[Frontiers in Bioscience 13, 1064-1071, January 1, 2008]

Discovery of alternative DNA structures: a heroic decade (1979-1989)

Sergei M. Mirkin

Department of Biology, Tufts University, Medford, MA 02155

TABLE OF CONTENTS

1. ABSTRACT

The first alternative DNA structure - left-handed Z-DNA – was described back in 1979. The discoveries of the cruciform DNA structure, three-stranded H-DNA, four-stranded G-quartets and stably unwound DNA followed in the next decade. Each alternative structure was formed by a specific DNA sequence, which as a rule was repetitive. Furthermore, these repetitive elements were situated at functionally important areas of various genomes, pointing to the biological significance of these structures. This chapter concentrates on the first period of studies of alternative DNA structures, beginning in 1979 and ending in 1989, which transformed our views on DNA structure and functioning.

2. INTRODUCTION

This special issue of the Frontiers in Biosciences is called "DNA Structures, Genome Instability and Human Disease". Its take-home message is that alternative, *i.e.* non-B, DNA structures affect major genetic transactions, such as DNA replication, transcription, recombination and repair, occasionally leading to genome instability and disease. It took almost thirty years of studies in many labs worldwide to make this idea plausible, while its final proof has yet to come. In this introduction, I take the liberty to describe only the initial period of research on alternative DNA structures, beginning in 1979 and ending around 1989, which could undoubtedly be called its heroic age.

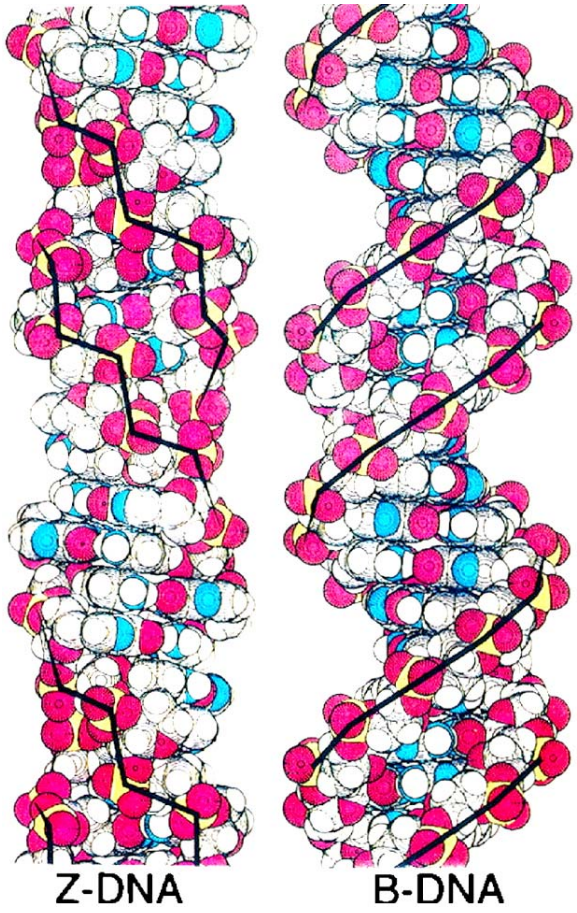

Figure 1. Side-by-side view of Z-DNA and B-DNA structures (59). The two DNA strands of each duplex are highlighted by solid black lines. The ``zigzag'' nature of the Z-DNA backbone is clearly seen.

Prior to 1979, DNA was generally considered as a uniform right-handed double helix, built of AT and GC base pairs, as was originally postulated by Watson and Crick (1). The main physiological version of this helix, called B-form (Figure 1), has the following characteristics: (i) two antiparallel sugar-phosphate backbones form a right helix, (ii) the planes of base pairs are perpendicular to the helix axis, (iii) the base pairs are centered over the helix axis, given rise to major and minor grooves, (iv) a helical turn consists of 10.5 base pairs, (v) a repetitive unit is mononucleotide, (vi) the form of deoxyribose is C2'-endo and (vii) individual nucleotides exist in anti-conformation (2, 3). The best characterized deviation from this structure was A-DNA, formed in water-alcohol solutions (4). In contrast to B-DNA, its sugars are in C3'-endo conformation, it has 11 base pairs per helical turn and its base pairs are inclined 20º toward, and shifted 5 Å from the helical axis, resulting in a ribbon-shaped helix with a deep and narrow major grove. Other deviations from the B-helix, including C-form with 9.3 bps per turn (5), D-form with 8.5 bps per turn (6), and T-form, a glucosylated structure with 8 bps per turn (7), were also described. All of them, however, existed in the realm of right-handed double helical family of structures.

