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## A Unified Model for the Origin of DNA Sequence- Directed Curvature

**Abstract:** The fine structure of the DNA double helix and a number of its physical properties depend upon nucleotide sequence. This includes minor groove width, the propensity to undergo the B-form to A-form transition, sequence-directed curvature, and cation localization. Despite the multitude of studies conducted on DNA, it is still difficult to appreciate how these fundamental properties are linked to each other at the level of nucleotide sequence. We demonstrate that several sequence-dependent properties of DNA can be attributed, at least in part, to the sequence-specific localization of cations in the major and minor grooves. We also show that effects of cation localization on DNA structure are easier to understand if we divide all DNA sequences into three principal groups: A-tracts, G-tracts, and generic DNA. The A-tract group of sequences has a peculiar helical structure (i.e., B\*-form) with an unusually narrow minor groove and high base-pair propeller twist. Both experimental and theoretical studies have provided evidence that the B\*-form helical structure of A-tracts requires cations to be localized in the minor groove. G-tracts, on the other hand, have a propensity to undergo the B-form to A-form transition with increasing ionic strength. This property of G-tracts is directly connected to the observation that cations are preferentially localized in the major groove of G-tract sequences. Generic DNA, which represents the vast majority of DNA sequences, has a more balanced occupation of the major and minor grooves by cations than A-tracts or G-tracts and is thereby stabilized in the canonical B-form helix. Thus, DNA secondary structure can be viewed as a tug of war between the major and minor grooves for cations, with A-tracts and G-tracts each having one groove that dominates the other for cation

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localization. Finally, the sequence-directed curvature caused by A-tracts and G-tracts can, in both cases, be explained by the cation-dependent mismatch of A-tract and G-tract helical structures with the canonical B-form helix of generic DNA (i.e., a cation-dependent junction model). © 2003 Wiley Periodicals, Inc. Biopolymers 69: 144–159, 2003

**Keywords:** A-tract; G-tract; generic DNA; A-form; B\*-form; bending; cation localization; sequence specific; electrostatic collapse; junction model

## INTRODUCTION

It is well known that the structural and physical properties of duplex DNA depend upon nucleotide sequence. These properties include minor groove width,<sup>1,2</sup> the propensity to undergo the B- to A-form transition,<sup>3–6</sup> and cation localization within the major and minor grooves.<sup>7–18</sup> Additionally, certain sequences can cause bending in the helical axis of duplex DNA.<sup>19–22</sup> Sequences that cause axial bending are also among those that stand out as having extraordinary cation localization properties, as well as atypical helical structures. The tantalizing connection between cation localization and helical axis bending has drawn much attention, but the underlying physical principles that presumably unite these phenomena have remained controversial.<sup>23–25</sup> The purpose of this article is to present a model that clarifies the role of cation localization in the sequence-dependent helical structure and sequence-dependent curvature of DNA. DNA axial bending is a complicated molecular phenomenon that can include both static and dynamic components.<sup>26</sup> In the following discussion our use of the terms “axial bending” and “curvature” refers to any phenomena (static or dynamic) that produce a time average deviation in the DNA helical axis from linearity.

For many years models put forth to explain the origins of sequence-specific variations in DNA structure focused almost exclusively on base stacking interactions, groove hydration patterns, and the preferred geometries of A · T vs G · C base pairs (e.g., propeller twist).<sup>1,27–32</sup> More recently, it has been proposed that cation localization within the grooves could also be a significant factor in sequence-specific helical structure.<sup>10,11,13,23,33</sup> We have even gone as far as to propose that the DNA major and minor grooves can be viewed “flexible ionophores.”<sup>17</sup> That is, the grooves of DNA dynamically narrow in response to the entry and localization of cations. This model has substantial support from both experimental and theoretical studies that show a positive correlation to exist between groove width and cation localization.<sup>17</sup> Nevertheless, it is still difficult to distinguish cause and effect, and to infer the degree to which cation local-

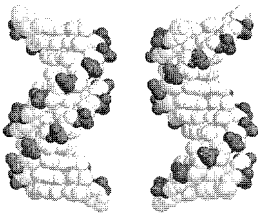
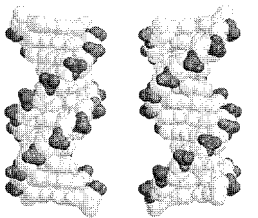
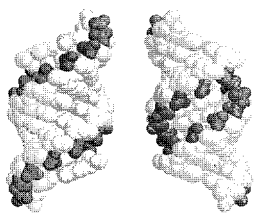
ization dictates helical structure, and moreover, sequence-directed curvature.

Here we demonstrate that by accepting the DNA grooves as flexible ionophores, and by dividing all possible DNA sequences into three principal groups, the sequence dependence of several DNA structural properties can be directly related to cation interactions. Specifically, the narrowness of the minor groove in A-tract DNA, DNA sequence-directed curvature, and in some cases, the sequence dependence of the B- to A-form transition, can be related to each other through what we now know about sequence-specific cation localization. In our model DNA helical structure is modulated by a *tug of war* between the two grooves for cation localization. Sequences with one groove that dominates the other for preferential cation localization tend to assume a helical structure other than the canonical B-form (e.g., B\*-form or A-form). Axial bending (i.e., sequence-directed curvature) is connected to sequence-specific cation localization by a model that is best described as the “cation-dependent junction model.” While these models do not prove unequivocally that cation localization is the main cause of all sequence-specific phenomena, we argue that the excellent agreement between our models and a wide variety of experimental observations demonstrates that the sequence-specific localization of cations can no longer be questioned in regard to its importance to DNA secondary structure, and may eventually prove to be the single most significant driving force of several sequence-specific phenomena.

## DEFINING THE THREE PRINCIPAL SEQUENCE MOTIFS OF DUPLEX DNA

For the present discussion of DNA structure we divide all nucleotide sequences into three groups: A-tracts, G-tracts, and generic DNA. Assignment of a particular DNA sequence element to one of these three groups depends upon both the known helical structure of the sequence in solution (or its propensity to assume a helical structure other than the canonical B-form) and the nature of cation localization within the

**Table I** Selected Physical Properties of the Three Principal DNA Sequence Motifs

A-tracts	Generic DNA	G-tracts
B*-Form	Helical Form Canonical B-Form	<i>propensity to assume A-Form</i>
		
minor groove	minor groove	minor groove
major groove	major groove	major groove
	Groove Width <sup>a</sup>	
Minor groove: narrow (2.8 Å) Major groove: wide (13.9 Å)	Minor groove: medium (5.9 Å) Major groove: medium (11.2 Å)	Minor groove: wide (11.2 Å) Major groove: narrow (2.4 Å)
	Sequence Restrictions	
At least four consecutive AA, TT or AT base-steps, but no TA base-steps. Example: Poly(dA)·Poly(dT)	Sequences containing both A·T and G·C base pairs. Any sequences not considered A-tracts or G-tracts. Example: The vast majority of genomic DNA sequences.	Sequences containing only G·C base pairs, and rich in GG base-steps. Example: Poly(dG)·Poly(dC)
	Cation Localization	
Minor groove: Monovalent cations are localized deep in the groove near the O2 atoms of thymines. Hydrated divalent cations are localized on top of the groove, bridging phosphate groups. Major groove: Not detected	Minor groove: Monovalent and divalent cations enter the groove as well as localize near the phosphates above the groove. Localization is favored at A·T base pairs.  Major groove: Cations localize near the N7 of guanine at GG, GA and GT base-steps.	Minor groove: Not detected.  Major groove: Cations localized near the N7 of guanine at GG base-steps, in both B-form and A-form, and presumably in all intermediate forms.

