Anomalous properties of adenine·thymine tracts

SIR—Nelson *et al.*[1] reported the single-crystal structure of DNA dodecamer containing an oligo(dA)·oligo(dT) tract. This tract is a model of $(dA)_n \cdot (dT)_n$ that apparently differs from ordinary B-type DNA by some unusual properties, for example: (1) conformational stability; (2) oligo(dA)·oligo(dT) abutting other sequences causes DNA bending; and (3) poly(dA)·poly(dT) does not associate into nucleosomes. What is the origin of these anomalous properties? The authors emphasize several distinctive structural features of the oligo(dA)·oligo(dT) tract: a high propeller twist (improving the intrastrand stacking); a narrow minor groove; and a system of bifurcated hydrogen bonds in the major groove, additional bond formation being promoted by the zero tilt angle. The first two features have been widely discussed[2], whereas the bifurcated bonds were revealed only recently[1,3].

It seems that experiments with duplexes, where some dA·dT pairs are replaced by dI·dC pairs, could clarify the relative role of bifurcated bonds in properties (1) and (2). Duplexes with alternating poly d(A-I)·poly d(C-T) sequences obviously cannot contain bifurcated bonds. Nevertheless, in fibres this polymer has the same B′-conformation as poly(dA)·poly(dT) and similarly is not converted into other structural forms[4]. The contribution of bifurcated bonds to DNA bending can be estimated from the data of Diekmann *et al.*[5]. When the A_5 tract is replaced by AIAIA which is accompanied by a loss of this particular system of hydrogen bonds, the anomalous electrophoretic mobility does not disappear, but decreases by about 20 per cent when A_5 is replaced by AAIAA[5,6], though here there is no tract of at least three successive adenines necessary to stabilize the bifurcated interaction according to Nelson *et al*[1]. The third anomalous property was explained[1] by an increased rigidity of the poly(dA)·poly (dT) caused by good stacking and bifurcated bonds. However, there is experimental evidence that poly(dA)· poly(dT) has approximately the same rigidity as poly d(A-T)·poly d(A-T) and less than poly d(G-C)·poly d(G-C) or a random DNA (see ref. 7), whereas in the latter three cases DNA can associate into nucleosomes.

Thus, the bifurcated bonds could hardly contribute much to the formation of DNA bending and conformational stability of polynucleotides in fibres. Two other factors, the overall energy balance (in particular, base-pair stacking) and the minor groove spine of hydration stabilizing the structure, suggest a qualitative explanation of several anomalies observed in $(dA)_n \cdot (dT)_n$ tracts[2,8]. The relationship between the polymer rigidity and the inability to associate into nucleosomes is not clear at present. Thus, additional experimental data are required for a final answer to the question about the determinants of $(dA)_n \cdot (dT)_n$ anomalous properties.

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NELSON AND KLUG REPLY — Chuprina and Abagyan omit to mention the main result of our paper[1], which is that the oligo(dA)·oligo(dT) tract is straight — there is no roll nor tilt at each base pair. This is relevant to the question of how free DNA containing phased runs of adenines is bent[2]: the implication is that bending must occur outside the adenine tracts. We then look at the structural features which might account for the unusual properties of poly(dA)·poly(dT). We find a high degree of propeller twist between the AT base pairs, which enables a system of bifurcated hydrogen bonds to be formed and leads to the very good base-stacking interactions which occur. (Here, of course, we cannot distinguish cause and effect.) Note that these stacking interactions would be only minimally affected by the introduction of an inosine into the adenine tracts, as an IC base pair, with only two hydrogen bonds, could accommodate a high degree of propeller twist. We have already noted that isolated examples of bifurcated hydrogen bonds have been found[3].

Two other points are raised. First, the experiments on rigidity of homopolymer poly(dA)·poly(dT)[4], quoted by Chuprina and Abagyan, rely on intercalation of dyes to determine the torsional and bending stiffness of the DNA. Not only do these dyes bind less tightly to the highly propeller-twisted AT-rich DNAs, but they also would force a decrease in the propeller twist that would destroy many of the special properties of poly(dA)·poly(dT). What is needed is a reliable determination of the persistence length of homopolymer poly(dA)·poly(dT) which would settle the question of its 'conformational rigidity'. Second, we have no evidence for the spine of hydration postulated by Chuprina to exist in the minor groove of homopolymer poly(dA)·poly(dT)[5]. This must await a higher-resolution X-ray analysis.

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Open reading frames and translational control

SIR—Bürglin *et al.* have pointed out the striking sequence homologies in the 5′ regions of mouse and *Xenopus* homoeobox messenger RNAs[1], especially between the Hox. 1.1 RNAs from mouse[2] and *Xenopus* (Xhox-36, ref. 3) and the Hox 2.3 RNAs from mouse[4], *Xenopus* (X1Hbox2, ref. 5) and man (HHO.C1, ref. 6). The importance of this sequence is indicated by the fact that it was conserved between different species as well as during a (probable) gene duplication event leading to the two closely similar genes, Hox 1.1 and Hox 2.3, in one species. Although Bürglin *et al.* have denied the significance of open reading frames (ORFs) in this region, we would like to present evidence suggesting the importance of these ORFs and their possible role in a translational control mechanism.

During the study of murine Hox 1.1 complementary DNA sequences extending further in the 5′ direction, we observed the conservation of an ORF between the murine and the *Xenopus* Hox 1.1 genes. The 5′ region of the murine Hox 1.1 RNA encodes a 23-amino-acid peptide starting at position −145 (counting backwards from the ATG codon). The *Xenopus* Hox 1.1 (Xhox-36) contains an ORF at position

ATG GGA CAG TCA GAT GGT GGC AGA TCA CGT GGC CCA GGC AGG CAG CTC	Xenopus	Hox 1.1
ATG --- --- --- --- --- TGG CGG TCA CGT GCC GCG GCG AGC --- ---	Mouse	Hox 1.1
Met --- --- --- --- --- Trp Arg Ser Arg Ala Ala Ala Ser --- ---	Mouse	Hox 1.1
Met Gly Gln Ser Asp Gly Gly Arg Ser Arg Gly Pro Gly Arg Gln Leu	Xenopus	Hox 1.1
AGT GTA AAG GAA AAA ATG GGG TTT TGC GTA AAT GTG GGG GTT TAG	Xenopus	Hox 1.1
TCC GTC CAA AAG AAA ATG GGG TTT GGT GTA AAT CTG GGG GTG TAA	Mouse	Hox 1.1
Ser Val Gln Lys Lys Met Gly Phe Gly Val Asn Leu Gly Val	Mouse	Hox 1.1
Ser Val Lys Glu Lys Met Gly Phe Cys Val Asn Val Gly Val	Xenopus	Hox 1.1

Conserved open reading frames upstream of the homoeobox-containing frame in the Hox 1.1 genes of mouse[2] and *Xenopus* (Xhox-36, ref. 3). The distance between the stop codon and the homoeobox reading frame initiator codon is 73 and 75 base pairs, respectively.