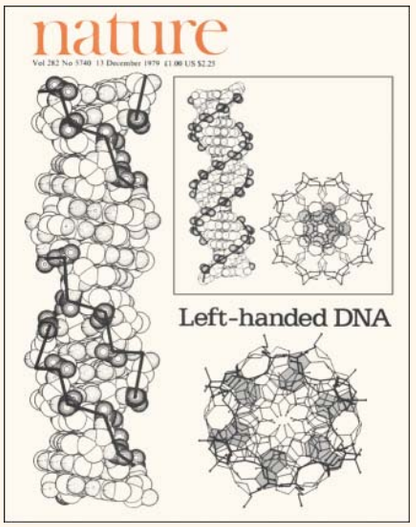



Figure 1 | **Discovery of Z-DNA.** Cover of *Nature*, showing the discovery of left-handed Z-DNA in side and end views, with the right-handed B-DNA in the enclosed box[1]. The shaded guanine residues in Z-DNA are near the outside of the helix in the end view, compared with their central location in B-DNA.

been anticipated. Instead of all the residues having bases organized in the *ANTI*-CONFORMATION, in this molecule every other base rotated around the glycosyl bonds so that the bases alternated in *anti*- and *SYN*-CONFORMATIONS along the chain. Also, there was a zigzag arrangement of the backbone of the molecule (hence, the name Z-DNA) that looked different from the smooth continuous coil seen in models of B-DNA (FIG. 1). Instead of having a helix with a major and minor groove, the base pairs were set off to the side, away from the axis, and only one groove was found that was analogous to the minor groove of B-DNA. The bases that form the major groove in B-DNA were reorganized in Z-DNA to form the convex outer surface.

The general response to this unusual structure was amazement, coupled with scepticism. However, after a brief period of excitement, the biological community largely ignored Z-DNA, as it did not seem to be required to explain anything. Recent research on this alternative conformation of the double helix has begun to show that Z-DNA has important biological functions.

## Conformation and stability

The relationship between Z-DNA and the more familiar B-DNA was indicated by the earlier work of Pohl and Jovin[2], who showed that the ultraviolet CIRCULAR DICHROISM of poly(dG-dC) was nearly inverted in 4 M sodium chloride solution. The suspicion that this was the result of a conversion from B-DNA to Z-DNA was confirmed by examining the RAMAN SPECTRA of these solutions and the Z-DNA crystals[3]. The conversion of B-DNA to Z-DNA was associated with a 'flipping over' of the base pairs so that they had an upside down orientation relative to that of B-DNA. This flipping over resulted both in the production of a *syn*-conformation in every other base and a change in the deoxyribose-ring pucker in alternate bases. The net result of this reorganization was that the phosphate groups were closer together in Z-DNA than in B-DNA. Hence, under standard cellular conditions, the electrostatic repulsion of these charged phosphate groups would push the molecule into the B-DNA conformation. In the presence of a high-salt solution, the electrostatic repulsion of the phosphate residues is vastly decreased, and Z-DNA becomes the stable conformation.

Several studies quickly showed that chemical modification, including cytosine methylation and many other cations, such as spermine and spermidine, would stabilize the Z conformation[4]. It emerged that the lowest energy-level ground state of DNA in a physiological solution was B-DNA, and that the Z conformation was a higher energy state. Because purines can form the *syn*-conformation without an energy penalty, it became apparent that the specific sequence of the base pairs was important in determining the energy that was required to change B-DNA to Z-DNA[5]. The sequences that most readily converted had alterations of purines and pyrimidines, especially alternations of C and G. This change also occurred easily with alternations of CA on one strand and TG on the other[6,7]. However, many other sequences were shown to be capable of forming Z-DNA[8].

This discovery stimulated a burst of research from chemists who were interested in studying DNA conformational changes. Although the ionic conditions that were suitable for stabilizing Z-DNA in most experiments were different from those present in a cell, spermine, spermidine and cytosine methylation, which also stabilize Z-DNA[4], are found *in vivo*. Furthermore, the discovery that negative supercoiling would stabilize Z-DNA indicated biological involvement[9]. Negative supercoiling requires energy and tends to unwind B-DNA. For example, in a plasmid with three negative supercoils, if one turn of the DNA helix changed from right-handed to left-handed, two negative supercoils would disappear and the energy of negative supercoiling would then stabilize a small segment of Z-DNA (FIG. 2). Supercoiling was known to be a part of biological systems, which indicated a connection between this alternative conformation and biological phenomena. Early experiments showed that the negative supercoiling of plasmids in prokaryotes would stabilize Z-DNA[10].

Many *in vitro* experiments were carried out to determine the energy that was required for a supercoiled plasmid with a particular sequence to flip from the B form to the Z form. The energetics of these conformations was studied for several different sequences[11]. This ultimately led Ho *et al.* to devise a computer program that made it possible to calculate the relative energy required to flip any sequence from the B form to the Z form[12].

Workers in several laboratories determined crystal and solution structures of DNA sequences in the Z conformation. These provided a great deal of detailed information about the Z conformation and, at the same time, many chemists discovered ways in which the Z conformation could be stabilized relative to the B conformation. Nonetheless, work on the biology of Z-DNA progressed slowly. By the mid-1980s, after several years of research, nothing definitive had emerged about Z-DNA function. During this period, although some notable studies supported a functional role for Z-DNA in transcription (see later), others showed that the influence of Z-DNA on transcription was dependent on the gene that was examined, which increased scepticism and decreased enthusiasm for studying the biological role of Z-DNA. Many people felt that Z-DNA was a non-functional