<sup>a</sup> Groove widths are reported as the distance between pairs of phosphorus atoms across the grooves from which 5.8Å was subtracted to account for the sum of van der Waals radii of the two phosphate groups.

major and minor grooves of the sequence. Below we present a description of the sequence requirements and properties of each group. A point-by-point comparison of some important and distinguishing features of the three sequence groups is also presented in Table I.

### A-Tracts Defined

Sequences within the A-tract group are at least four base pairs in length, containing only AA (equivalently

TT) or AT base-steps, and no TA base-steps (5′–3′ sequence notation is implied throughout). For example, AAAAA, AATTT, and AAAAT are all considered to be A-tract sequences of five base pairs in length. The sequence element TTAAA, however, is not an A-tract because it contains a TA base-step. The most peculiar and intensely studied feature of A-tract sequences is their propensity to cause helical axis bending when incorporated into otherwise *non*-A-tract DNA (i.e., generic or G-tract).<sup>34</sup> An axial bend of around 13°–18° can be produced by a single A-

tract.<sup>35,36</sup> It is from comprehensive studies of sequence-directed curvature by A-tracts that we have such a precise definition for sequences that belong in this group.<sup>19,22</sup>

In the present discussion, A-tracts also include the homopolymer duplex poly(dA) · poly(dT), whereas in some contexts a length limit is added to the definition of an A-tract. Poly(dA) · poly(dT) is a useful member of this group because of the large number of physical studies that have characterized this duplex, including fiber diffraction studies.<sup>37–39</sup> The helical structure of A-tract DNA is often referred to as the B\*-form because it is of the B-form family, yet it is distinct from that of the canonical B-form. Characteristic features of the B\*-form helix include an unusually narrow minor groove and high base propeller twist.<sup>29,38,39</sup> The preference of A-tracts for the B\*-form helix fundamentally sets these sequences apart from generic DNA.

Another distinctive property of A-tract DNA is the nature of cation association with these sequences. Over the past several years both experimental and theoretical studies have revealed that monovalent cations are localized in the minor groove of A-tracts, both deep in the groove and near the top of the groove,<sup>9,11,13,14,40</sup> and this localization is clearly favored with respect to localization in the minor groove of *non*-A-tract sequences.<sup>13,15,17,41</sup> Divalent cations also appear to preferentially associate with the A-tract minor groove, but in this case localization is restricted to the top of the groove, where fully (or partially) hydrated divalent cations straddle the narrow A-tract minor groove and are bound between phosphate groups on opposite sides of the groove.<sup>40,42</sup> It is important to emphasize that evidence also exists for a *lack* of cation localization in the major grooves of A-tracts,<sup>16–18</sup> which is a characteristic that also distinguishes A-tracts from our two other classes of DNA sequences. The preferential localization of cations in the minor groove of A-tracts can, in the most simple terms, be attributed to the electronegative adenine N2 and thymine O2 atoms than line the A-tract minor groove, and the lack of electropositive amino protons, that are present in minor groove of GC sequences.<sup>9,43</sup> Similarly, the lack of cation localization in the A-tract major groove can be principally attributed to the electropositive amino proton of adenine that is located near the center of the major groove at every A · T base pair.

## G-Tracts Defined

The group of sequences we will define as G-tracts are in several ways the antitheses of A-tracts. G-tracts are

homo-GC in base composition and particularly rich in GG base-steps (equivalently CC base-steps). G-tracts localize cations in the major groove, but not in the minor groove. G-tracts have a propensity to undergo the B- to A-form structural transition *in solution*, which is induced by increased cation association or reduced water activity.<sup>44–50</sup> Similar to A-tracts, a G-tract placed between two sections of *non*-G-tract DNA causes a bend in the helical axis of the DNA duplex. However, this bending apparently requires divalent or multivalent cations, whereas axial bending by A-tracts does not.<sup>21,51</sup> We note that the term “G-tract” has been used on occasion by others to describe various GC-rich sequences. Our definition is not necessarily the same as these, and is likely more restrictive.

We have assigned several characteristics to G-tract DNA, yet specific sequence rules for G-tract membership are not as well defined as they are for A-tracts. This relaxed definition is primarily due to the less-developed state of knowledge on G-tracts in comparison to A-tracts. The following are a few example sequences that experiments have shown to possess one or more properties required for membership in the G-tract group: GGGCCC, GGGCC, GGCC, CCCC-GGGG, CCCGCGGG, and poly(dG) · poly(dC). Collectively, these closely related sequences exhibit all the required properties of G-tracts, and we predict that subsequent experiments will demonstrate that each of these and additional sequences possess all our requisite G-tract properties. The homopolymer duplex is again a valuable member of this group owing to the large number of structural and physical studies that have utilized this duplex. The sequence GGGCCC, and its truncated subsequences GGGCC and GGCC, have been shown to cause axial bending in DNA polymers in the presence of MgCl<sub>2</sub>.<sup>21,51,52</sup> The sequences CCCCGGGG, CCCGCGGG, and poly(dG) · poly(dC) have demonstrated a propensity to undergo the B- to A-form helical transition in solution.<sup>45–47,49,50</sup> In the case of duplex poly(dG) · poly(dC), conversion to the A-form has been shown to occur at 1M NaCl,<sup>53</sup> whereas micromolar concentrations of Co(NH<sub>4</sub>)<sub>6</sub><sup>3+</sup> have been shown to convert the duplexes d(CC-CCGGGG) and d(CCCGCGGG) to the A-form helix.<sup>49,50,54</sup> We note that Subirana and co-workers recently found that in the crystal state d(CCCGCGGG) adopts the A-form helix *and* causes substantial helical axis bending when flanked by B-form regions.<sup>55</sup> A substantial number of other GC-rich sequences also preferentially crystallize in the A-form helix,<sup>56,57</sup> and such sequences may eventually be shown to satisfy our definition of G-tracts. However, at present we restrict the G-tract group to those sequences for which

either the B- to A-form transition or helical axis bending has already been demonstrated to occur in solution.