Admittedly, there were early indications that DNA might be a bit more versatile than the Watson-Crick double helix. For example, alternative schemes of base pairings, such as Hoogsteen and reverse Hoogsteen were discovered in early crystal studies of nucleotides (8). Three- (9, 10) and four-stranded (11) DNA structures formed by specific deoxyribonucleotides were detected in biophysical experiments of the 1960s. Finally, there were preliminary indications that the handedness of poly (dG-dC) helix may change from right to left under high ionic strength (12). These and related observations did not attract prime attention, however, due to the general belief that normal B-DNA is by far the most thermodynamically favorable structure under physiological conditions.

3. ALTERNATIVE DNA STRUCTURES

These conventional views on DNA structure began to unravel after the discovery of negative DNA supercoiling (13) and DNA topoisomerases (14) that modulated topological properties of circular DNA. The subsequent discovery of DNA gyrase in 1976 (15) became the major breakthrough in the field by demonstrating that negative DNA supercoiling can be crucial for DNA functioning and organism viability (16). This was soon followed by the realization that changes in DNA structure, topologically equivalent to helix unwinding, would be energetically favorable in negatively supercoiled DNA (17) and the accumulation of experimental data on hypersensitivity of supercoiled DNA to the enzymes and chemicals specific towards single-stranded DNA (18).

Notably, the whole concept of B-DNA was based on the low-resolution fiber diffraction data, or various indirect biochemical and biophysical assays, rather than from studies of DNA crystals at the atomic resolution. By the irony of fate, the first DNA crystal solved in 1979 (19) delivered a shocking left-handed DNA helix that looked nothing like B-DNA (Figure 1). This structure, called Z-DNA, was formed by specific sequences composed of alternating purines and pyrimidines and had the following structural characteristics: (i) the sugar pucker for pyrimidines was C2'-endo, as in B-DNA, but it changed to C3'-endo for purines, (ii) the configuration of the glycosidic bond for purines was *syn*, as opposed to *anti* in B-DNA, (iii) the alternation of the C2'-endo, *anti*-conformation for pyrimidines with the C3'-endo, *syn*-conformation for purines resulted in a zig-zag sugar-phosphate backbone, hence the name Z-DNA, (iv) its repetitive unit was dinucleotide, rather than mononucleotide, which is typical for B-DNA, (v) it had only one deep and narrow groove, corresponding to the minor groove in B-DNA, (vi) its helical turn was 12 bps.

While in linear DNA, Z-conformation was formed under rather exotic high-salt conditions, it was found to form under physiological conditions in negatively supercoiled DNA (20, 21). Indeed by introducing negative DNA twist, it serves as a true sink for the superhelical tension, relaxing roughly1.8 supercoils per 10 bps. Furthermore, antibodies developed against Z-DNA interacted with multiple sites at active eukaryotic genes,

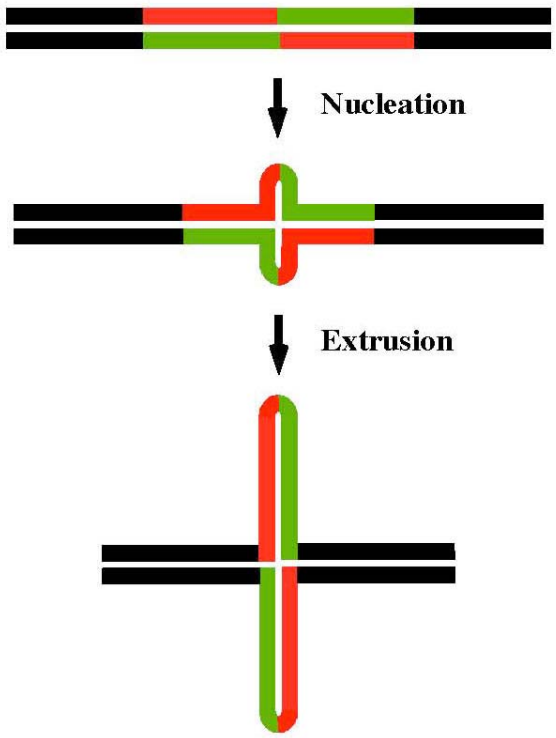

Figure 2. Formation of the DNA cruciform. The complementary halves of an inverted repeat are colored in red and green; flanking DNA is shown in black. The nucleation step is followed by the cruciform extrusion via the process of branch migration.

pointing to its potentially significant biological role (22, 23). Thus, Z-DNA was hypothesized to regulate eukaryotic gene expression from the very moment of its discovery (24).

At about the same time, formation of a different alternative, cruciform-like DNA structure (Figure 2) was detected in superhelical DNA *in vitro* using nuclease S1, which was specific to single-stranded DNA (25, 26) and, later on, 2-dimensional gel-electrophoresis of DNA topoisomers (27). This structure is also formed by specific DNA sequences, called inverted repeats, in which DNA bases that are equidistant from the symmetry center in a DNA strand are complementary to each other. To form a cruciform, approximately 10 bps at the center of symmetry of an inverted repeat must unwind in the pre-nucleation step. This event allows nucleation to occur due to the intrastrand hydrogen bond formation near the center of symmetry. Once nucleation has occurred, branch migration drives the process of extrusion of the cruciform (28). Since inverted repeats are enormously overrepresented in both pro- and eukaryotic DNA (29, 30), DNA cruciforms were readily detected in practically every DNA studied *in vitro*.