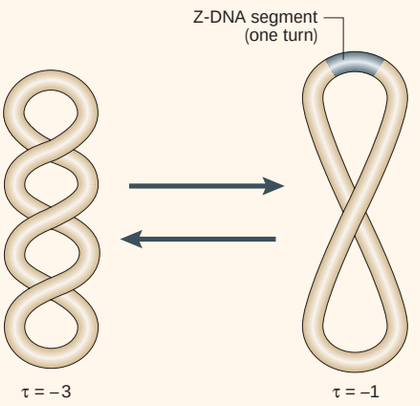
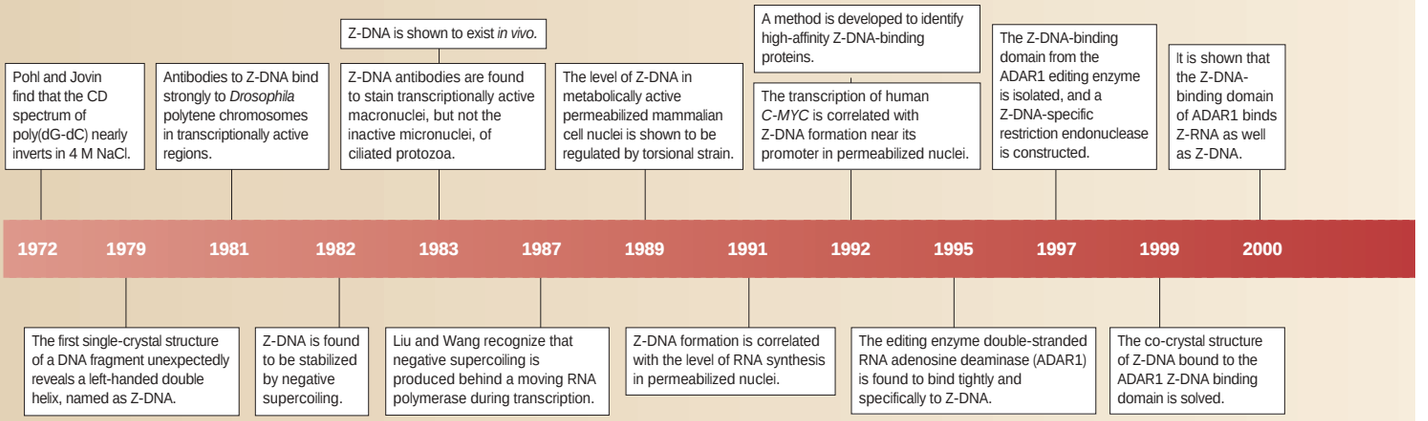



Figure 2 | **Negative supercoiling stabilizes Z-DNA.** A double helical plasmid with three negative supercoils (τ) is shown on the left. The energy of supercoiling is proportional to the square of the number of supercoils. On the right, one turn switches the DNA from right- to left-handed, and two negative supercoils are removed. The change of negative supercoiling energy stabilizes Z-DNA formation.

Timeline | From Z-DNA structure to function




conformational phenomenon and this attitude became relatively widespread[13]. So, although chemists continued to find Z-DNA interesting, by the end of the 1980s the biology of Z-DNA was not receiving attention from researchers, and its study had largely disappeared, except in the Rich laboratory.

## Transcription and Z-DNA

In fact, the groundwork towards showing a biological role for Z-DNA came from immunological research that was carried out years before the downturn of interest in this area. Unlike B-DNA, Z-DNA is highly antigenic; both polyclonal[14] and monoclonal[15] antibodies can be raised to Z-DNA molecules. The characterization of these antibodies led to the discovery that Z-DNA-specific antibodies are found in human autoimmune diseases, especially in systemic lupus erythematosus[16]. Antibodies to Z-DNA also provided a useful tool for characterizing chromosome organization. They bound specifically to the interband regions of the *Drosophila* polytene chromosomes, and this binding was particularly strong in the puff regions, which are the sites of enhanced transcriptional activity[17]. Others found the same staining patterns in unfixed POLYTENE CHROMOSOMES[18]. However, some studies have shown antibody binding outside these regions[19].

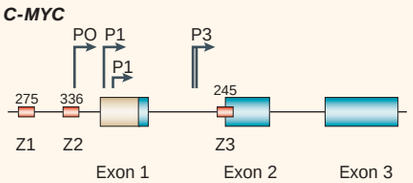


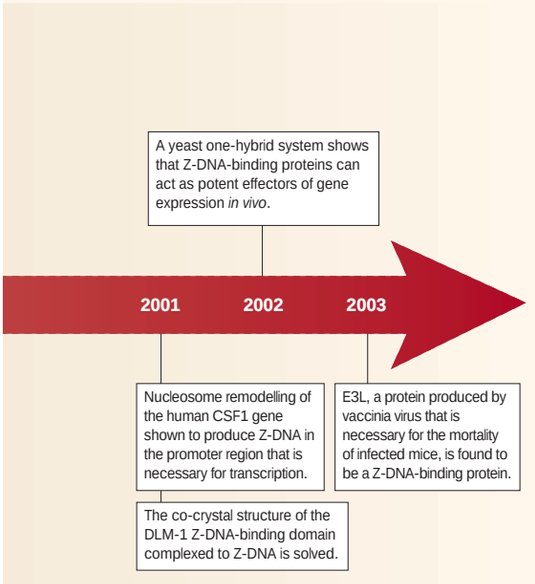

Figure 3 | **Transcription stabilizes Z-DNA.** Metabolically active permeabilized nuclei were used to show that *C-MYC* transcription is associated with the formation of Z-DNA in three restriction fragments, which are labelled Z1–Z3 (numbers above the boxes show the size of the fragments). These are all found near the *C-MYC* promoters, which are labelled P0–P3 (REF. 26). When transcription is turned off, the continued action of topoisomerases relaxes the Z-DNA, which disappears entirely after ~15 minutes. Therefore, Z-DNA is formed transiently in association with transcription.

Further studies in protozoa also indicated a link to transcription. Ciliated protozoa have two nuclei: the macronucleus, which is the site of transcription, and the micronucleus, which contains DNA that is involved in sexual reproduction. Anti-Z-DNA antibodies stained the macronucleus of the ciliated protozoan *Stylonychia*, but not its micronucleus[20].