Our stipulation that G-tract sequences localize divalent cations in the major groove is motivated by the observation that cation localization in this groove promotes the B- to A-form transition,<sup>49,50,54</sup> and could thereby cause axial bending.<sup>33</sup> The relationship between cation localization, the B- to A-form transition and axial bending by G-tracts is discussed in detail below. At this point we simply state that G-tract sequences favor cation localization in the major groove, which places substantial restrictions on possible G-tract sequences. The sequence dependence of divalent cation localization in the DNA grooves has been characterized in solution by NMR spectroscopy studies of  $Mn^{2+}$  localization along duplex oligonucleotides of various sequences.<sup>7,8,18</sup> It has been shown that major groove cation localization requires a base-step of the form GN (N being any of the four nucleotides). Furthermore, the relative propensity of these four base-steps to localize divalent cations in duplex DNA has been determined to be  $GG \geq GA \geq GT \gg GC$ .<sup>7,8</sup> The condensation of cations onto DNA duplexes that necessarily occurs during crystallization makes it difficult to determine the sequence-dependent cation association rules for the solution state based upon x-ray crystal structures. For example, hydrated divalent cations are almost always found in the major groove of the sequence element CGCG in crystal structures of the Drew–Dickerson dodecamer, d(CGCGAATTCGCG),<sup>16</sup> yet the base-steps CG and GC are not a favored site for divalent cation localization in solution.<sup>7</sup> Thus, we stipulate that G-tract sequences must be rich in GG base-steps (equivalently CC base-steps) to maximize major groove cation localization *in solution*. This is not to say that GC or CG base-steps cannot be tolerated at all within a G-tract, just as a single AT base-step can be tolerated within an A-tract. An important consequence of our provisional sequence definition for this group is that G-tracts are potentially the most unfavorable sequences for minor groove cation localization, as their minor grooves are lined with the relatively electropositive protons of the guanine amino group, making the G-tract minor groove the antithesis of the A-tract minor groove.

### Generic DNA Defined

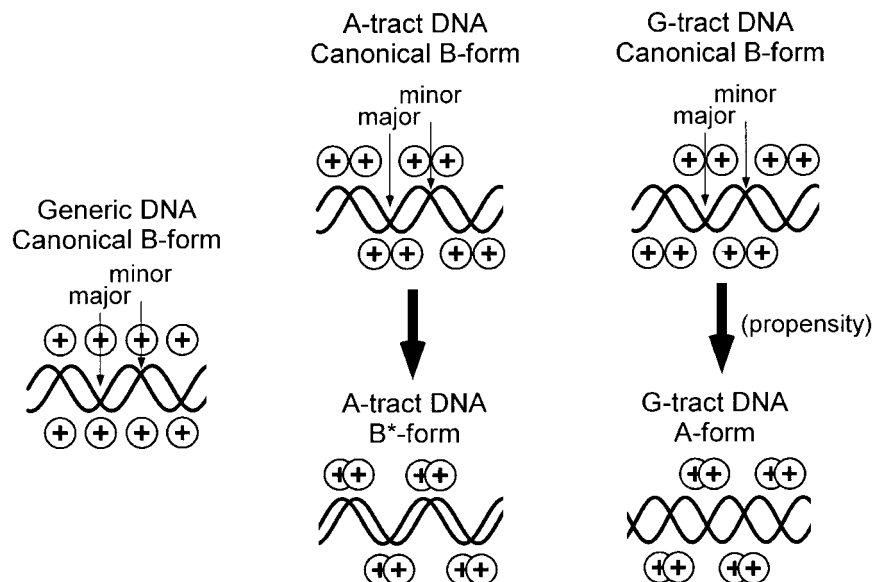
Generic DNA represents the vast majority of all possible DNA sequences. Most generic DNA contains of a mixture of A · T and G · C base pairs. A generic DNA sequence element can be of any length, with the

restriction that no subsequence within a given generic DNA sequence can be defined as either an A-tract or a G-tract. Generic DNA, by definition, also includes any sequences that are not included within the definitions given above for A-tract or G-tract sequences, including many homo-AT and homo-GC sequences (e.g., ATAT and GCGC). The structure of generic DNA is well represented by the canonical B-form helix under most solution conditions (e.g. neutral pH, moderate ionic strength). For generic DNA sequences the degree of cation localization in the major and minor grooves varies along the helix in response to local variations in base composition and sequence. However, in the context of the present discussion, cations can be viewed as transiently occupying both the major and minor grooves of generic DNA with a more or less equal preference (Table I).

### DNA SECONDARY STRUCTURE, A TUG OF WAR BETWEEN THE GROOVES FOR CATION LOCALIZATION

The identification of cation localization sites in minor groove of A-tracts inspired the proposal that the narrowness of the B\*-form minor groove is a result of electrostatic attractions between cations in the groove and phosphates groups on both sides of the groove.<sup>10–13</sup> The proposal of groove narrowing due to cation localization is related to the more general proposal that DNA structure can be modulated by a local screening of phosphate repulsions, which has been referred to as the “electrostatic collapse” model.<sup>23,58,59</sup> The B- to A-form transition of G-tract DNA in solution has also been proposed to be a “narrowing” of the major groove in response to cation localization.<sup>33,54</sup> In this case, major groove narrowing more precisely refers to the decreased phosphate-phosphate distances across the major groove that are associated with the B- to A-form transition.

This parallel between A-tracts and G-tracts is quite satisfying. A-tracts localize cations in the minor groove and adopt a helix with an unusually narrow minor groove. G-tracts localize cations in the major groove and adopt a helical form with an unusually narrow major groove. Thus, the observation that A-tracts represent B\*-form-philic sequences and G-tracts represent A-form-philic sequences<sup>3,4</sup> could be due to their respective localization of cations in one particular groove. However, there is an additional parallel between A-tracts and G-tracts that also correlates with observed helical preferences. A-tract major grooves are apparently the most unfavorable major



**FIGURE 1** A schematic representation of the tug of war model for DNA secondary structure. Left: Generic DNA sequences share cation localization in the major and minor grooves in a manner that stabilizes the canonical B-form helix. Middle: A-tract sequences localize cations more favorably in the minor groove *and* less favorably in the major groove, in comparison to generic DNA sequences. Cations localized in the minor groove pull phosphates groups closer together, with little resistance from cations in the major groove. The resulting B\*-form helix has a narrower minor groove with enhanced cation localization, while the B\*-form major groove is slightly widened and even less able to localize cations. Right: G-tract sequences localize cations more favorably in the major groove *and* less favorably in the minor groove, in comparison to generic DNA sequences. These differences in cation localization give G-tract DNA a greater propensity to adopt the A-form helix than generic DNA. Upon transition to the A-form, the G-tract major groove has an even greater ability to localize cations, while the minor groove is even less able to localize cations.

grooves for cation localization, as are the minor groove of G-tracts.<sup>17</sup> Here we propose that in considering the effects of localized cations on DNA secondary structure, the *differential* localization of cations between the two grooves of a given sequence may be even more important than the absolute degree of cation localization within one particular groove. In other words, we propose that local helical structure of duplex DNA is largely the result of a tug of war between the major and minor grooves for cation localization (Figure 1). In this model, A-tracts adopt the B\*-form helix because the A-tract minor groove dominates the A-tract major groove for cation localization. Similarly, G-tracts are A-form-philic because the G-tract major groove dominates the G-tract minor groove (under certain conditions) for cation localization.