Initial hopes that DNA cruciforms would be ubiquitous *in vivo*, however, appeared to be overly optimistic. The free energy of intracellular DNA supercoiling is insufficient to warrant cruciform formation by short inverted repeats. For long inverted repeats, intracellular supercoiling is sufficient for the cruciform formation, but the process is forbidden kinetically (31-33). Lucky exceptions from this rule were the very AT-rich DNA cruciforms: these efficiently formed *in vivo*, since their nucleation energy was lower and their kinetics was much faster than that of GC-rich structures (34-36). As for the possible biological role, the remarkable similarity between the base of DNA cruciforms and Holliday junctions triggered many speculations on the role of cruciform DNA in genetic recombination (37).

In the early 1980s, it has also become clear that various homopurine-homopyrimidine stretches are hypersensitive to the nuclease S1, when in active chromatin, or when cloned into supercoiled plasmids (18, 38, 39). Besides being homopurine-homopyrimidine, these sequences had little in common and the nature of structural transition leading to nuclease sensitivity remained elusive, particularly because many of them were rather GC-rich. Using 2-dimensional electrophoretic analysis of DNA topoisomers, two independent groups have demonstrated that these sequences undergo a pH-dependent structural transition when in superhelical DNA (40, 41). There were, however, significant differences in the experimental data and interpretations between those groups. Subsequent studies of led to unraveling of a totally novel, three-stranded DNA structure, called H-DNA (Figure 3A), which can be formed by various homopurine-homopyrimidine sequences as long as they are mirror repeats (42). An instant support for this model came from fine chemical and nuclease probing of homopurine-homopyrimidine repeats in supercoiled plasmids (43-47).

The major element of H-DNA is an intramolecular triple helix. To build this structure, a DNA strand from one half of a homopurine-homopyimidine repeat folds back, forming a triplex with the duplex half of the repeat, while its complement remains single stranded. This extensive single-stranded DNA segment accounts for the S1 sensitivity. As can be seen from Figure 3A, the two complementary DNA strands are not linked in this structure, *i.e.* formation of H-DNA is topologically equivalent to an unwinding of the entire homopurine-homopyrimidine stretch. Therefore, it is favored in negatively supercoiled DNA. In the initially described structure, the third strand in the triplex was pyrimidine hence it was called H-y. H-y form is built from TA*T and CG*C^+ triads (Figure 3B), in which pyrimidines from the third strand form Hoogsteen hydrogen bonds with the purines of the duplex. The requirement for cytosine protonation makes this structure stable under acidic pH.

Just a year later, an isoform of H-DNA, which was stable at physiological pH in the presence of divalent cations, was discovered (48). In the latter structure, the strand donated to the triplex was homopurine, and the structure was built of CG*G and TA*A triads, where purines from the third strand form reverse Hoogsteen hydrogen bonds with the purines in the duplex (Figure 3B). Since S1 hypersensitive homopurine-homopyrimidine repeats were initially discovered in the upstream regulatory