A real breakthrough came with the work of Liu and Wang[21], in 1987, on the interaction of the RNA polymerase complex with the DNA duplex during transcription. They suggested that the complex does not rotate around the helical DNA, but instead plows straight through. Because the ends of the DNA molecule are fixed, the DNA behind the moving polymerase is unwound and subjected to negative torsional strain, which is known to stabilize Z-DNA. In front of the moving polymerase, positive torsional strain develops.

Further evidence came from the work of Ho and colleagues, who first studied the distribution of sequences that favoured Z-DNA formation in 137 human genes. They found a high concentration of these sequences near the transcription start site[22]. In more recent experiments, Ho scanned human chromosome 22 and estimated that ~80% of its genes have sequences that favour Z-DNA formation near the transcription start site (P. S. Ho, personal communication). As these were not present in many pseudogenes, Z-DNA-forming sequences near the transcription start site might have a functional role.

To study the association between Z-DNA and transcription more directly, Rich collaborated with Wittig and colleagues to use a technique developed by Cook at Oxford University. Mammalian cells were encapsulated in agarose microbeads and mild detergent treatment lysed the cytoplasmic membrane and permeabilized the nuclear membrane, but left the nucleus otherwise intact. The resulting 'entrapped' nuclei replicated DNA at nearly the *in vivo* rate and were able to carry out transcription[23]. Using biotinylated monoclonal antibodies against Z-DNA, the level of Z-DNA was determined in these nuclei, and was shown to be regulated by torsional strain[24]. Moreover, an increase in the transcriptional activity of the embedded nuclei resulted in a parallel increase in the amount of bound Z-DNA[25]. Using a short ultraviolet (UV) laser pulse for protein–DNA cross linking, they linked the biotinylated anti-Z-DNA antibodies to DNA. This made it possible to isolate DNA restriction fragments that were bound to the antibody and probe them for specific nucleotide sequences. They found that three regions near the promoters of the *C-MYC* gene formed Z-DNA when *C-MYC* was expressed (FIG. 3), and the Z-DNA-forming nucleotides were identified. However, these regions quickly reverted to B-DNA if *C-MYC* transcription was switched off[26,27]. Nonetheless,




the constitutively expressed actin control retained its Z-DNA at all times. This showed a correlation between transcription and Z-DNA conformation, which has also been found in other genes[28].

So, the negative torsional strain induced by the movement of RNA polymerase[21] stabilized Z-DNA formation near the transcription start site. Although topoisomerases tried to relax the DNA, the continued movement of RNA polymerases generated more negative torsional strain than the topoisomerases could relax. However, on the cessation of transcription, topoisomerase action rapidly converted the DNA back to the B conformation. So, Z-DNA was seen as a metastable conformation that formed and disappeared depending on physiological activities.

How is Z-DNA formation initiated in transcription? One answer was afforded by the recent work of Liu *et al.* who studied the chromatin remodelling system SWI/SNF[29]. This complex of proteins helps to turn on genes by unwrapping DNA from nucleosomes. Liu *et al.* studied the human colony stimulating factor 1 gene (*CSF1*), which is turned on by this system. Unwrapping the nucleosome leaves the DNA negatively supercoiled as a result of the way in which it is wrapped around the nucleosome. Liu *et al.* discovered that the released DNA was in the Z conformation. It had been known for several years that Z-DNA could not form nucleosomes[30]. So, the nucleosome cannot reform and the site is kept open, which allows the accumulation of other transcription factors and the initiation of transcription by RNA polymerase. They showed that transcription was triggered by Z-DNA formation. Given the prevalence of sequences that favour Z-DNA formation near transcription sites, it is possible that this mechanism is widespread[22].

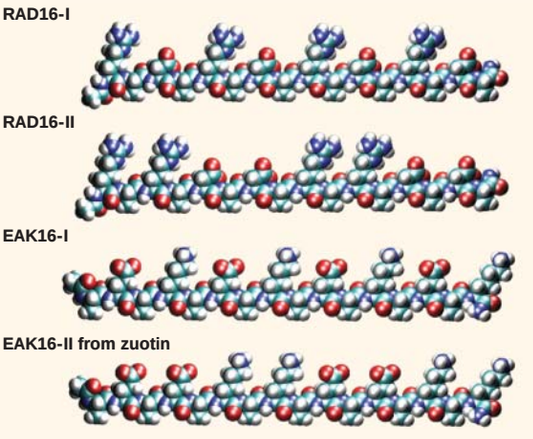
## Proteins that bind Z-DNA

***Identifying binding proteins.*** If Z-DNA were to have biological functions, it seemed highly likely that there would be a class of proteins that would bind specifically to it. Several attempts were made to isolate such proteins, using columns and other techniques. Early attempts met with limited success but did result in the important and serendipitous discovery that is described in BOX 1.

The problem with Z-DNA-binding proteins was devising a method that would make it possible to isolate proteins that bound selectively to Z-DNA with high affinity. Herbert developed a powerful technique for identifying Z-DNA-binding proteins with the exclusion of proteins that could bind to B-DNA[31]. The method used a gel-shift assay with radioactive-labelled chemically stabilized Z-DNA in the presence of a ~20,000-fold excess of B-DNA and single-stranded DNA. This technique detected proteins that bound specifically and tightly to Z-DNA and led to the isolation, in 1995, of a Z-DNA-binding nuclear-RNA-editing enzyme[32] called double-stranded RNA adenosine deaminase (ADAR1).

ADAR1 acts on double-stranded segments that are formed in pre-mRNA, binding to the duplex and selectively deaminating adenosine to yield inosine. Ribosomes interpret inosine as guanine, so it can alter the amino-acid sequence of a DNA-encoded protein. A typical substrate of this enzyme is an RNA duplex in which an exon is paired with a region of an intron. The deaminase edits several pre-mRNAs, including a glutamate receptor that is expressed in the central nervous system (CNS)[33]. The receptor is an ion channel, and a glutamine residue near the centre of the channel is changed through editing to arginine; its positive charge restricts the entry of calcium ions, a change that is essential for CNS function. Another substrate is the serotonin receptor[33]. In all of these cases, the functional properties of the edited protein (with the amino-acid alteration) are detectably different from those of the unedited protein. The editing enzyme is found in all metazoa and acts to increase the functional diversity of proteins that are transcribed from a given locus.

