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DNA Kinks Available…If Needed

In this issue of *Structure*, Lankas and coworkers (Lankas et al., 2006) use molecular dynamics simulations of highly strained 94 base-pair DNA circles to show DNA kinking (unstacking of base pairs). The significance of such kinks is surprisingly controversial.

One might think that 53 years after the elucidation of the basic elements of DNA structure, little mystery or controversy would remain concerning the physical properties of the familiar double helix (Figure 1A). Think again. For example, who would have imagined that DNA gets longer when it is wound more tightly (Gore et al., 2006; Lionnet et al., 2006)? Or consider the question of why DNA is so much stiffer than other biopolymers. Does DNA resist bending because of the spine of stacked base pairs in the core of the molecule (Mills and Hagerman, 2004) or because bending causes expensive crowding of negatively charged phosphates (Podesta et al., 2005)? We don't know. In fact, researchers studying the fundamental physical properties of DNA are far from tying up loose ends and moving on.

It is in this context that the issue of DNA kinking has recently surfaced, and the fascinating molecular dynamics simulations of Lankas et al. (2006) are salient. The DNA double helix consists of a stack of planar base pairs between two spiraling sugar-phosphate backbones. Base pairs prefer to stack on one another rather than being exposed to water. A DNA kink occurs when stacked base pairs become unstacked (Figure 1B). Such a kinked site allows strained DNA to make a sharp turn. DNA kinking has long been predicted (Crick and Klug, 1975), and it has been directly observed by X-ray crystallography when DNA is bent tightly around a protein surface (Luger et al., 1997; Schultz et al., 1991). Protein-induced DNA kinks can act to widen either the major or the minor groove (Figure 1C), while Crick and Klug predicted that kinking to widen the major groove would be favored in free DNA (Crick and Klug, 1975).

So what's the big deal with DNA kinking? It turns out that it has been very productive to model the behavior of long DNA molecules by dispensing with all the atomic detail and treating the double helix as an elastic rod with a persistence length of 150 base pairs (the distance over which the average deflection of the polymer axis is one radian). By definition, such elastic rods don't kink. How good are such simplified models at predicting the physical properties of real DNA chains? The answer depends to some extent upon the length of the chain. For DNA chains significantly longer than one persistence length, the experimental behavior of DNA in assays such as stretching with an optical tweezers (Bustamante et al., 1994) or forming circles by enzymatic end-joining (Shore et al., 1981) is nicely predicted by simple models. However, in 2004 Cloutier and Widom experimentally tested the enzymatic cyclization of short (~100 base pair) DNA chains and reported the complete failure of the theory: DNA circles appeared to form three orders of magnitude faster than predicted (Cloutier and Widom, 2004). Maybe short DNA molecules are subject to kinking and are not so stiff after all. Maybe DNA kinks, though rare, must be included in predictions. The issue is important because DNA loops of ~100 base pairs are common in gene regulation, most notably in bacteria. Will such DNA loops readily form with spontaneous kinking to relieve the strain, or are accessory kinking proteins really needed to facilitate such loops (Becker et al., 2005)?

Inquisitive DNA researchers immediately set to work to ponder the implications of the Cloutier and Widom experiments. A team of theoreticians showed that including more microscopic detail to supplement rod models predicts enhanced apparent flexibility of short DNAs without invoking kinks (Czapla et al., 2006). Meanwhile, Du and colleagues calculated that allowing rare DNA kinks might explain facilitation of short DNA circles, but including the possibility of such kinks degraded fits to cyclization data for longer DNA chains (Du et al., 2005). These investigators then performed their own cyclization experiments with short DNA chains and, in so doing, reported an apparent technical flaw in the study of Cloutier and Widom. It involved the failure of a crucial assumption about the rates of end "un-joining" for intramolecular versus intermolecular reactions in the absence of a joining enzyme. Though a tiny detail, accounting for this issue in their experiments led Du et al. (2005) to the very opposite conclusion: the traditional elastic rod theory of DNA predicts DNA cyclization very nicely down to 105 bp…no kinking required.

While this was going on, the heroic molecular dynamics simulations of Lankas et al. (2006) were undertaken. All-atom simulations (including solvent molecules and ions) are computationally intense, all the more so because of the massive number of atoms in realistic DNA circles and the need for simulations of tens of nanoseconds to detect large macromolecular motions. Ninety-four base-pair DNA circles were modeled with or without twisting strain. The authors simulated at least 80 nanoseconds of DNA molecular motion for each of four starting conditions. Remarkably, three of the four trajectories predicted the formation of at least one DNA kink. Some of the observed kinks were as anticipated (i.e., base-pair unstacking to widen the major groove and narrow the minor groove; Figure 1D). One example was more dramatic, involving unstacking of three consecutive base pairs with one pair completely broken. Although kinks occurred most frequently at weak pyrimidine/purine stacks, the authors were surprised that the most common kinks were inexplicably at 5′-CG-3′ steps, rather than predicted 5′-TA-3′ or 5′-CA-3′ steps. The result is a beautifully executed and technically admirable in silico treatment of the controversial kinking issue. To the extent that the simulation force field is to be trusted, kinks appear to be an inherent DNA response to strong bending and twisting strain.

Is kinking an essential property of DNA that is necessary to explain the probability of protein-free DNA loops, or do traditional theories of smoothly curving polymers

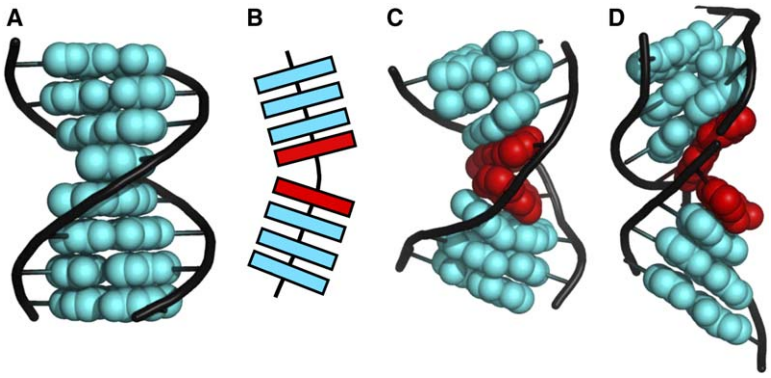

Figure 1. DNA Kinks

(A) The undistorted DNA double helix. Atoms of the stacked base pairs are shown as cyan spheres. The sugar-phosphate backbones are shown as black wires.

(B) Schematic illustration of base pair unstacking to form a kink. Kinked base pairs are red, and the curved helix axis is indicated.

(C) Example of a DNA kink that widens the minor groove induced upon DNA bending by the *E. coli* catabolite activator protein (Schultz et al., 1991).

(D) Example of a DNA kink that widens the major groove predicted from the molecular dynamics simulations of strained DNA circles by Lankas et al. (2006).

suffice to model the flexibility of short segments of DNA? We still don't know! What Lankas et al. show us with their remarkable simulations is that DNA kinks are theoretically available if the experimentalists end up needing them. We'll see if they do.

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