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Bullied no more

— When DNA shoves proteins around —

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Most studies of protein-DNA interactions take a protein-centric perspective—giant proteins "bully" a static DNA polymer into a recognizable configuration [1]. The structure of the protein is considered the primary determinant in the interaction, and DNA is considered, by comparison, merely a passive substrate. There are likely several reasons for this view, but the most important reason, perhaps, is that static crystal structures, which are the most vivid and compelling pictures we have, contain only a short fragment of DNA. The mechanistic explanations for protein-DNA recognition, therefore, usually arise from the structure of the protein. But protein structure does not tell the whole story.

We propose that to understand protein-DNA interactions, a more holistic perspective must be taken. Protein-DNA interactions involve not just the protein, but also what we now know are incredibly dynamic DNA molecules, and the equally dynamic solvent molecules and counterions that surround them. Here we consider the ways that DNA topology can affect protein-DNA interactions, and focus, in particular, on the local, sequence-specific properties of DNA that do not occur when DNA is in the relaxed B-form as it is found in nearly all DNA crystal structures and is employed in the overwhelming majority of biophysical and biochemical studies of DNA structure and protein-DNA binding. DNA in cells is not inert like the linear B-form used in such experiments and it does not have naked ends. Instead, DNA in cells has topology, and topology affects: curvature, twist, kinking, base flipping, denaturation, and counterion concentrations, in addition to the likelihood that two DNA helices come together to form DNA juxtapositions.

1 Background

Until recently, the most common theoretical and computational models of DNA with sufficient length to consider topology have reduced the complexity of DNA by assuming it behaves as an isotropic elastic polymer [2]. Under small torsional deformations, the so-called worm-like chain models have been in good agreement with single molecule experiments [3], but the models break down over larger torsional deformations—ranges well within the biological realm. Because the models assume constant values for the effective diameter, persistence length, and charge density of DNA, they ignore local non-linear effects, and the differences between positive and negative supercoiling.

Although the worm-like chain models work well to explain average effects of long DNA molecules, as they do for single molecule manipulation experiments at low torsional forces [4], an average behavior under less than biologically relevant forces can obfuscate localized variations in behavior. DNA in the cellular milieu is looped, kinked, base-flipped, denatured, writhed,

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twisted, and has helices juxtaposed. It may even form Pauling-like DNA (P-DNA) under extreme overwinding, which is not unlikely during transcription and replication. Without any influence from proteins, these structural deformations create landmarks that promote protein-DNA interactions either through direct contact or through modulation of the electrostatics.

Until recently [5, 6, 7, 8], the worm-like chain models have neglected any localized, sequence-dependent, non-linear deformations and any potential asymmetric internal energies of DNA, that result from DNA bending and torsional stress and surely play a key role in protein-DNA interactions [9]. Thus, as useful as the worm-like chain models have been for understanding behavior of a long, unconstrained polymer without ions, water, or force, more detailed atomistic simulations are required to approach the broad, unanswered questions regarding protein-DNA recognition.

2 All atom molecular dynamics simulations of overwound and underwound DNA

To elucidate the localized, sequence-dependent, non-linear deformations and any potential asymmetric internal energies of DNA that result from DNA bending and torsional stress and surely play a key role in protein-DNA interactions, we studied the effects of torsional stress on DNA structure in the absence of writhe [10]. Using all atom molecular dynamics (MD), we simulated 19 systems of DNA helices with twist angles ranging from 25.714° to 49.091° in explicit solvent and 500 mM NaCl.

Contrary to the assumptions of elastic polymer models and in agreement with the findings of Harris *et al.* [11], we found significant differences in the dynamic structural response of DNA to underwinding and overwinding. The twist deficit caused by underwinding the helix was completely absorbed in the regions of base flipping and denaturation allowing the remainder of the helix to relax back to the torsionally relaxed B-form structure [10]. Consequently, the helix became partitioned into regions of localized structural failure and relaxed B-form. As a result, the average base pair step parameters, as well as the major groove width, minor groove width, and helical diameter, approach the B-form values predicted by NMR and X-ray crystallography data for this sequence [12, 13].