### Box 1 | Discovery of self-assembling peptides from the study of zuotin




Zhang developed a simple gel-retardation assay using stabilized methylated Z-DNA [d(5mCG)n] to purify the first naturally occurring left-handed Z-DNA-binding protein. A yeast protein called zuotin was found to bind Z-DNA in the presence of a 400-fold molar excess of right-handed B-DNA[46]. Zuotin had an interesting repetitive sequence motif — AEAEAKAKAEAEAKAK — which has been extensively developed by Zhang to create a class of simple β-sheet peptides that are self-complementary as a result of the presence of both positive and negative side chains on one side of the β-sheet and hydrophobic side chains on the other[47].

This serendipitous discovery of a self-complementary peptide inspired Zhang to design several new members of this peptide class, which form 3-dimensional (3D) nanofibre scaffolds that can be used in 3D cell culture[48–50]. The four self-complementary peptides shown here — RDA16-I, RAD16-II, EAK-I and EAK16-II (the segment from yeast zuotin) — form stable β-sheet structures in water and undergo spontaneous assembly to form nanofibre scaffolds. These nanofibre scaffolds hold large volumes of water (>99.5% water content). Tissue cells can be embedded in a 3D nanofibre scaffold in which they can establish molecular gradients that often mimic the *in vivo* environment.

Other related self-assembling peptide systems have also been designed, which range from 'molecular switch' peptides that undergo marked conformational changes[51] to 'molecular carpet' peptides for surface engineering to peptide nanotubes and nanovesicles[52–55], all of which originated with the Z-DNA-binding zuotin discovery.

### A Z-DNA-binding domain

Proteolytic dissection of the editing enzyme ADAR1 made it possible to isolate a domain from the N-terminus, called $Z\alpha_{ADAR1}$, which contains all the Z-DNA-binding properties that are associated with the editing enzyme[34]. This domain was used by Kim *et al*. to create a conformationally specific restriction endonuclease that would only cut Z-DNA[35,36].

Several experiments were carried out to illustrate the interaction of the $Z\alpha_{ADAR1}$ domain with DNA in solution. If the dodecamer $d(CG)_6$ was put in solution, it produced the typical circular-dichroism spectrum of B-DNA. As $Z\alpha_{ADAR1}$ was added to the physiological solution, the spectrum gradually changed, which reflected conversion to the Z form[37]. This showed that the Zα domain of ADAR1 was capable of stabilizing the dodecamer in the Z conformation, which was probably generated by BROWNIAN MOTION that twisted the dodecamer fragment. After flipping into the Z-DNA conformation, the $Z\alpha_{ADAR1}$ domain binds to the DNA and prevents it returning to the B conformation. In later experiments, a similar phenomenon was shown in a longer DNA molecule in which the $d(CG)_6$ segment was inserted between two longer segments with sequences that would not easily form Z-DNA[38]. In such cases, the $Z\alpha_{ADAR1}$ would bind to the central region, holding it in the Z conformation, whereas the flanking regions remained in the B conformation. Therefore, the binding energy was great enough to hold a small segment of DNA in the Z conformation and also provided enough energy to stabilize the two B–Z junctions.

Oh and Kim developed a yeast one-hybrid system to study Z-DNA-binding proteins *in vivo*[39]. They discovered that when $Z\alpha_{ADAR1}$ that has been fused to an activation domain binds to Z-DNA near a promoter, it enhances the transcription of the reporter gene. However, even without the activation domain, a level of transcriptional activation remains. These findings are consistent with the suggestion of Liu *et al.*[29] that Z-DNA formation near the promoter would stimulate transcription.

Schwartz *et al*. discovered that the purified $Z\alpha_{ADAR1}$ domain from ADAR1 could be co-crystallized with $d(CG)_3$. The crystal structure, solved at a resolution of 2.1 Å (REF. 40), showed that the DNA was identical in form to that seen in the first crystal of Z-DNA[1]. The 70-amino-acid binding domain was found to adopt a helix–turn–helix β-sheet motif (winged helix) in which the recognition helix and the β-sheet were bound to five successive phosphate groups in the zigzag backbone of Z-DNA. Also, it recognized the *syn*-conformation of guanine. So, the $Z\alpha_{ADAR1}$ domain is designed to specifically recognize the structural features of Z-DNA — it is specific for the conformation, not the sequence. The winged-helix protein motif is also widely present in sequence-specific DNA-recognition proteins, which bind to the bases in the major groove of B-DNA. The use of this domain for binding to the Z-DNA conformation shows an interesting adaptation of a widely used DNA-binding tertiary-protein fold.

It is likely that the Z-DNA-binding domain of ADAR1 targets the Z-DNA-forming regions of certain transcriptionally active genes, as only they have Z-DNA. The use of an exon–intron duplex in the editing process means that editing has to be rapid, as the introns are rapidly removed by the splicing apparatus. $Z\alpha_{ADAR1}$ seems to be active *in vivo* in the editing of certain transcripts in which it might target the gene[41]; however, its role in double-stranded RNA (dsRNA) editing is not resolved. Interestingly, dsRNA can also adopt a Z-RNA conformation, which might be a substrate for ADAR1 (BOX 2).

### Other Z-DNA-binding proteins

The co-crystal structure of $Z\alpha_{ADAR1}$ and Z-DNA made it possible to identify those amino acids that are important for Z-DNA recognition. A computer search rapidly discovered other proteins with similar sequence motifs, such as DLM1, which is upregulated in tissues that are in contact with tumours as well as during the interferon response[42]. The co-crystal structure of the Z-DNA-binding domain of DLM1 ($Z\alpha_{DLM1}$) and $d(CG)_3$ was solved at a resolution of 1.85 Å, and showed that this second protein domain recognizes Z-DNA in a manner similar to that of $Z\alpha_{ADAR1}$ (REF. 43).