The tug of war model for DNA helix structure predicts that the “winning” groove will enjoy enhanced cation localization as the result of a structural transition away from the canonical B-form, whereas the “loosing” groove may become even less able to localize cations (Figure 1). A relatively simple way to

test the validity of this prediction is to compare the electrostatic surface potentials (ESP) of the major and minor grooves for an A-tract and a G-tract in different helical structures. Such calculations were perhaps the first indication that cation localization would prove to be sequence specific.<sup>43</sup> Results obtained from ESP calculations we have carried out for the A-tract duplex  $d(A_{12}) \cdot d(T_{12})$  and the G-tract duplex  $d(G_{12}) \cdot d(C_{12})$  are presented in Table II, and graphically in Figure 2. These calculations illustrate several points in support of the tug of war model. Upon conversion from the canonical B-form to the B\*-form helix, the average ESP of the A-tract minor groove decreases (i.e., becomes more electronegative), while the average ESP of the A-tract major groove is virtually unchanged. Upon conversion from the canonical B-form to the A-form helix, the average ESP of the G-tract major groove decreases substantially, while the average ESP of the G-tract minor groove increases (i.e., becomes more electropositive). Thus, ESP calculations support the basic premise of the tug of war model that the transition of an A-tract from the canonical B-form to

**Table II** Electrostatic Surface Potentials<sup>a</sup> in the Grooves of A-Tract and G-Tract DNA

Duplex Sequence	Helical Type	Groove	Electrostatic Surface Potential (kT/e)		
			Average	Minimum <sup>b</sup>	Maximum
d(A <sub>12</sub> ) · d(T <sub>12</sub> )	B* <sup>c</sup>	Minor	−7.0	−27.5	0.4
		Major	−3.3	−31.6	22.4
	B	Minor	−5.7	−28.1	1.4
		Major	−3.2	−16.9	10.0
d(G <sub>12</sub> ) · d(C <sub>12</sub> )	A	Minor	−2.9	−28.7	21.0
		Major	−5.7	−24.6	9.6
	B	Minor	−4.8	−24.7	12.4
		Major	−3.4	−19.0	12.8

<sup>a</sup> Electrostatic potentials were obtained by nonlinear Poisson–Boltzmann calculations using atomic charges and van der Waals parameters from the Kollman group,<sup>99</sup> with the Delphi program (ver. 3.0) as implemented in Insight II (Accelrys).

<sup>b</sup> Absolute minimum and maximum values of individual points in a grid were found in the range from −434 to 646 kT/e.

<sup>c</sup> Atomic coordinates were obtained from a fiber diffraction model of poly(dA)·poly(dT).<sup>39</sup>

the B\*-form further enhances differential cation localization in favor of the minor groove, and the transition of a G-tract from the canonical B-form to the A-form further enhances differential cation localization in favor of the major groove (Figure 1). While these calculations do show a trend in ESP that would be expected to accompany cation-induced structural transitions, we emphasize that results from ESP calculations are not definitive indicators of cation localization. The precise placement of chelating groups on the bases, which is highly sequence and helical-form specific, must also be taken into account in relating ESP to cation localization.<sup>60</sup>

The correlation observed between cation localization within A-tracts and the narrow minor groove of A-tracts is not an open-and-shut case of cause and effect. This correlation is still open to the question: “Which comes first, cation localization or a narrow groove?”<sup>17</sup> An alternative to the electrostatic collapse model is to accept that the unusually narrow minor groove of the B\*-form helix is an intrinsic property of A-tract sequences that does not require cation localization. In this case, cation localization by A-tracts must then be seen as merely a result of a preexisting unusually electronegative minor groove within the A-tract. Such a model for the B\*-form helix is more in line with traditional models for sequence-dependent DNA structure, which have focused on short-range base–base interactions.<sup>1,28–32</sup> Whether or not cation localization in the minor groove is necessary for the B\*-form helix of A-tracts has recently been addressed by molecular dynamics (MD) simulations of DNA with explicit water and cations.<sup>25,61,62</sup> Wilson and co-workers have presented results from two different simulations that support cation localization as the

origin of groove narrowing in A-tracts.<sup>61,62</sup> In their first study the width of the minor groove over an A-tract sequence element was monitored as a function of groove occupancy by monovalent cations.<sup>61</sup> These investigators found that if a cation is localized either deep in the minor groove or near the top (i.e., bridging phosphate groups across the groove), the minor groove was narrower than that of canonical B-form DNA. However, if a cation was not localized at either of these positions, the minor groove was actually wider than that of canonical B-form DNA. A second study by the same group investigated the effects of neutralizing phosphate charges (i.e., *in silico* chemical modification) across the minor groove of a DNA sequence element that normally has a relatively wide minor groove (i.e., a *non*-A-tract).<sup>62</sup> MD simulations of this modified duplex showed a substantial narrowing of the minor groove with respect to the unmodified duplex. This was taken as additional evidence that the minor groove of B-form DNA will narrow to that of B\*-form DNA if phosphate repulsions are screened by localized cations (or chemical modifications), and that the minor groove principally remains wider than that of B\*-form in *non*-A-tract DNA due to the lack of localized cations in the minor groove. The ultimate conclusion of Wilson and co-workers is that if one controls for the effects of cations, then there is no effect of sequence on groove width (i.e., differences in groove width arise solely from sequence-specific differences in cation distributions). It should be noted that McConnell and Beveridge reached the opposite conclusion, that cation distributions are not related to or correlated with groove narrowing,<sup>25</sup> based upon a nearly identical simulation of the same (unmodified) DNA duplex studied by Wilson and



co-workers. This apparent discrepancy may arise from differences in definitions of free and groove-localized cations applied by the two groups. McConnell and Beveridge restricted their definition of groove-localized cations to those near the floor of the groove, whereas Wilson and co-workers used a more expansive definition that also includes cations higher up from the floor of the minor groove and bridging phosphates across the groove.