The structural response of DNA to overwinding was found to be quite different from that of underwound DNA [10]. For $0 < \sigma \leq 0.28$, DNA behaved like an elastic rod: twist increased linearly with increased σ, and other base pair step parameters varied with σ as well. Thus, all the previous conclusions and results using worm-like chain models are valid, but only for the regime of low to moderate overwinding. At $\sigma > 0.28$, a localized region of DNA showed a structural failure and transitioned into P-DNA and the rest of the DNA relaxed back to B-form. This bimodal distribution between utter structural failure and normal helix is just like what was seen for underwound DNA.

For overwound DNA, all atom MD simulations of twisted DNA showed that, up to the P-DNA threshold, overwinding DNA increases counterion condensation in the major and minor grooves. For underwound DNA, however, the structural deformations that relieve torsional stress—base flipping and denaturation—and allow the remaining helix to relax to B-form DNA, also reduce, on average, the effect of negative supercoiling on local counterion concentrations. Not surprisingly, counterion concentrations in those regions of an underwound helix that relaxed to B-DNA structure were similar to those of the $\sigma = 0.0$ helix. Although these trends describe the average relationship between counterion concentrations and σ, there was notable local variance in counterion concentrations in areas where the DNA structure failed. Such alterations in counterion concentrations could effectively increase the influence of changes in σ and, specifically,

the structural deformations of DNA in the cellular milieu. In regions of P-DNA structure, counterions were interspersed with the flipped out bases and concentrated near the intertwined backbones. Where bases flipped out or denatured in underwound helices, lower concentrations of counterions were found. This may be explained by the reduced negative charge density of the flattened helix in these regions and possibly by the range of motion of the extruded base.

A long-standing question in the study of protein-DNA interactions is how a protein finds its DNA binding sequence in a sea of competitor DNA sequence when the base composition information is buried inside the helix. Pauling and Corey [14] recognized that the outward orientation of the bases in his proposed structure permitted the bases "to interact vigorously with other molecules." In a similar manner, base flipping and denaturation caused by negative supercoiling provide a way to display sequence. Indeed, base flipping and denaturation were strictly sequence dependent in our study [10]. Base pairs located in regions of the sequence known to be rigid [12, 13] were more likely to exhibit base flipping and denaturation. The finding that base flipping was a feature in methyltransferase-DNA binding Klimasauskas1994,Reinisch1995 led Roberts to postulate that base flipping was an "ancient evolutionary discovery," and he predicted that it is much more prevalent in biology than previously thought [15]. DNA underwinding-mediated sequence-dependent base flipping may account for some protein recognition of DNA sequence just as underwinding-mediated DNA denaturation may occur at initiation sites for DNA transcription and replication.

3 Conclusions and Future Directions

In this article, we have focused on how the structural deformations of DNA in the cellular milieu might influence protein-DNA interactions. We reviewed how simulations of torsionally stressed DNA that can lead to juxtapositions, and may serve as loci for protein-DNA interactions. In the end, we presented five ideas:

1. Results of worm-like chain models are valid, but only for low to moderate overwinding.
2. Underwound and extremely overwound DNA partitions into regions of localized structural failure and relaxed B-form.
3. These structural deformations may occur naturally as consequences of DNA metabolism.
4. The influence of these structural deformations in the cellular milieu is extended by the effect of the deformations on counterion concentrations.
5. These structural deformations may play a role in sequence-specific protein recognition of DNA.

Moving beyond elastic polymer models finally permits studies that delve into the role of sequence in the stability of the helical structure under bending and torsional stress. MD simulations of underwound and overwound DNA have shown that some base pair steps are more rigid than others, leading to higher likelihoods of denaturation and base flipping. How biologically relevant sequences, like the TATA box, oriC, etc., respond to the torsional stress, could reveal new mechanisms for sequence-specific protein-DNA binding, specifically a much bigger role for DNA and a lesser role for proteins. Furthermore, we propose that modulation of counterion concentrations because of supercoiling, denaturation, base flipping, and P-DNA not only provides an additional exquisite potential mechanism to control DNA structure and geometry, but also provides means for proteins to find DNA and locate preferred sequences. So instead of the passive polymer being pushed around by the protein bullies, DNA pushes back to regulate its own biology.

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