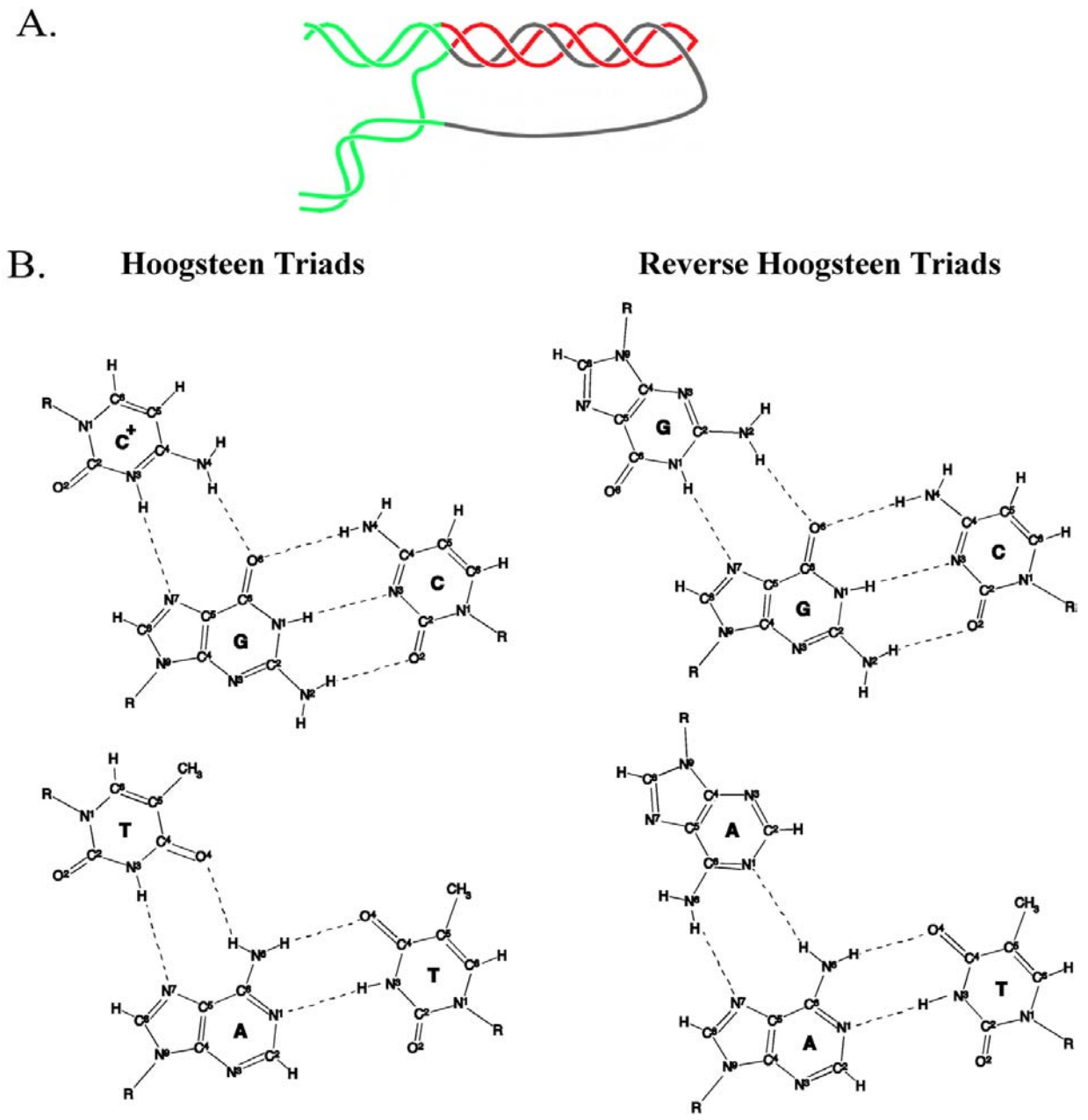

Figure 3. H-DNA. **A.** The structure of an intramolecular triplex. The two complementary strands of a homopurine-homopyrimidine repeat are colored in red and gray, while flanking DNA is colored green. The structure is called H-y when the red strand is homopyrimide, and H-r if when it is homopurine. One can see that the red and gray strands in this structure are not linked, *i.e.* formation of H-DNA is topologically equivalent to an unwinding of the entire homopurine-homopyrimidine repeat. **B.** H-y form is built from TA*T and CG*C^+ triads, in which pyrimidines in the third strand form Hoogsteen hydrogen bonds with the purines of the duplex. H-r form and is built of CG*G and TA*A triads, where purines from the third strand form reverse Hoogsteen hydrogen bonds with the purines in the duplex.

parts of eukaryotic promoters, H-DNA was hypothesized to serve as a transcription regulatory signal in eukaryotes (42).

As it turned out, three-stranded DNA was just the first example of multistranded DNA structures. A four-stranded structure appeared to be formed by single-stranded DNA containing tandemly arranged runs of guanines. The original observation that was made for the G-rich element in the immunoglobulin switch region (49) was soon expanded to telomeric DNA repeats (50, 51) and poly (dG) runs (52). The building elements of this structure are stacked G-tetrads that are stabilized by monovalent cations,