Another Z-DNA-binding protein is E3L, which is found in poxviruses, including the vaccinia virus. These large DNA viruses reside in the cytoplasm of cells and produce several proteins that help to abort the interferon response of the host cell. E3L is a small protein that is necessary for pathogenicity[44]. If vaccinia virus is given to a mouse, the mouse dies within one week. However, a virus that has a mutated or missing E3L is no longer pathogenic for the mouse, even though the virus can still reproduce in cell culture[44]. The protein has an N-terminal domain with a sequence that is characteristic of Z-DNA

#### Box 2 | Z-RNA

The discovery of the structure of left-handed Z-DNA naturally led to the question of whether RNA could also form this conformation. In 1982, chemically modified oligoribonucleotides indicated that this was a possibility[56], and further nuclear magnetic resonance, circular dichroism and absorption spectroscopy studies strongly indicated that Z-RNA could be formed in high-ionic-strength solutions[57,58]. More detailed structural information was obtained from X-ray crystallographic studies with chimeric hexamers that were made of alternating CG residues in which the two central CG residues were ribonucleotides, whereas the flanking pair of nucleotides were deoxyribonucleotides. These and other structural studies showed that the conformation of Z-RNA was similar to that of Z-DNA[59,60]. Z-RNA was also found to be immunogenic and, although Z-RNA specific antibodies could be isolated[61], some antibodies recognized both Z-RNA and Z-DNA. Staining experiments with Z-RNA-specific antibodies showed that the antibody bound to fixed protozoan cells that were visualized by immunofluorescence microscopy. The antibodies were mostly found in the cytoplasm, which indicated that some cytoplasmic sequences in fixed cells existed as Z-RNA[62]. Cytoplasmic microinjection of anti-Z-RNA antibodies was found to inhibit cell multiplication[63].

The experiments on Z-RNA were mostly carried out in the late 1980s, and since 1990 there have been no further publications. However, a possible physiological role for Z-RNA was suggested recently by Brown, and Lowenhaupt *et al*., who found that the Z-DNA-binding domain of the editing enzyme double-stranded RNA adenosine deaminase (ADAR1) could bind to Z-RNA and Z-DNA[64] with similar affinity. It had been known for some time that certain RNA viruses that replicate in the cytoplasm undergo considerable changes in sequence, which were probably the consequence of hyper-editing by ADAR1. Sequence analysis of the virus found in measles encephalitis showed that the RNA undergoes many edits: adenines are replaced by guanines, and uracils by cytosines. So, this virus has been extensively hyper-edited by the editing enzyme[64]. Full length ADAR1 that contains the Z-DNA-binding domain is upregulated by the interferon response of the cell, which is triggered by the measles virus. Furthermore, ADAR1 accumulates in the cytoplasm in which the measles virus replicates. So, it is possible that the $Z\alpha_{ADAR1}$ binds to negatively torsionally strained double-stranded RNA, which might form during viral replication, targeting the editing enzyme to this site. The Z-DNA/Z-RNA-binding domain might have a role in the attempts of cells to inactivate the invading virus. More experimental data are needed to test this hypothesis.

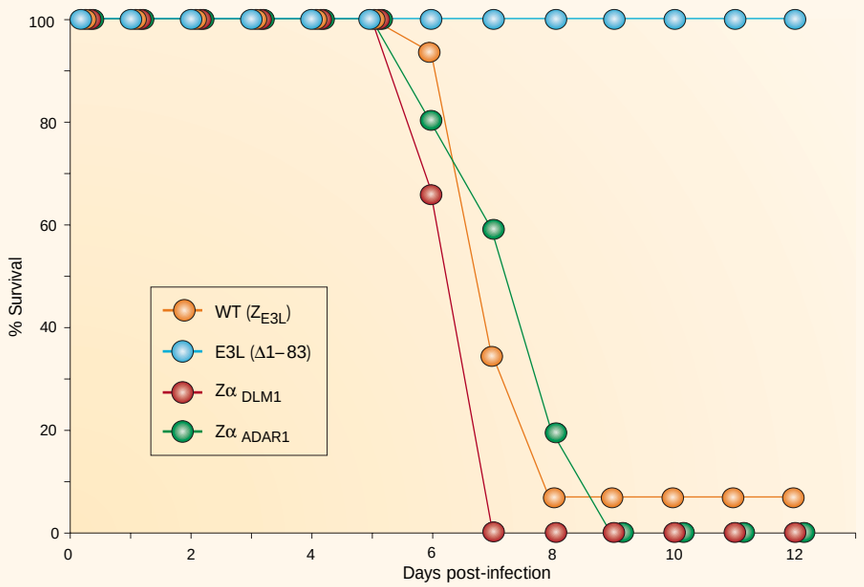



Figure 4 | **The lethality of mice following intracerebral inoculation of 100 viral plaque-forming units of vaccinia virus constructs**[45]**.** No lethality is associated with the deletion of the 83 N-terminal residues of the E3L protein (Δ1–83). However, the wild-type (WT) virus and the two chimeric viruses in which the wild-type E3L N-terminal domain has been substituted by either $Z\alpha_{DLM1}$ or $Z\alpha_{ADAR1}$ are all equally pathogenic. The wild type and the two chimeric E3L molecules have residues in common, which are important in binding Z-DNA in the two co-crystal structures that have been solved. Reproduced with permission from REF. 45.

binding ($Z_{E3L}$) and a C-terminal domain that has a dsRNA-binding motif. In infected cells, E3L is exported to the nucleus where it accumulates.