The solution state and theoretical studies discussed above that show a direct correlation between cation localization and helical structure (i.e., B\*-form, A-form) are perfectly supportive of the tug of war model for G-tracts and A-tracts. Results obtained from fiber diffraction studies provide additional, though perhaps more subtle, support for this model. It is well documented that DNA derived from natural sources, which is predominantly of generic DNA sequence, will undergo the B- to A-form transition under mildly dehydrating conditions (i.e., 75% relative humidity or below, depending on counterions).<sup>63</sup> Poly(dG) · poly(dC), possibly the most A-form-philic duplex,<sup>3,6</sup> is locked in the A-form helix in the fiber state at even 100% relative humidity.<sup>64</sup> Poly(dA) · poly(dT), on the other hand, resists adopting the A-form helix even at very low relative humidity.<sup>65</sup> This difference in the behavior of poly(dA) · poly(dT) and poly(dG) · poly(dC) is consistent with our tug of war model, since cations are localized in the minor groove *and* lacking in major groove of A-tracts, which would make A-tract DNA extremely resistant to opening the minor groove and closing the major groove (necessary for the B- to A-form transition). Further support for this model is provided by the fact that poly(dI) · poly(dC) also resists adopting the A-form helix in fibers at low relative humidity.<sup>65</sup> This observation is particularly interesting because this homopolymer duplex has the major groove of poly(dG) · poly(dC) and the minor groove of poly(dA) · poly(dT). Thus, in the tug of war model for the B- to A-form transition, the resistance of poly(dI) · poly(dC) to adopting the A-form helix is ascribed to the localization of cations in the minor groove, since poly(dI) · poly(dC) should also localize cations in the major groove.

The stark contrast between the propensities of A-tracts and G-tracts to undergo the B- to A-form transition, and the fact that poly(dI) · poly(dC) resists this transition, brings up the question of how much the B- to A-form transition is driven by cation localization in the major groove vs a *lack* of cation localization in the minor groove. Data relevant to this question has recently appeared as a result of a study by McLaughlin and co-workers of DNA duplexes containing structural analogs of A · T base pairs in which the elec-

tronegative atoms N3 of adenine and O2 of thymine were replaced by nonpolar carbon atoms (i.e., 3-deazaadenine · 3-methyl-2-pyridone base pairs).<sup>66</sup> The N3 of adenine and O2 of thymine are normally part of the minor groove floor, and these atoms have been suggested to be an essential component of cation localization in the minor groove.<sup>9,61</sup> McLaughlin and co-workers found that a DNA duplex containing four tandem substitutions of their unnatural A · T analogues produced a helix that is more A-like than B-like.<sup>66</sup> This observation was attributed to changes in groove hydration. However, in the context of the tug of war model, this reported change in helical structure could also be ascribed to changes in cation localization within the minor groove, implying that cations in the minor groove are an important factor in keeping B-form DNA from converting to the A-form helix.

The adoption of the canonical B-form by generic DNA sequences can also be rationalized in terms of the tug of war model. Generic DNA sequences will have a more even distribution of cations between the two grooves than either A-tracts or G-tracts (Table I). Thus, according to our model, the secondary structure of generic DNA sequences should also fall between that of B\*-form and A-form. With cations sharing both grooves more evenly, the favored helical structure of generic DNA should have a wider minor groove than B\*-form, as it does in the canonical B-form. Additionally, generic DNA sequences should be more prone to undergoing the B- to A-form transition than poly(dA) · poly(dT), but not as prone as poly(dG) · poly(dC), which is also the case. Of course local sequence-specific variations in helix structure will occur in generic DNA for sequences with varying degrees of cation localization. However, these variations (e.g., variations in groove width) are in most cases so subtle that ultrahigh resolution crystal structures are required to discern them from the canonical B-form helix.<sup>17,40,67</sup>

## CONNECTING CATION LOCALIZATION TO SEQUENCE-DIRECTED CURVATURE

We first discuss the role of cation localization in the origin of G-tract sequence-directed curvature because, from our perspective, this is easier to appreciate for G-tracts than for A-tracts. Thus far, G-tract-induced helical axis bending has been characterized for only a few GC-rich sequences, and most extensively for GGGCCC. Gel electrophoresis mobility studies of DNA polymers with GGGCCC flanked by generic