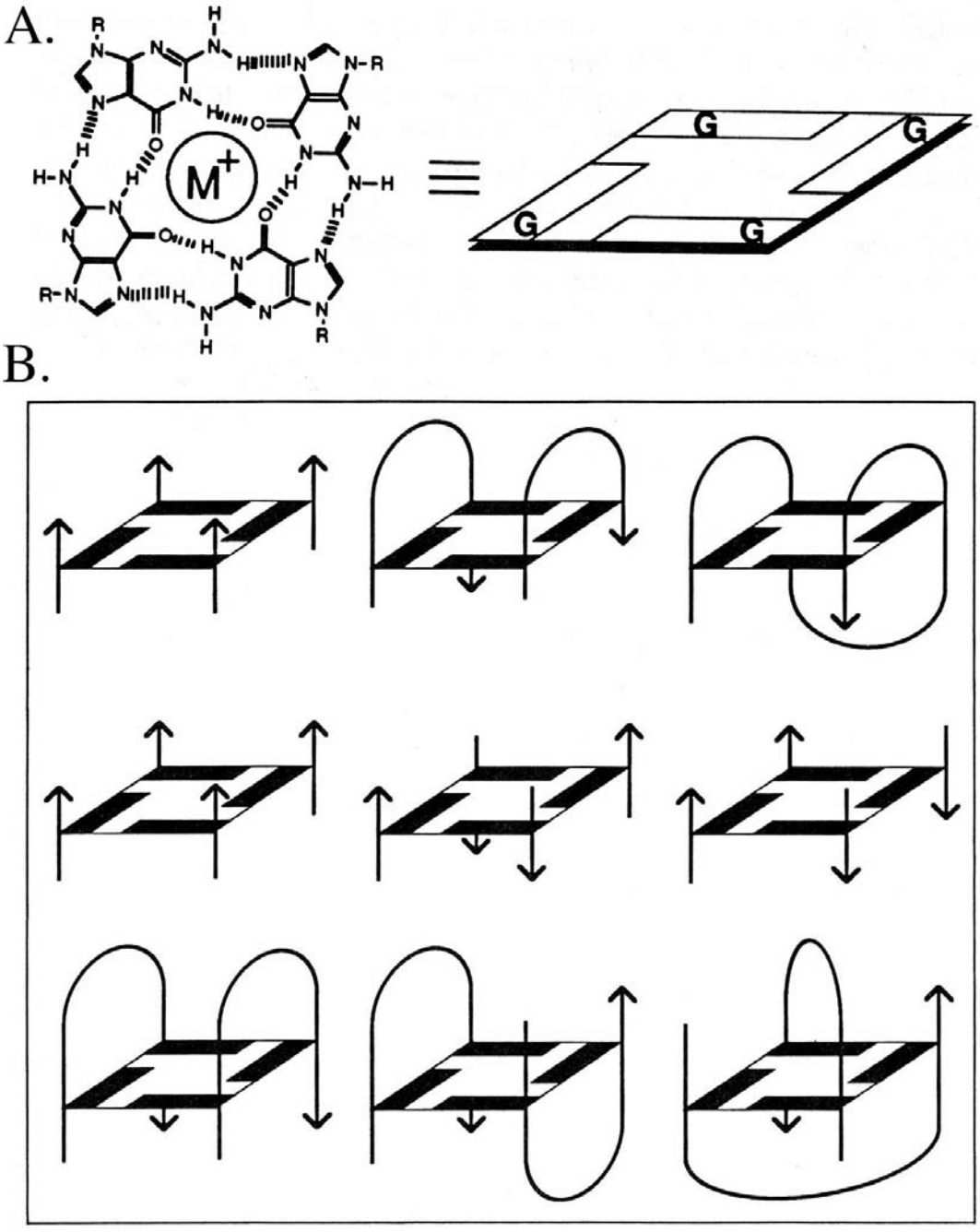

Figure 4. G-quartet structures (modified from 53). **A.** The G-quartet is a cyclic arrangement of four guanine bases forming two hydrogen bonds with each other. Monovalent cations, such as sodium or potassium, fit nicely into a pocket at the center of the quartet. **B.** Polymorphism of G-quartet structures. These structures are formed through the association of four, two or one DNA strands (upper panel). The relative arrangement of adjacent strands can be parallel, alternating parallel or adjacent antiparallel (middle panel). The connecting loops can be arranged head-to-head, head-to-tail, or diagonally.

such as sodium and potassium (Figure 4A), giving the name G-quartet structure. In fact, this structure could be quite polymorphic (reviewed in (53): it can be formed by the association of one, two or four G-rich DNA strands in various orientations relative to each other (Figure 4B). From a biological standpoint, a possibility that four parallel DNA segments can associate in a sequence-specific manner could be a plausible solution for the chromosome alignment in meiosis (49). At the same time, an ability of a single-stranded telomeric repeat to form an intramolecular G-quartet was widely discussed as a way of capping of the chromosome ends (50, 51).

Finally, long AT-rich sequences with a peculiar bias in the distribution of adenines and thymines between the two DNA strands appear to form yet another unusual structure, called DNA Unwinding Element (DUE) (54, 55). Under the influence of negative DNA supercoiling, these sequence elements undergo a transition into a stably unwound conformation. Surprisingly, this conformational transition was non-cooperative, *i.e.* the length of an unwound area gradually increased with an increase in the supercoiling density. DNA unwinding elements were often found at the replication origins and were, thus, implicated in the initiation of DNA replication in pro- and eukaryotes (56, 57).

4. CONCLUDING REMARKS

In retrospect, it is quite striking that after a quarter century reign of the right-handed double helix, a plethora of non-B DNA structures were discovered within just a decade-long time interval. Remarkably, molecular models of these structures withstood the test of time even though most of them, with the exception of Z-DNA, were based on a limited amount of indirect data. At the same time, as readers will see from the following chapters, the first, naïve biological ideas about these structures have undergone considerable revision. Most importantly, we have come to realize that these structures appear on a transient basis in various genetic processes, instead of being steady-state components of the genome. This special issue discusses the current views on structure and biology of unusual DNA structures, including those described above plus the slipped-strand structure that was discovered more recently (58). As readers will learn, these structures are involved in various aspects of genome functioning and appear to be responsible for various aspects of genome instability, implicated in dozens of human hereditary and non-hereditary disorders. As someone who happened to do research in this field from its very beginning, I feel a paternal pride when looking at its current advances and hope that the readers of this issue will share my excitement.