### Viral pathogenicity

To investigate the pathogenicity of the vaccinia virus in the mouse, and its relationship to the possible Z-DNA-binding activities of E3L, a collaboration was set up between the Rich laboratory and the Jacobs laboratory. Chimeric viruses were created in which the N-terminal domain of vaccinia E3L ($Z_{E3L}$) was removed. $Z_{E3L}$ has sequence similarities to $Z\alpha_{ADAR1}$ and $Z\alpha_{DLM1}$. Two chimeric viruses were created in which the two known Z-DNA-binding domains were substituted for $Z_{E3L}$. In carrying out these domain swaps, a little more than a dozen amino acids in the domain remained unchanged; these were the residues that were known to bind Z-DNA in the co-crystals. However, more than 50 other residues were changed. The chimeric viruses were as pathogenic as the wild type[45]; however, when the N-terminal residues of vaccinia E3L were deleted, the mice survived (FIG. 4). Other experiments were carried out in which mutations were introduced into both chimeric and wild-type viruses; if Z-DNA binding was weakened by these mutations, viral pathogenicity was reduced[45]. It was postulated that the $Z_{E3L}$ domain in the nucleus of the infected cell might bind to the Z-DNA that formed near the transcription start site of certain genes, which would impair the ability of the host cell to carry out transcription and so inhibit the anti-viral response[45].

It is likely that a small molecule or drug can be made that will bind to the Z-DNA-binding pocket of the E3L molecule. If such a molecule rescued mice that were infected with vaccinia virus, it might also be active in humans. This molecule could be developed to eliminate the harmful side-effects of vaccination. More importantly, the E3L protein of the closely related variola virus, which is the agent of smallpox, is almost identical to the vaccinia E3L[45]. So, small molecule drugs that bind to E3L might make it possible to develop a therapy for smallpox.

This is the first example in which a Z-DNA-binding protein has been found to be involved in viral pathogenesis. If other viruses use a similar mechanism to downregulate the host response, then these proteins might be potential targets for anti-viral drugs.

### Conclusions

The Z-DNA conformation has been difficult to study because it does not exist as a stable feature of the double helix. Instead, it is a transient structure that is occasionally induced by biological activity and then quickly disappears. The discovery and biological activity of Z-DNA-specific binding proteins point the way to a broader understanding of its biological roles. One active area of research will be the comparison of the occurrence of Z-DNA-forming sequences and Z-DNA-binding proteins between prokaryotes and eukaryotes; already, there are indications that sequences that form Z-DNA are less frequent in prokaryotes (P. S. Ho, personal communication). What we have seen so far is just the beginning, but it has provided insights that are likely to stimulate more research into this unusual left-handed version of the DNA double helix.

## Glossary

ADAR1
The editing enzyme double-stranded RNA adenosine deaminase, which converts adenine to inosine in pre-mRNA. This enzyme has an N-terminal domain that binds tightly to Z-DNA.

*ANTI*- AND *SYN*-CONFORMATIONS
Nucleic-acid bases can rotate about the glycosyl bond. The Watson–Crick hydrogen-bonding atoms point away from the sugar in the *anti*-conformation (as in B-DNA), and have the opposite orientation in the *syn*-conformation. Purines can form the *syn*-conformation more easily than pyrimidines.

BROWNIAN MOTION
The random kinetic thermal motion of molecules.

DNA FIBRE X-RAY DIFFRACTION ANALYSIS
X-rays are scattered by electrons and if a molecule has regular periodicities, they will be detected by the diffraction pattern. In this technique, DNA molecules are orientated so that their long axes are parallel. Although the diffraction pattern can provide some information about the molecule, the conclusions are often tentative because the number of reflections is relatively small.

CIRCULAR DICHROISM
This method measures the difference in absorption of right and left circularly polarized light as it passes through a solution containing molecules that absorb at that wavelength. The circular-dichroism spectrum is plotted as a function of wavelength.

POLYTENE CHROMOSOME
A chromosome that has duplicated many times and has remained laterally associated so that it is visible, as seen in *Drosophila* salivary glands.

RAMAN SPECTRUM
Measures the vibrations of molecules that are usually influenced by the conformation of a molecule. This can be obtained from crystalline materials as well as materials in solution.

SINGLE-CRYSTAL X-RAY DIFFRACTION
In this technique, a molecule is crystallized to produce many repetitions that are organized in a regular three-dimensional array. This produces X-ray diffraction with a large number of reflections. Solution of the crystal structure can establish the conformation of the molecule because large amounts of redundant data are collected.

