Spare the Rod: The Surprising Response of DNA in a Tight Situation

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DNA is justly famous as a high-density, nonvolatile information-storage medium for cells. But this versatile molecule also participates in its own packaging, duplication, and control as a result of its subtle mechanochemical behavior. One key physical property of DNA that is central to processes ranging from DNA packing in viruses and cells to transcriptional regulation is its susceptibility to bending and twisting. Over the last few decades, a picture of the mechanical behavior of DNA has emerged, one that regards the molecule in a coarse-grained way as simply an elastic rod. This simple mathematical model has scored many impressive successes, embracing a wide range of single-molecule and more traditional experimental data. But in this issue of *PNAS* and elsewhere, Cloutier and Widom provide convincing evidence that the mechanical resistance of short DNA fragments to severe bend and twist deformations is dramatically weaker than the expectations of the elastic rod model [1, 2], a discovery with significance for biologists and physical scientists alike.

Textbooks tell us that DNA is a stiff polymer. To make that statement quantitative, we can model DNA mathematically as a rod made of a continuum elastic material (also called the "wormlike chain" or "persistent chain" model), with an elastic constant representing the rod's resistance to bending. Setting this bending stiffness constant to $\xi \approx 50\,\mathrm{nm}$ (in units of the thermal energy $k_\mathrm{B}T$) matches the observed gross behavior of very long DNA. For example, electron micrographs of DNA from lysed bacteria show it following a wandering path, whose tangent vector varies significantly on the scale ξ as shown in fig 1. Because ξ is much greater than the diameter of the DNA duplex ($\approx 1\,\mathrm{nm}$), we say that DNA is stiff. A simple generalization of the elastic rod model also accounts for the resistance of DNA to twisting, by introducing a second elastic constant. More advanced approaches introduce sequence dependence, bend anisotropy and other refinements, but retain the essential character of the elastic rod model, and in particular, its simple harmonic form for the deformation energy of the molecule.

Now in its sixth decade [3], the elastic rod model of a polymer has proven to be amazingly good at integrating classical biochemical measurements on DNA with the latest single-molecule results. On the other hand, puzzling results have been accumulating for some time. For example, *in vivo* experiments on the activity of bacterial genes that are repressed via DNA looping show vigorous repression even when the distance between the repressor binding sites is considerably smaller than ξ [4, 5, 6]. One could try to explain these results by invoking the presence of proteins that bend the DNA, thus reducing the free energy cost associated with repressor binding. Other *in vitro* experiments reveal similar puzzles. For example, recent experiments using atomic-force microscopy revealed a higher probability for high-curvature configurations than would be expected on the basis of conventional DNA elasticity [7].

But by far the clearest, simplest, and most inescapable evidence for a spontaneous breakdown of harmonic elasticity in DNA have recently come from a fairly traditional biochemical assay. In experiments published last year, Cloutier and Widom measured the propensity for DNA of various lengths to cyclize (form closed loops). To quantify this property, they measured the "Jacobson–Stockmayer J factor," essentially the effective concentration of one end of the DNA in the vicinity of the other as shown in fig. 2. DNA fragments much longer than ξ yield a small value of J, as the two ends wander through a large volume [8]. At the opposite extreme, fragments shorter than ξ were also expected to give a small value of J, due to the high elastic energy cost of bending the DNA duplex into a tight ring. What the experiments found, however, was that random-sequence DNA fragments of length 32 nm give J factors at least 1000 times greater than expected on the basis of the elastic rod model [1]. That

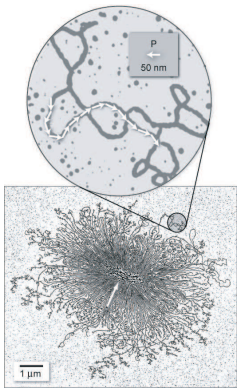

Figure 1: DNA from a bacterium that has been lysed by osmotic shock. The bacterial genome that once occupied a small region in the center of the figure now extends in a series of loops from the core structure. Right to left, about 10 μm. (Electron micrograph by Ruth Kavenoff.) The enlarged region divides the DNA into a collection of segments each with a length of the persistence length.

is, taking the value for the bending stiffness constant ξ deduced from many different experiments on longer DNAs, and extrapolating the model to the shorter-length regime of the new experiments which are of special biological interest, leads to a prediction of the J factor that differs by orders of magnitude from what Cloutier and Widom observed. This elegant experiment thus implied that the resistance of DNA to bending on such short scales is much smaller than that predicted by the elastic rod model. Sequence-dependence effects cannot explain this phenomenon [9, 10].

