## Nucleic acid structure

# New twists to left-handed DNA

*from Stephen Neidle*

THAT DNA appeared a relatively monotonous molecule in structural terms was perhaps an inevitable outcome of the classical Watson–Crick model, for it seemed that the regularity of the anti-parallel right-handed double-helix imparted a precise structural equivalence to all residues in a DNA sequence. The past three years have, however, seen profound changes in our view of nucleic acid structure, and a more complex and subtle picture is emerging. Of particular interest has been the discovery of the quite unexpected left-handed Z-DNA helix. Crystallographic studies of $d(CpG)_n$ oligonucleotides[1,2] with $n=2,3$ and fibre diffraction analysis of poly(dC-dG) (ref.3) first revealed this profound structural deviation from the classical picture, and ready correlations have been found in a stream of studies of Z-DNA structure and reactivity, in solution (see *News and Views* **292**, 292; 1981), the latest installment of which is reported in this week's *Nature* (see p.632).

In early studies on the Z structure in solution poly(dC-dG) was mostly used as it readily displays a salt-induced right- to left-handed helix transition. Wells and his colleagues[4] pioneered the insertion of varying lengths of $d(CpG)_n$ tracts into recombinant plasmids, and showed them to be remarkably sensitive and versatile probes of Z-DNA properties, especially when embedded in natural right-handed DNA sequences. A closed-circular DNA plasmid is negatively supercoiled when it contains normal right-handed helical DNA but when parts are converted to a left-handed form by the insertion of d(CpG) there is a loss of superhelicity which can be readily monitored by changes in either superhelical density or electrophoretic mobility. The inserted $d(CpG)_n$ segments have to be substantially longer than 10 base pairs (possible more than 20) for this relaxation of negative supercoiling to occur[5]; but as the process is energetically favourable, it can occur at a much lower salt concentration than that required for the B→Z transition in linear poly(dC-dG) (refs 6,7) and may indeed be driven by torsional forces alone.

Recent experiments have dispelled any lingering doubts over whether the relaxed $d(CpG)_n$ plasmid really contains left-handed DNA. The use of antibodies raised against Z-DNA has shown that the antibody combining site for a CG-modified pLP32 plasmid is clearly at the insertion site[8].

Z-helix formation appears not to be confined to $d(CpG)_n$ sequences — Arnott *et al.*[3] showed that poly (dG-dT)·poly(dA-dC) could equally well adopt a left-handed Z structure in a semi-crystalline fibre sample. However, Wells *et al.* have found[9] that this polynucleotide will only form a left-handed helix, in the most extreme environmental conditions. Furthermore, they were unable to observe the transition with an almost perfect $d(TpG)_{31}$ sequence either alone or when inserted into a plasmid. These findings are important since such a 62 bp sequence has been found in a eukaryotic DNA, from the 3′ side of the mouse immunoglobulin κ gene. If Z-DNA does indeed have a biological role, in, say, gene regulation and signalling, one might expect that such a sequence would readily adopt a left-handed form.

The controversy concerning the Z-DNA potential of $d(TpG)_n$ sequences is further fuelled by a report in this week's *Nature* (p.632). Haniford and Pulleyblank used a fundamental property of closed-circular DNAs containing a left-handed region embedded within a normal right-handed helix — the degree of supercoiling (and hence the linking number) is altered compared with the all-right-handed form. A mixture of such DNAs was constructed from the plasmid pDPL6 by cloning varying lengths of $d(TpG)_n$ sequence into it. These modified plasmids (topoisomers) had differing degrees of supercoiling, depending on the amount of left-handedness introduced. Gel electrophoresis of both the topoisomers and unmodified plasmids after the same degree of partial supercoil relaxing had been applied suggested an abrupt structural transition in the $d(TpG)_n$ region after a certain degree of unwinding had been produced in the plasmid as a whole.

Detailed examination of a plasmid with a 60 bp d(TpG) insert showed that the superhelical DNA turns in it were unwound to roughly twice the extent of sections of DNA sequence with hairpin-loops. The torsional free energy for initiation of this B→Z transition was calculated to be quite high, almost twice that required for stabilization of the Z form, and possibly higher than that required for $d(CpG)_n$ sequences. The previous failure to observe Z-DNA with $d(TpG)_n$ inserts[9] may thus be due to the use of a plasmid with a superhelix density insufficient for the initiation of the transition, suggesting this sequence is less readily driven to the Z form than the $d(CpG)_n$ one. There is little doubt that this difference originates in differences in the water molecules and ionic environments surrounding these sequences compared with natural DNA; but a full explanation is not yet possible.

The continuing search for a definite biological role for Z-DNA has recently revealed some potentially significant findings. Following the location of regions in *Drosophila* polytene chromosomes that react with Z-DNA antibodies[10], at least four specific Z-DNA-binding proteins, have been detected in *Drosophila*[11], using affinity chromatography. The proteins not only interact with, but actually stabilize Z-DNA sequences; such proteins would enable $d(TpG)_n$ sequences to flip from right- to left-handed helices rather more readily than do simple salts. Thus, polyarginine stabilizes the poly(dC-dG)·poly(dC)·dG Z helix at physiological ionic strengths[12], rather than the > 2.5 molar salt concentration normally required.

At the more detailed sequence level two recent reports using specific hybridization probes have revealed that potential Z-forming $d(TpG)_n$ sequences in eukaryotic DNA are widespread. Such results do not, however, prove that these regions are in, or can indeed change to, a Z-form helix. First, Hamada and Kakunaga[13] have found a $d(TpG)_{25}$ region in an intron of a human cardiac muscle actin gene. Use of their $d(TgG)_n$-specific hybridization probe on restriction fragments of human DNA revealed a very large number of such sequences — an estimated $10^5$ copies — in the genome. This result is, in retrospect, not altogether surprising, in view of the large amount of Z-DNA banding found previously in *Drosophila*[10].

Second, Walmsley *et al.*[14] have examined the genome of *S. cerevisiae* yeast with a $d(TpG)_n$ hybridization probe. Their finding of at least 30 regions of $d(TpG)_n$ is in itself not surprising but they then went on to isolate the 1.4 kilobase chromosome ends (telomeres), which themselves hybridized with the putative Z-DNA probe. Furthermore, the telomeres, when cloned with the linear plasmid having chromosomal ends from the protozoa *Tetrahymena*, retained hybridization properties, which were carried through after restriction enzyme cutting of the plasmid into separate *Tetrahymena* and yeast telomere-containing fragments. They conclude that both these eukaryotic telomeres contain $d(TpG)_n$ tracts, which, if one accepts that these are involved in regulatory processes, implies features common to these very different eukaryotes, at least for the initiation of replication at chromosomal ends.

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