5. ACKNOWLEDGEMENTS

I would like to acknowledge the pivotal role of Maxim Frank-Kamenetskii, who was my mentor in 1980s and introduced me to the area of DNA structures. I am indebted to Alexander Vologodskii, Alexander Lukashin and Vadim Anshelevich for explaining to me many aspects of nucleic acids biophysics. My first studies in the field resulted from the terrific collaboration with Victor Lyamichev and Igor Panyutin: I cannot find words strong enough to describe their contribution. Our appreciation of the alternative DNA structures would be impossible if we were not standing on the shoulders of Alex Rich, David Lilley and Bob Wells. I am forever grateful to many wonderful students and postdoctoral fellows who have chosen to work in my lab to unraveled many striking features of alternative DNA structures. Last, but not the least, I thank NIH and NSF for their continuous support of my research.

6. REFERENCES

1. Watson, J. D. & F. H. Crick: Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. *Nature*, 171, 737-738 (1953)
2. Lagridge, R., H. R. Wilson, C. V. Hooper, M. H. F. Wilkins & L. D. Hamilton: The molecular configuration of deoxyribonucleic acid. I. X-Ray diffraction study of a crystalline form of the lithium salt. *J Mol Biol*, 2, 19-37 (1960)
3. Lagridge, R., D. A. Marvin, W. E. Seeds, H. R. Wilson & L. D. Hamilton: The molecular configuration of deoxyribonucleic acid. II. Molecular models and their Fourier transforms. *J Mol Biol*, 2, 38-64 (1960)
4. Fuller, W., M. H. Wilkins, H. R. Wilson & L. D. Hamilton: The Molecular Configuration of Deoxyribonucleic Acid. Iv. X-Ray Diffraction Study of the A Form. *J Mol Biol*, 12, 60-76 (1965)
5. Marvin, D. A., M. Spencer, M. H. Wilkins & L. D. Hamilton: The molecular configuration of deoxyribonucleic acid. III. X-ray diffraction study of the C form of the lithium salt. *J Mol Biol*, 3, 547-565 (1961)
6. Arnott, S., R. Chandrasekaran, D. W. Hukins, P. J. Smith & L. Watts: Structural details of double-helix observed for DNAs containing alternating purine and pyrimidine sequences. *J Mol Biol*, 88, 523-533 (1974)
7. Mokul'skii, M. A., K. A. Kapitonova & T. D. Mokul'skaya: Secondary structure of phage T2 DNA. *Mol Biol*, 6, 716-31 (1972)
8. Hoogsteen, K.: The crystal and molecular structure of a hydrogen-bonded complex between 1 methylthymine and 9 methyladenine. *Acta Cryst*, 16, 907-16 (1963)
9. Riley, M. & B. Maling: Physical and chemical characterization of two- and three-stranded adenine-thymine and adenine-uracil homopolymer complexes. *J Mol Biol*, 20, 359-389 (1966)
10. Morgan, A. R. & R. D. Wells: Specificity of the three-stranded complex formation between double-stranded DNA and single-stranded RNA containing repeating nucleotide sequences. *J Mol Biol*, 37, 63-80 (1968)
11. Ralph, R. K., W. J. Connors & H. J. Khorana: Secondary structure. and aggregation in deoxyguanosine oligonucleotides. *J Am Chem Soc*, 84, 2265-2266 (1962)
12. Pohl, F. M. & T. M. Jovin: 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)
13. Vinograd, J., J. Lebowitz, R. Radloff, R. Watson & P. Laipis: The twisted circular form of polyoma viral DNA. *Proc Natl Acad Sci USA*, 53, 1104-1111 (1965)
14. Wang, J. C.: Interaction between DNA and an Escherichia coli protein omega. *J Mol Biol*, 55, 523-533 (1971)
15. Gellert, M., K. Mizuuchi, M. H. O'Dea & H. A. Nash: DNA gyrase: an enzyme that introduces superhelical turns into DNA. *Proc Natl Acad Sci USA*, 73, 3872-3876 (1976)
16. Gellert, M., M. H. O'Dea, T. Itoh & J. Tomizawa: Novobiocin and coumermycin inhibit DNA supercoiling catalyzed by DNA gyrase. *Proc Natl Acad Sci USA*, 73, 4474-4478 (1976)
17. Vologodskii, A. V., V. V. Anshelevich, A. V. Lukashin & M. D. Frank-Kamenetskii: Statistical mechanics of