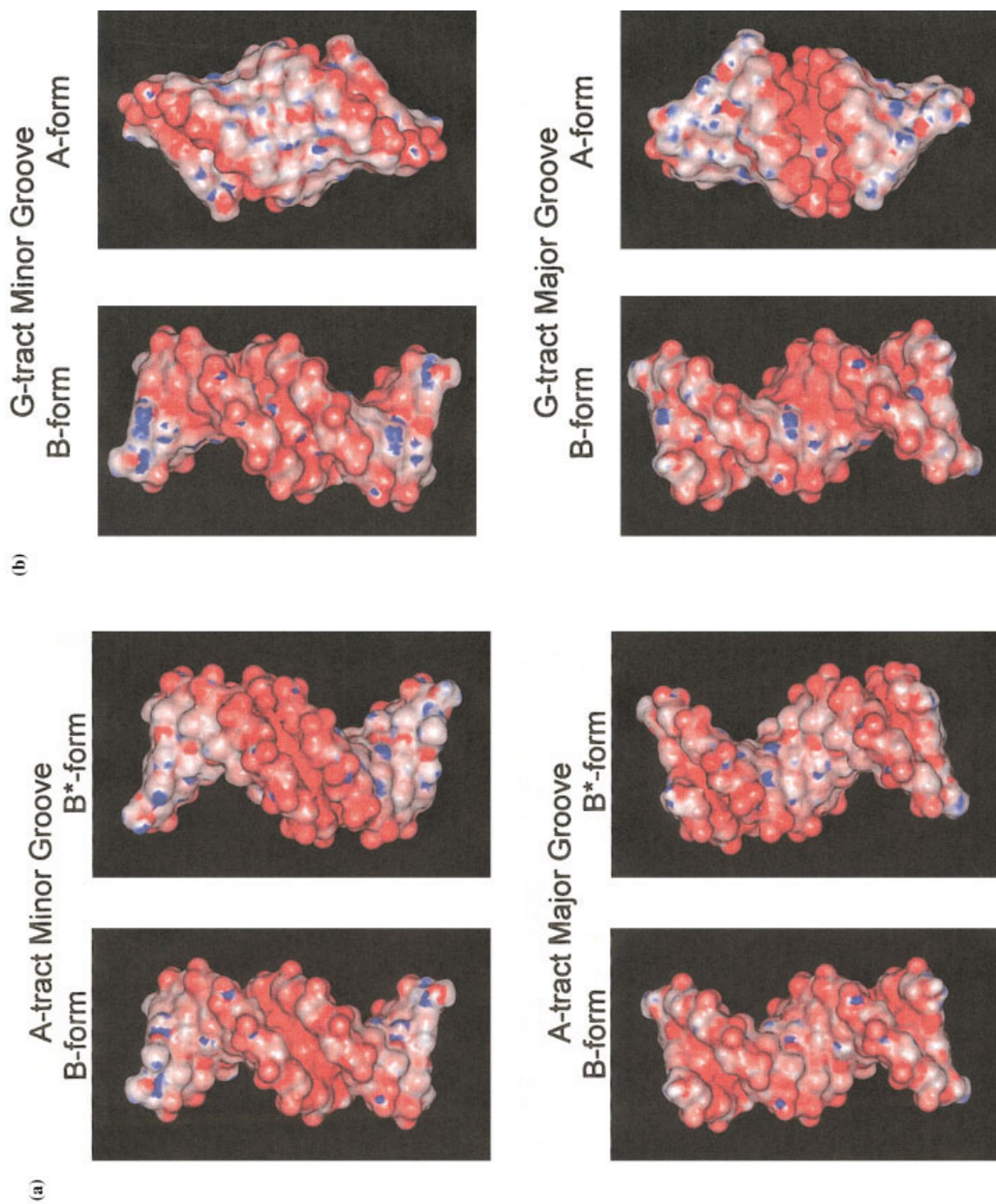


FIGURE 2

DNA sequences have shown that the amount of bend caused by this G-tract increases with divalent cation concentration (for both  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$ ).<sup>21,68</sup> The helical axis bending induced by this same G-tract was determined to be toward the major groove, with the bend center located at the center of the sequence element.<sup>21</sup> Based upon the NMR studies discussed above, GGGCCC preferentially localizes cations in the major groove, and this has been connected to the propensity for similar sequences to undergo the B- to A-form transition.<sup>49,50,54</sup> Thus, sequence-directed curvature can be connected to cation localization, if we can connect sequence-directed curvature to the B- to A-form transition.

A rigorous theoretical analysis of DNA–cation interactions by Rouzina and Bloomfield investigated the potential for highly mobile cations to cause helical axis bending.<sup>33</sup> A principal conclusion of this study was that divalent cations localized in the major groove cause the phosphate groups on both sides of the groove to move towards these cations, thereby narrowing the width of the major groove. This was considered consistent with a purely electrostatic model for cation-induced helical axis bending in which the DNA helix bends toward localized cations due to an asymmetric screening of phosphate repulsions (discussed below). Rouzina and Bloomfield went further to suggest that the closing of the major groove around localization cations could also be viewed as a B- to A-form transition. This proposal provided a plausible connection between cation localization in G-tracts, sequence-directed curvature by G-tracts, and the B- to A-form transition.

Two decades ago Wells and co-workers first proposed that helical axis bending would occur at the junction between an A-form and a B-form section of a contiguous DNA duplex.<sup>69</sup> Based upon computer modeling studies, a bend of  $26^\circ$  was predicted to occur at this junction. Olson and co-workers have also constructed a series of models with a section of A-form DNA flanked on both sides by B-form helices that reveal axial bending at both B–A junctions and bending in the overall helical axis.<sup>70</sup> The junction model can be viewed more generally as a model that illustrates how axial bending of a DNA duplex can

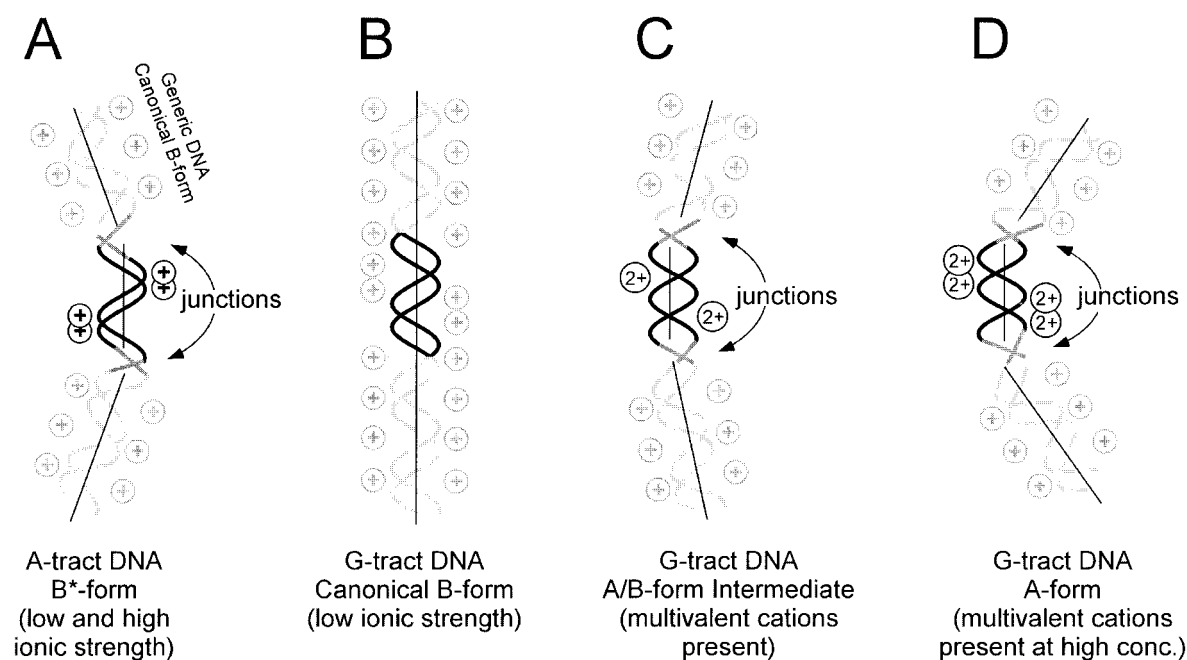
occur at any place where there is a junction between the canonical B-form and another helical form. Furthermore, the degree of bending should be proportional to the extent to which this localized helical structure deviates, by some measure, from the canonical B-form.

Recent analyses of numerous DNA x-ray crystal structures have shown that the B-form helix and the A-form helix are connected by a continuous series of intermediate helical structures,<sup>57,70</sup> and the energy potential across this structural continuum appears to be relatively flat.<sup>71</sup> Putting these observations together with the junction models of Wells and Olson for DNA bending, and the fact that cations can promote the A-form transition in G-tracts, we propose that the cation-induced helical axis bending reported for the G-tract GGGCCC<sup>21,68</sup> is a direct result of this G-tract progressing across the B- to A-form structural continuum with increasing ionic strength, while the helix structure of the flanking generic DNA sequences are relatively unaffected by cation concentration (Figure 3). Thus, the junction model can connect G-tract sequence-directed curvature to sequence-specific cation localization through a cation-dependent B- to A-form transition.

