New and Notable

Melting under Stress

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How does DNA fold into the compact structure of a chromosome? How do daughter strands separate after replication? How can one explain the action of proteins that bind to the DNA far away from their point of action? How can small local structural changes influence the global folding of the largest macromolecule in the living cell? The answers to those kinds of questions lie within the flexible polymer behavior of DNA. The properties of long DNA molecules have intrigued the biophysical community ever since the discovery of the double-helical structure of B-DNA 51 years ago (Watson and Crick, 1953), and although many of the biological functions of DNA could be explained from the x-ray structure, those which depend on the large-scale conformation still remain obscure to a great extent. Sucato and co-workers, in this issue, address one important consequence of DNA long-range folding: namely, how stress-induced local denaturation can change the global structure of a supercoiled DNA.

That long DNAs can fold into different types of conformation, even though they all share the B-helix structure, became evident in the early 1960s with the discovery of different forms of covalently closed circular DNA. Vinograd et al. (1965) showed that a DNA circle under internal torsional strain forms a compact ''supercoil'', distributing its elastic energy into writhe (winding of the helix axis around itself) and twist (the actual local under- or overwinding of the double helix). Nowadays, it is well established that negative torsional strain, directed against the sense of the B-helix, occurs in almost all native DNA as long as it is topologically constrained, so that the two ends of the DNA segment cannot freely rotate with respect to each other. This is not only true for circular DNA, but also for any DNA segments whose ends are anchored to some cellular structure. The global folding of the supercoil can have important biological implications, since it determines directly the interaction between distant DNA segments, as occurs in transcriptional regulation.

Torsional strain also can change the DNA structure locally. Transitions that decrease the number of B-helix turns, such as conversion to left-handed Z-form, cruciform extrusion, or denaturation, will be favored in negatively supercoiled DNA. A region that undergoes such a local transition will have different elastic properties. As an example, a denatured segment consisting of two DNA single strands will be very ''soft'' in regard to bending and local twisting. Such a soft spot may have dramatic effects on global supercoil folding, since it decreases the overall twist and creates a local bending hinge. Thus, long-range interactions in DNA may be strongly affected by local structural transitions. This has recently been confirmed experimentally for the case of DNA bending (Bussiek et al., 2002). A better theoretical understanding of this local-global structure coupling would significantly advance our insight into the biological implications of supercoiling.

In their contribution to this issue, Sucato and co-workers take an important step toward this goal. They use a computer model, in which the DNA is approximated as a flexible chain consisting of short rigid segments connected by twisting and bending springs. The validity of this approximation for the quantitative description of supercoil structure and thermodynamics hadalready been demonstrated by previous work, to a great extent from the Schurr laboratory. Denaturation is modeled by inserting into the chain one or two springs that are very soft in regard to bending and twisting. A Monte Carlo algorithm is then used to generate an equilibrium ensemble of supercoil conformations, from which average structural and thermodynamic quantities can be computed.

This study complements and interprets earlier experimental work by Bauer, Benham, and co-workers (1993, 1995), who used gel electrophoresis to analyze thermal denaturation of circular DNAs with different superhelix densities. One important earlier finding of Benham was that regulatory regions should be more sensitive to twist-induced denaturation, and that this effect could be used in the search for regulatory sites in sequence databases (Benham 1996). There, Benham had presented a thermodynamic theory of twist-induced denaturation, but the effect of the altered DNA mechanical properties on the structure had not been considered.

Sucato et al. (2004) not only present a model from which such effects can be determined, they also obtain in silico measurements of quantities such as the radius of gyration, fluctuations in writhe, and the temperature dependence of the supercoiling free energy. In the latter case, they show that the earlier results derived from gel electrophoretic data have to be reinterpreted to obtain the correct value.

To interpret their data, Bauer, Benham, and co-workers (1993, 1995) invoked a crucial assumption, specifically that the denatured region in a supercoil does not contribute significantly to the total writhe, predicting that the denaturation serves only to decrease the apparent linking number of the supercoil. A superhelix containing some turns of denatured helix would then show the same global structural properties, and run at the

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same position in a gel, as one without the denatured regions, but with a correspondingly lower superhelix density. Sucato et al. (2004) showed that this assumption, which is crucial for the gel electrophoretic analysis, but unproven so far, is a rather good approximation.

The authors then asked how the global folding changes upon denaturation. Surprisingly, the soft denatured region is most often found in positions where the interwound superhelix forms branches, unlike permanently curved regions, which tend to localize in the apex of the superhelix. It would be quite interesting to test this prediction experimentally.

All in all, the Monte Carlo analysis presented here advances our understanding of long-range DNA folding significantly and should prove its value especially in cases such as transcription initiation, where local denaturation accompanies the formation of the open complex.

The next step, of course, will be to model not just a soft spot in the DNA, but to make formation of this soft spot twist-dependent. This would couple the twisting energy with local denaturation. Hysteresis effects, as have already been predicted by Benham (1996), and the concomitant structural changes could then be directly observed in the Monte Carlo model. Further studies of the interaction of denaturation-induced structural changes with the folding of the rest of the DNA, and therefore, with the action of transcription factors, should provide substantial insights into the role of ''DNA mechanics'' in gene regulation.

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