supercoils and the torsional stiffness of the DNA double helix. *Nature*, 280, 294-298 (1979)
18. Larsen, A. & H. Weintraub: An altered conformation detected by S1 nuclease occurs at specific region in active chick globin chromatin. *Cell*, 29, 609-622 (1982)
19. Wang, A. H., G. J. Quigley, F. J. Kolpak, J. L. Crawford, J. H. van-Boom, G. van-der-Marel & A. Rich: Molecular structure of a left-handed double helical DNA fragment at atomic resolution. *Nature*, 282, 680-666 (1979)
20. Peck, L. J., A. Nordheim, A. Rich & J. C. Wang: Flipping of cloned d (pCpG)n.d (pGpC)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)
21. Singleton, C. K., J. Klysik, S. M. Stirdivant & R. D. Wells: Left-handed Z-DNA is induced by supercoiling in ohysiological ionic conditions. *Nature*, 299, 312-316 (1982)
22. Lipps, H. J., A. Nordheim, E. M. Lafer, D. Ammermann, B. D. Stollar & A. Rich: Antibodies against Z DNA react with the macronucleus but not the micronucleus of the hypotrichous ciliate stylonychia mytilus. *Cell*, 32, 435-441 (1983)
23. Nordheim, A., M. L. Pardue, E. M. Lafer, A. Moller, B. D. Stollar & A. Rich: Antibodies to left-handed Z-DNA bind to interband regions of Drosophila polytene chromosomes. *Nature*, 294, 417-422 (1981)
24. Rich, A., A. Nordheim & A. H.-J. Wang: The chemistry and biology of left-handed Z-DNA. *Annu Rev Biochem*, 53, 791-846 (1984)
25. Panayotatos, N. & R. D. Wells: Cruciform structures in supercoiled DNA. *Nature*, 289, 466-470 (1981)
26. Lilley, D. M. J.: The inverted repeat as a recognizable structural feature in supercoiled DNA. *Proc Natl Acad Sci USA*, 77, 6468-6472 (1980)
27. Lyamichev, V. I., I. G. Panyutin & M. D. Frank-Kamenetskii: Evidence of cruciform structures in superhelical DNA provided by two-dimensional gel electrophoresis. *FEBS Lett*, 153, 298-302 (1983)
28. Vologodskii, A. V. & M. D. Frank-Kamenetskii: Theroretical study of cruciform states in superhelical DNA. *FEBS Lett*, 143, 257-260 (1982)
29. Cox, R. & S. M. Mirkin: Characteristic enrichment of DNA repeats in different genomes. *Proc Natl Acad Sci USA*, 94, 5237-5242 (1997)
30. Schroth, G. P. & P. S. Ho: Occurrence of potential cruciform and H-DNA forming sequences in genomic DNA. *Nucleic Acids Res*, 23, 1977-1983 (1995)
31. Lyamichev, V., I. Panyutin & S. Mirkin: The absence of cruciform structures from pAO3 plasmid DNA in vivo. *J Biomol Struct Dyn*, 2, 291-301 (1984)
32. Courey, A. J. & J. C. Wang: Cruciform formation in a negatively supercoiled DNA may be kinetically forbidden under physiological conditions. *Cell*, 33, 817-829 (1983)
33. Sinden, R. R., S. S. Broyles & D. E. Pettijohn: Perfect palindromic lac operator DNA sequence exists as a stable cruciform structure in supercoiled DNA in vitro but not in vivo. *Proc Natl Acad Sci USA*, 80, 1797-1801 (1983)
34. Dayn, A., S. Malkhosyan, D. Duzhy, V. Lyamichev, Y. Panchenko & S. Mirkin: Formation of (dA-dT)n cruciforms in Escherichia coli cells under different environmental conditions. *J Bacteriol*, 173, 2658-2664 (1991)
35. McClellan, J. A., P. Boublikova, E. Palecek & D. M. J. Lilley: Superhelical torsion in cellular DNA responds directly to environmental and genetic factors. *Proc Natl Acad Sci USA*, 87, 8373-8377 (1990)
36. Haniford, D. B. & D. E. Pulleyblank: Transition of a cloned d (AT)n.d (AT)n tranct to a cruciform in vivo. *Nucleic Acids Res*, 13, 4343-4346 (1985)
37. Leach, D. R. & F. W. Stahl: Viability of lambda phages carrying a perfect palindrome in the absence of recombination nucleases. *Nature*, 305, 448-451 (1983)
38. Mace, H. A. F., H. R. B. Pelham & A. Travers: Association of an S1 nuclease-sensitive structure with short direct repeats 5' of Drosophila heat shock genes. *Nature*, 304, 555-557 (1983)
39. Hentchel, C. C.: Homocopolymer sequences in the spacer of a sea urchin histone gene repeat are sensitive to S1 nuclease. *Nature*, 295, 714-716 (1982)
40. Lyamichev, V. I., S. M. Mirkin & M. D. Frank-Kamenetskii: A pH-dependent structural transition in the homopurine-homopyrimidine tract in superhelical DNA. *J Biomol Struct Dyn*, 3, 327-38 (1985)
41. Pulleyblank, D. E., D. B. Haniford & A. R. Morgan: A structural basis for S1 nuclease sensitivity of double-stranded DNA. *Cell*, 42, 271-280 (1985)
42. Mirkin, S. M., V. I. Lyamichev, K. N. Drushlyak, V. N. Dobrynin, S. A. Filippov & M. D. Frank-Kamenetskii: DNA H form requires a homopurine-homopyrimidine mirror repeat. *Nature*, 330, 495-497 (1987)
43. Hanvey, J. C., M. Shimizu & R. D. Wells: Intramolecular DNA triplexes in supercoiled plasmids. *Proc. Natl. Acad. Sci. USA*, 85, 6292-6296 (1988)
44. Htun, H. & J. E. Dahlberg: Single strands, triple strands and kinks in H-DNA. *Science*, 241, 1791-1796 (1988)
45. Johnston, B. H.: The S1-sensitive form of d $(C\text{-}T)_n$.d $(A\text{-}G)_n$: chemical evidence for a three-stranded structure in plasmids. *Science*, 241, 1800-1804 (1988)
46. Vojtiskova, M., S. Mirkin, V. Lyamichev, O. Voloshin, M. Frank-Kamenetskii & E. Palecek: Chemical probing of the homopurine.homopyrimidine tract in supercoiled DNA at single-nucleotide resolution. *FEBS Lett*, 234, 295-299 (1988)
47. Voloshin, O. N., S. M. Mirkin, V. I. Lyamichev, B. P. Belotserkovskii & M. D. Frank-Kamenetskii: Chemical probing of homopurine-homopyrimidine mirror repeats in supercoiled DNA. *Nature*, 333, 475-476 (1988)
48. Kohwi, Y. & T. Kohwi-Shigematsu: Magnesium ion-dependent triple-helix structure formed by homopurine-homopyrimidine sequences in supercoiled plasmid DNA. *Proc Natl Acad Sci USA*, 85, 3781-3785 (1988)
49. Sen, D. & W. Gilbert: Formation of parallel four-stranded complexes by guanine-rich motifs in DNA and its implications for meiosis. *Nature*, 334, 364-366 (1988)
50. Sundquist, W. I. & A. Klug: Telomeric DNA dimerizes by formation of guanine tetrads between hairpin loops. *Nature*, 342, 825-829 (1989)
51. Williamson, J. R., M. K. Raghuraman & T. R. Cech: Monovalent cation-induced structure of telomeric DNA: the G-quartet model. *Cell*, 59, 871-880 (1989)
52. Panyutin, I. G., O. I. Kovalsky & E. I. Budowsky: Magnesium-dependent supercoiling-induced transition in (dG)n.(dC)n stretches and formation of a new G-structure by (dG)n strand. *Nucleic Acids Res*, 17, 8257-71 (1989)