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1. Wang, A. H. J. *et al.* Molecular structure of a left-handed double helical DNA fragment at atomic resolution. *Nature* **282**, 680–686 (1979).
2. Pohl, F. M. & 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**, 375–396 (1972).
3. Thamann, T. J., Lord, R. C., Wang, A. H. J. & Rich, A. High salt form of poly(dG-dC)•poly(dG-dC) is left handed Z-DNA: raman spectra of crystals and solutions. *Nucl. Acids Res.* **9**, 5443–5457 (1981).
4. Behe, M. & Felsenfeld, G. Effects of methylation on a synthetic polynucleotide: the B–Z transition in poly(dG–m5dC)•poly(dG–m5dC). *Proc. Natl Acad. Sci. USA* **78**, 1619–1623 (1981).
5. Rich, A., Nordheim, A. & Wang, A. H.-J. The chemistry and biology of left-handed Z-DNA. *Ann. Rev. Biochem.* **53**, 791–846 (1984).
6. Nordheim, A. & Rich, A. The sequence $(dC–dA)_n$•$(dG–dT)_n$ forms left-handed Z-DNA in negatively supercoiled plasmids. *Proc. Natl Acad. Sci. USA* **80**, 1821–1825 (1983).
7. Haniford, D. B. & Pulleyblank, D. E. Facile transition of poly[d(TG) x d(CA)] into a left-handed helix in physiological conditions. *Nature* **302**, 632–634 (1983).
8. Feigon, J., Wang, A. H.-J., van der Marel, G. A., van Boom, J. H. & Rich, A. Z-DNA forms without an alternating purine–pyrimidine sequence in solution. *Science* **230**, 82–84 (1985).
9. Peck, L. J., Nordheim, A., Rich, A. & Wang, J. C. Flipping of cloned d(pGpG)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**, 4560–4564 (1982).
10. Haniford, D. B. & Pulleyblank, D. E. The *in vivo* occurrence of Z-DNA. *J. Biomol. Struct. Dyn.* **1**, 593–609 (1983).
11. Ellison, M. J., Kelleher, R. J., Wang, A. H.-J., Habener, J. F. & Rich, A. Sequence-dependent energetics of the B–Z transition in supercoiled DNA containing nonalternating purine–pyrimidine sequences. *Proc. Natl Acad. Sci. USA* **82**, 8320–8324 (1985).
12. Ho, P. S., Ellison, M. J., Quigley, G. J. & Rich, A. A computer aided thermodynamic approach for predicting the formation of Z-DNA in naturally occurring sequences. *EMBO J.* **5**, 2737–2744 (1986).
13. Marx, J. Z-DNA: still searching for a function. *Science* **230**, 794–796 (1985).
14. Lafer, E. M., Moller, A., Nordheim, A., Stollar, B. D. & Rich, A. Antibodies specific for left-handed DNA. *Proc. Natl Acad. Sci. USA* **78**, 3546–3550 (1981).
15. Moller, A. *et al.* Monoclonal antibodies recognize different parts of Z-DNA. *J. Biol. Chem.* **257**, 12081–12085 (1982).
16. Lafer, E. M. *et al.* Z-DNA specific antibodies in human systemic lupus erythematosus. *J. Clin. Invest*. **71**, 314–321 (1983).
17. Nordheim, A. *et al.* Antibodies to left-handed Z-DNA bind to interband regions of *Drosophila* polytene chromosomes. *Nature* **294**, 417–422 (1981).
18. Lancillotti, F., Lopez, M. C., Arias, P. & Alonso, C. Z-DNA in transcriptionally active chromosomes. *Proc. Natl Acad. Sci. USA* **84**, 1560–1564 (1987).
19. Arndt-Jovin, D. J. *et al.* Left-handed Z-DNA in bands of acid-fixed polytene chromosomes. *Proc. Natl Acad. Sci. USA* **80**, 4344–4348 (1983).
20. Lipps, H. J. *et al.* Antibodies against Z-DNA react with the macronucleus but not the micronucleus of the hypotrichous ciliate *Stylonychia mytilus*. *Cell* **32**, 435–441 (1983).
21. Liu, L. F. & Wang, J. C. Supercoiling of the DNA template during transcription. *Proc. Natl Acad. Sci. USA* **84**, 7024–7027 (1987).
22. Schroth, G. P., Chou, P.-J. & Ho, P. S. Mapping Z-DNA in the human genome: computer aided mapping reveals a non-random distribution of potential Z-DNA forming sequences in human genes. *J. Biol. Chem*. **267**, 11846–11855 (1992).
23. Jackson, D. A., Yuan, J. & Cook, P. R. A gentle method for preparing cyto- and nucleo-skeletons and associated chromatin. *J. Cell Sci*. **90**, 365–378 (1988).
24. Wittig, B., Dorbic, T. & Rich, A. The level of Z-DNA in metabolically active, permeabilized mammalian cell nuclei is regulated by torsional strain. *J. Cell. Biol*. **108**, 755–764 (1989).
25. Wittig, B., Dorbic, T. & Rich, A. Transcription is associated with Z-DNA formation in metabolically active permeabilized mammalian cell nuclei. *Proc. Natl Acad. Sci. USA* **88**, 2259–2263 (1991).
26. Wittig, B., Wolfl, S., Dorbic, T., Vahrson, W. & 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**, 4653–4663 (1992).
27. Wolfl, S., Wittig, B. & Rich, A. Identification of transcriptionally induced Z-DNA segments in the human *C-MYC* gene. *Biochim. Biophys. Acta* **1264**, 294–302 (1995).
28. Wolfl, S., Martinez, C., Rich, A. & 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**, 3664–3668 (1996).
29. Liu, R. *et al.* Regulation of CSF1 promoter by the SWI/SNF-like BAF complex. *Cell* **106**, 309–318 (2001).
30. Garner, M. M. & Felsenfeld, G. Effect of Z-DNA on nucleosome placement. *J. Mol. Biol*. **196**, 581–590 (1987).
31. Herbert, A. G. & Rich, A. A method to identify and characterize Z-DNA binding proteins using a linear oligodeoxynucleotide. *Nucl. Acids Res*. **21**, 2669–2672 (1993).
32. Herbert, A., Lowenhaupt, K., Spitzner, J. & Rich, A. Chicken double-stranded RNA adenosine deaminase has apparent specificity for Z-DNA. *Proc. Natl Acad. Sci. USA* **92**, 7550–7554 (1995).
33. Bass, B. L. RNA editing by adenosine deaminases that act on RNA. *Annu. Rev. Biochem.* **71**, 817–846 (2002).
34. Herbert, A. *et al.* A Z-DNA binding domain present in the human editing enzyme, double-stranded RNA adenosine deaminase. *Proc. Natl Acad. Sci. USA* **94**, 8421–8426 (1997).