The relationship between helical axis bending by A-tracts and sequence-specific cation localization has proven more difficult to establish. Early in the study of A-tract sequence-directed curvature, Crothers and co-workers proposed that helical axis bending occurred at the junctions between the B\*-form helix of A-tracts and the canonical B-form helix of flanking non-A-tract sequences.<sup>19</sup> This proposal was essentially the junction model of Wells, except that the helical form creating a junction with the canonical B-form was the B\*-form rather than the A-form. Crothers and co-workers later demonstrated that the bend center of an individual A-tract is located near the center of this sequence element, with the direction of the bend being toward the minor groove of the A-tract.<sup>72</sup> These results did *not* necessarily imply that the helical axis within an A-tract is bent. The actual bend could be the combined effect of two approximately equal bends located at the junctions between the A-

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**FIGURE 2** Graphical representations of electrostatic surface potentials (ESP) calculated at the solvent accessible surface<sup>100</sup> of model A-tract and G-tract DNA duplexes, each in two helical forms. The A-tract model is the duplex  $(\text{dA})_{12} \cdot (\text{dT})_{12}$ . The G-tract model is the duplex  $(\text{dG})_{12} \cdot (\text{dC})_{12}$ . Major groove and minor groove views, for the same models, are related to each other by a  $180^\circ$  rotation about the helical axis. Colors on the DNA surfaces proceed from red,  $-8 \text{ kT/e}$ , to blue,  $+3 \text{ kT/e}$ , with increasing electrostatic potential. Additional information concerning the ESP calculations is presented in Table II.



**FIGURE 3** Schematic representations of the cation-dependent junction model for the origin of sequence-directed curvature by A-tracts and G-tracts. (A) An A-tract sequence element is stabilized in the B\*-form helix, at low and high ionic strength, by cations localized in the minor groove. The helical mismatch between the B\*-form helix and the canonical B-form helix of the flanking generic DNA causes a bend in the overall helical axis according to the junction model.<sup>19</sup> (B) In the presence of monovalent cations at low ionic strength, a G-tract sequence element assumes the canonical B-form helix. There is no significant difference between the helical structure of the G-tract and the flanking generic DNA. Thus, no bend is produced in the overall helical axis. (C, D) In the presence of divalent cations the helical structure of a G-tract progresses from the B-form to the A-form across the A/B-form structural continuum. Higher divalent cation concentrations cause greater deviation of the G-tract helix towards the A-form. Helical axis bending by the G-tract is also produced according to the junction model.<sup>69,70</sup> DNA segments containing A-tract and G-tract sequence elements are drawn in black; generic DNA sequences are drawn in gray. Cations drawn in black represent those responsible for stabilizing a DNA helical structure other than the canonical B-form; gray cations represent those associated with canonical B-form DNA.

tract (i.e., B\*-form) and the flanking generic DNA (i.e., canonical B-form) sequence elements (Figure 3).

Crystal structures of DNA oligonucleotides containing A-tract sequences caused a controversy to erupt over the origin of bending by A-tracts, as these structures revealed A-tract segments to have a straight helical axis.<sup>29,73–75</sup> This observation was not inconsistent with the junction model, which only requires that there be a difference in the helical parameters of A-tracts and generic DNA. Unfortunately, the extent to which axial bending is caused by the helical mismatch between B\*-form and canonical B-form DNA is not easy to appreciate in crystal structures, because the direction of helical axes for the flanking canonical B-form regions are not obvious due to their short length of only two to four base pairs in most A-tract crystal structures. Thus, the origin of A-tract bending was overlooked (or misin-

terpreted) in crystal structures for almost two decades. Meanwhile, several proposals were put forth to explain why the crystal structures were “in disagreement” with gel electrophoresis studies of A-tract-induced bending. There were two principal camps formed around this debate. One camp proposed that the act of crystallization somehow removed the bend in A-tracts,<sup>76,77</sup> while the other camp held that A-tracts are always straight (in solution and in crystals) and that observed axial bending is caused by the intrinsic bends of intervening sequences.<sup>78</sup>

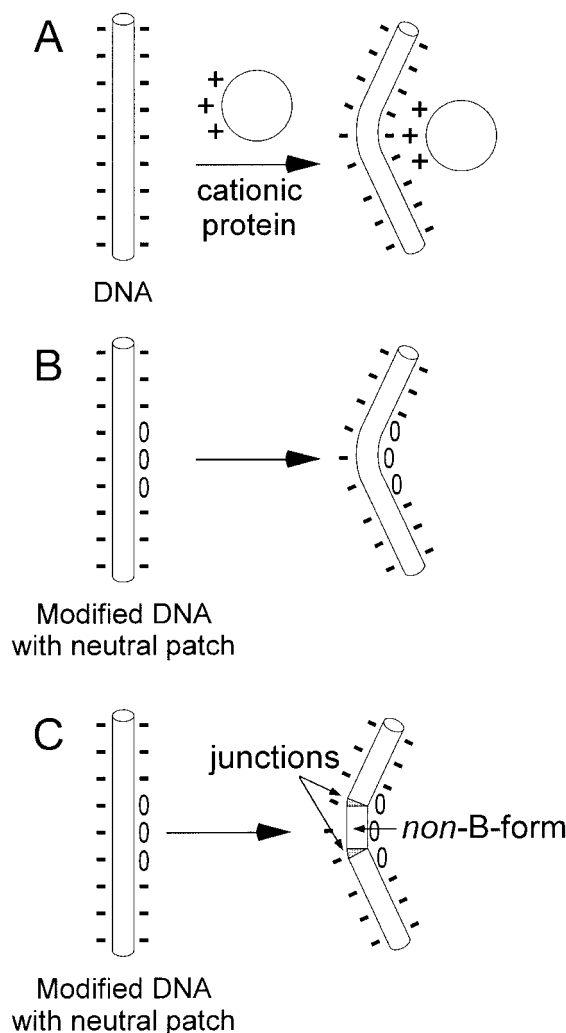
Important data and fresh insights into the origin of A-tract bending have been obtained through recent NMR studies and by revisiting A-tract crystal structures. During the past several years, advancements in techniques for molecular structure determination by NMR spectroscopy have allowed the structure of

DNA in solution to be analyzed for the presence of helical axis bending.<sup>79,80</sup> Results of such studies for an A-tract sequence flanked by generic DNA sequences are similar to the results of crystallographic studies in that the helical axis bending within the A-tract is small compared to the bend measured for A-tracts in solution.<sup>79</sup> However, appreciable helical axis bending is observed at the junctions between the A-tract and the generic sequences.<sup>79</sup> Molecular dynamics simulations of A-tracts have produced similar results.<sup>81</sup> Recently, Zappora and co-workers have solved the puzzle of why A-tract bending was for so long not observed in crystal structures.<sup>82</sup> Using computer modeling and a high-resolution crystal structure of duplex d(ACCGAATTCGGT), these investigators demonstrated that if multiple copies of their structure are joined together as one continuous duplex, the helical axis of this extended duplex is clearly bent at the junctions between the B\*-form and that canonical B-form segments. The magnitude of the observed bend is in excellent agreement with solution state studies. Furthermore, this result essentially confirms the validity of the junction model, at high resolution, as first proposed by Crothers to explain the origin of axial bending by A-tracts.<sup>19</sup>