In the experiments reported in this issue of *PNAS*, Cloutier and Widom have elaborated on this surprising feature of short-length-scale DNA mechanics by cyclizing DNA fragments in one-basepair increments, so as to probe the free energy consequences of requiring the two ends of the DNA to come into twist registry, as is needed for ligation. The authors show that the suppression due to the twist mismatch is also much smaller than the result obtained by extrapolating the elastic rod model to this length-scale regime [11, 9]. Remarkably, these experiments have gone where no single-molecule experiment has gone before, directly probing the length-scale regime that is crucial for describing biological processes such as regulation by DNA loop formation or the wrapping of DNA around histones to form the nucleosome.

In retrospect, perhaps we should not be surprised at these results. Certainly the idea that DNA could deviate markedly from simple elastic behavior is not new. For example, Francis Crick and Aaron Klug found an allowed sharply-kinked conformation in 1975 [12]. Recent molecular dynamics simulations of DNA minicircles have also begun to show the appearance of such localized defects: After twenty nanoseconds of simulation time, the minicircles spontaneously adopt elongated conformations with localized kinks [13]. Finally, DNA under superhelical tension has long been known to be susceptible to strand separation [14, 15]; conversely, transient breakdown of the DNA duplex can assist in the formation of structures that would otherwise require a prohibitive twist-energy cost.

Recent theoretical work has begun to incorporate these insights, creating a new framework for thinking about the short-length-scale mechanical properties of DNA [16, 17, 18, 19, 20]. It turns out upon closer examination that the classic successes of the elastic rod model all involve the behavior of DNA on length scales of 100 nm or longer. But in a wicked twist, it turns out that on length scales longer than ξ, *any* microscopic model of DNA elasticity—even one admitting sharp kinks—will give predictions resembling those of the naive harmonic theory! Thus, to peek behind the elastic rod model requires experiments like those of Cloutier and Widom that explicitly probe the short-length-scale behavior of DNA. The new models posit the transient appearance of localized weak spots in the DNA in a way that can be naturally incorporated into the usual statistical-mechanics formalism in a way that is anal-

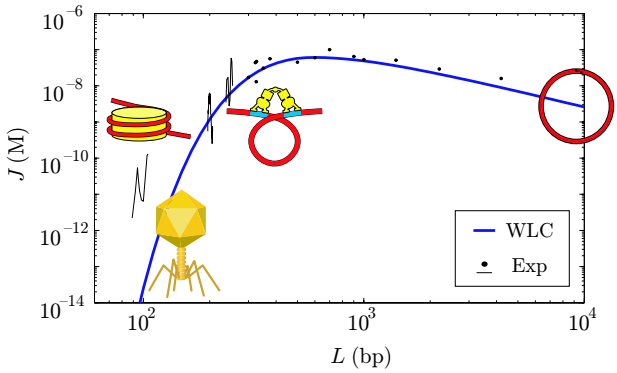

Figure 2: J-factors for cyclization of DNA molecules of different length. The new data of Cloutier and Widom presented in the present volume of PNAS, is seen as the oscillatory region around 100 basepairs (other data is from refs. [8, 10] and elsewhere). The icons show the length scale associated with the bending of DNA in particular biological examples such as DNA packing in bacteriophage ($\approx$ 100bp circles) and nucleosomes ($\approx$ 80bp loop), during transcriptional regulation ($\approx$ 400bp loop) and the lambda genome ($\approx$ 50,000bp loop).

ogous to the theoretical treatment of the DNA overstretching transition [21]. They show how the classic results—once taken as evidence for the naive elastic-rod model—merge seamlessly into the new results from cyclization and atomic force microscopy [7, 22].

The mechanics of DNA has long provided a common meeting ground for biologists, physicists, engineers, and mathematicians. The recent experiments of Cloutier and Widom reveal that after half a century of careful study, this resourceful molecule is still offering us new surprises, with new lessons for all of those disciplines.

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