35. Kim, Y.-G., Kim, P. S., Herbert, A. & Rich, A. Construction of a Z-DNA-specific restriction endonuclease. *Proc. Natl Acad. Sci. USA* **94**, 12875–12879 (1997).
36. Kim, Y. G., Lowenhaupt, K., Schwartz, T. & Rich, A. The interaction between Z-DNA and the Zab domain of dsRNA adenosine deaminase characterized using fusion nucleases. *J. Biol. Chem*. **274**, 19081–19086 (1999).
37. Berger, I. *et al.* Spectroscopic characterization of a DNA-binding domain, Zα, from the editing enzyme dsRNA adenosine deaminase: evidence for left-handed Z-DNA in the Zα-DNA complex. *Biochemistry* **37**, 13313–13321 (1998).
38. Kim, Y.-G. *et al.* The Zab domain of the human RNA editing enzyme ADAR1 recognizes Z-DNA when surrounded by B-DNA. *J. Biol. Chem*. **275**, 26828–26833 (2000).
39. Oh, D.-B., Kim, Y.-G. & Rich, A. Z-DNA-binding proteins can act as potent effectors of gene expression *in vivo*. *Proc. Natl Acad. Sci. USA* **99**, 16666–16671 (2002).
40. Schwartz, T., Rould, M. A., Lowenhaupt, K., Herbert, A. & Rich, A. Crystal structure of the Zα domain of the human editing enzyme ADAR1 bound to left-handed Z-DNA. *Science* **284**, 1841–1845 (1999).
41. Herbert, A. & Rich, A. Role of binding domains for dsRNA and Z-DNA in the *in vivo* editing of minimal substrates by ADAR1. *Proc. Natl Acad. Sci. USA* **98**, 12132–12137 (2001).
42. Fu, Y. *et al*. Cloning of *DLM-1*, a novel gene that is up-regulated in activated macrophages, using RNA differential display. *Gene* **240**, 157–163 (1999).
43. Schwartz, T., Behlke, J., Lowenhaupt, K., Heinemann, U. & Rich, A. Structure of the DLM-1–Z-DNA complex reveals a conserved family of Z-DNA-binding proteins. *Nature Struct. Biol*. **8**, 761–765 (2001).
44. Brandt, T. A. & Jacobs, B. L. Both carboxy- and amino-terminal domains of the vaccinia virus interferon resistance gene, *E3L* are required for pathogenesis in a mouse model. *J. Virol.* **75**, 850–856 (2001).
45. Kim, Y.-G. *et al.* A role for Z-DNA binding in vaccinia virus pathogenesis. *Proc. Natl Acad. Sci. USA* **100**, 6974–6979 (2003).
46. Zhang, S., Lockshin, C., Herbert, A., Winter, E. & Rich, A. Zuotin, a putative Z-DNA binding protein in *Saccharomyces cerevisiae*. *EMBO J.* **11**, 3787–3796 (1992).
47. Zhang, S., Holmes, T., Lockshin, C. & Rich, A. Spontaneous assembly of a self-complementary oligopeptide to form a stable macroscopic membrane. *Proc. Natl Acad. Sci. USA* **90**, 3334–3338 (1993).
48. Zhang, S. *et al.* Self-complementary oligopeptide matrices support mammalian cell attachment. *Biomaterials* **16**, 1385–1393 (1995).
49. Holmes, T., Delacalle, S., Su, X., Rich, A. & Zhang, S. Extensive neurite outgrowth and active neuronal synapses on peptide scaffolds. *Proc. Natl Acad. Sci. USA* **97**, 6728–6733 (2000).
50. Kisiday, J. *et al.* Self-assembling peptide hydrogel fosters chondrocyte extracellular matrix production and cell division: implications for cartilage tissue repair. *Proc. Natl Acad. Sci. USA* **99**, 9996–10001 (2002).
51. Zhang, S. & Rich, A. Direct conversion of an oligopeptide from a β-sheet to an α-helix: a model for amyloid formation. *Proc. Natl Acad. Sci. USA* **94**, 23–28 (1997).
52. Zhang, S. *et al.* Biological surface engineering: a simple system for cell pattern formation. *Biomaterials* **20**, 1213–1220 (1999).
53. Vauthey, S., Santoso, S., Gong, H., Watson, N. & Zhang, S. Molecular self-assembly of surfactant-like peptides to form nanotubes and nanovesicles. *Proc. Natl Acad. Sci. USA* **99**, 5355–5360 (2002).
54. von Maltzahn, G., Vauthey, S., Santoso, S. & Zhang, S. Positively charged surfactant-like peptides self-assemble into nanostructures. *Langmuir* **19**, 4332–4337 (2003).
55. Zhang, S. Building from bottom-up. *Materials Today* **6**, 20–27 (2003).
56. Uesugi, W., Shida, T. & Ikehara, M. Synthesis and properties of CpG analogues containing an 8-bromoguanosine residue. Evidence for Z-RNA duplex formation. *Biochemistry* **21**, 3400–3408 (1982).
57. Hall, K., Cruz, P., Tinoko, I., Jovin, T. M. & van de Sande, J. H. 'Z-RNA' — a left-handed RNA double helix. *Nature* **311**, 584–586 (1984).
58. Davis, P. W., Hall, K., Cruz, P., Tinoco, I. & Neilson, T. The tetraribonucleotide rCpGpCpG forms a left-handed Z-RNA double helix. *Nucleic Acids Res.* **14**, 1279–1291 (1986).
59. Teng, M. K., Liaw, Y. C., van der Marel, G. A., van Boom, J. H. & Wang, A.-H. Effects of the O2' hydroxyl group on Z-DNA conformation: structure of Z-RNA and (araC)-[Z-DNA]. *Biochemistry* **28**, 4923–4928 (1989).
60. Davis, P. W., Adamiak, R. W. & Tinoco, I. Z-RNA: the solution NMR structure of r(CGCGCG). *Biopolymers* **29**, 109–122 (1990).
61. Hardin, C. C., Zarling, D. A., Wolk, S. K., Ross, W. S. & Tincoc, I. Characterization of anti-Z-RNA polyclonal antibodies: epitope properties and recognition of Z-DNA. *Biochemistry* **27**, 4169–4177 (1988).
62. Zarling, D. A., Calhoun, C. J., Hardin, C. C. & Zarling, A. H. Cytoplasmic Z-RNA. *Proc. Natl Acad. Sci. USA* **84**, 6117–6121 (1987).
63. Zarling, D. A., Calhoun, C. J., Feuerstein, B. G. & Sena, E. P. Cytoplasmic microinjection of immunoglobulin Gs recognizing RNA helices inhibits human cell growth. *J. Mol. Biol*. **211**, 147–160 (1990).
64. Brown, B. A., Lowenhaupt, K., Wilbert, C. M., Hanlon, C. B. & Rich, A. The Za domain of the editing enzyme dsRNA adenosine deaminase binds left-handed Z-RNA as well as Z-DNA. *Proc. Natl Acad. Sci. USA* **97**, 13532–13586 (2000).

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