53. Williamson, J. R.: G-quartet structures in telomeric DNA. *Annu Rev Biophys Biomol Struct*, 23, 703-730 (1994)
54. Sheflin, L. G. & D. Kowalski: Mung bean nuclease cleavage of a dA + dT-rich sequence or an inverted repeat sequence in supercoiled PM2 DNA depends on ionic environment. *Nucleic Acids Res*, 12, 7087-7104 (1984)
55. Kowalski, D., D. A. Natale & M. J. Eddy: Stable DNA unwinding, not "breathing," accounts for single-strand-specific nuclease hypersensitivity of specific A+T-rich sequences. *Proc Natl Acad Sci USA*, 85, 9464-9468 (1988)
56. Umek, R. M. & D. Kowalski: The ease of DNA unwinding as a determinant of initiation at yeast replication origins. *Cell*, 52, 559-567 (1988)
57. Umek, R. M., M. H. Linskens, D. Kowalski & J. A. Huberman: New beginnings in studies of eukaryotic DNA replication origins. *Biochim Biophys Acta*, 1007, 1-14 (1989)
58. Pearson, C. E. & R. R. Sinden: Alternative structures in duplex DNA formed within the trinucleotide repeats of the myotonic dystrophy and fragile X loci. *Biochemistry*, 35, 5041-53 (1996)
59. Herbert, A. & A. Rich: The biology of left-handed Z-DNA. J Biol Chem 20, 11595-11598 (1996)

Key Words: B-DNA, Z-DNA, H-DNA, G-quartet, DNA Unwinding Element (DUE), left-handed DNA, triplex DNA, quadruplex DNA, Review

Send correspondence to: Dr Sergei M. Mirkin, Department of Biology, Tufts University, Medford, MA 02155, U.S.A., Tel: 617-627-4794, Fax: 617-627-3805, E-mail:Sergei.Mirkin@tufts.edu

http://www.bioscience.org/current/vol13.htm