As discussed above, support is building for the proposal that cation localization in the minor groove is necessary for A-tracts to assume the B\*-form helix. However, minor groove cation localization may not be all that is required for A-tracts to adopt the B\*-form helix. Other helical parameters of the B\*-form (e.g., base pair roll, slide, inclination, buckle) differ from the canonical B-form and contribute substantially to bending at the junction between these two forms.<sup>82</sup> Nevertheless, the unusual helical parameters of B\*-form DNA apparently require a narrow minor groove.<sup>83–85</sup> Thus, when cation localization in A-tracts is unequivocally shown to be required for the narrow minor groove, this will firmly establish cation localization as an essential component of A-tract sequence-directed curvature (Figure 3).

### THE CATION-DEPENDENT JUNCTION MODEL VS TWO ALTERNATIVE MODELS FOR SEQUENCE-DIRECTED CURVATURE

Mirzabekov and Rich proposed over twenty years ago that the DNA helix will spontaneously bend toward a positively charged protein (e.g., histones) as a result of the asymmetric screening of DNA phosphate repulsions by the protein (Figure 4).<sup>58</sup> Manning et al. provided the first theoretical support for this electro-



**FIGURE 4** (A) The electrostatic collapse model for DNA axial bending due to the asymmetric reduction of phosphate repulsions by a cationic protein.<sup>58,59</sup> (B) The related electrostatic collapse model for DNA bending due to phosphate charge neutralization by covalent chemical modification.<sup>86</sup> (C) An alternative junction-based model for the origin of DNA bending by covalent neutralization of phosphate charges. Figure adapted from Strauss and Maher, 1994.<sup>86</sup>

static collapse model for DNA bending based upon a greatly simplified two-dimensional model of DNA.<sup>59</sup> Maher and co-workers later put this hypothesis to an elegant experimental test by substituting phosphate groups with neutral methylphosphonate analogs in the backbones of DNA duplexes.<sup>86</sup> To mimic the effects of a cationic protein bound on one side of the DNA, methylphosphonate linkages were incorporated at specific positions in both strands, creating a neutral patch on one face of the double helix (Figure 4). These modified duplex DNA polymers exhibited axial bending at the location of the neutral methylphospho-

nate linkages, and the direction of bending was toward the neutral patch.<sup>86</sup> This observation was considered supportive of the electrostatic collapse model.

Soon after Maher and co-workers demonstrated DNA bending by asymmetric phosphate neutralization, cation localization sites were discovered in the minor groove of A-tract DNA.<sup>9,12,13</sup> The combination of these two events led to a new model for the origin of sequence-directed curvature by A-tracts. The DNA helix was proposed to be bent toward cations localized in the minor groove, with bending being coupled to a closing of the minor groove around the cations.<sup>12,13,23</sup> This model for A-tract sequence-directed curvature is conceptually very similar to the model explored by Rouzina and Bloomfield to explain the role of cation localization in sequence-directed curvature by G-tracts.<sup>33</sup> In the case of G-tracts, as discussed above, bending toward cations in the major groove can also be viewed as a local transition of the DNA helix to the A-form. Thus, the original electrostatic collapse model and the cation-dependent junction model proposed here seem to be equally satisfying explanations for the origin of G-tract-induced curvature. However, only the latter model appears consistent with A-tract-induced curvature.

It is conceptually easy to imagine an A-tract helix simply bending toward cations localized in the minor groove. Taken at face value, this model predicts that axial curvature will be at a maximum near the center of an A-tract, where cations are most favorably localized.<sup>12,13,23</sup> Considering the data accumulated over recent years from theoretical and experimental studies of A-tracts in solution, we must conclude that this model does not explain the origin of A-tract sequence-directed curvature. As discussed above, results from NMR spectroscopy and molecular dynamics simulations have confirmed that the helical axis of A-tracts is essentially as straight in solution as it is in crystals, and the majority of A-tract-induced bending occurs at the junctions between A-tracts and non-A-tract (i.e., generic or G-tract) DNA.<sup>25,80</sup> A fundamental limitation of the simple electrostatic collapse model is that it does not take into account the fact that DNA is not freely or even isotropically pliable.<sup>87–89</sup> Forces produced by electrostatic attractions between phosphates and localized cations will not necessarily produce DNA deformations that are directly in line with these force vectors. As Olson and co-workers recently demonstrated, the DNA helix often responds to protein and ligand binding by deforming along the B- to A-form continuum.<sup>70</sup> Why should localized cations, which presumably have less potential to exert a bending force on DNA than many cationic proteins, bend DNA into structures other than the same energetically

favorable helical conformations? How DNA responds to an electrostatic force, or any force, is largely governed by the chemical structure of DNA and the associated energy landscape of its helical structures. These are not problems for the cation-dependent junction model of A-tract bending. If we accept that DNA helical structure deviates from the canonical B-form as a result of sequence-specific cation localization, then axial bending naturally follows according to the junction model as put forth by Wells and Crothers (Figure 3).

The simple electrostatic collapse model has also turned out to be less than satisfactory as an explanation of helical axis bending in covalently charge-modified DNA polymers. The modified DNA polymers used in the initial phosphate neutralization experiments of Maher and co-workers contained a racemic mixture of (*R<sub>p</sub>*)- and (*S<sub>p</sub>*)-methylphosphonate substitutions. Subsequent studies of DNA with pure (*R<sub>p</sub>*)-methylphosphonate substitutions actually showed reduced bending compared to DNA with racemic substitutions,<sup>90</sup> an observation that is difficult to rationalize by simple electrostatic collapse. Recently, Manning, Olson and co-workers have conducted all-atom simulations for a series of DNA duplexes containing six neutralized phosphates on one face of the helix that are pure (*R<sub>p</sub>*), pure (*S<sub>p</sub>*), and various patterns of mixed (*R<sub>p</sub>*)/(*S<sub>p</sub>*)-methylphosphonate substitutions.<sup>91</sup> This study confirmed the basic proposal of the original electrostatic collapse model, that the DNA helix has a tendency to bend toward a charge-neutralized face. However, this study also demonstrated that DNA deformations (e.g., bending) induced by charge neutralization conform to the previously recognized preferential modes of protein-induced DNA deformation, and this deformation is also very sensitive to the exact stereochemistry of the modification.

Modified DNA has also been prepared with a variety of cationic side chains covalently tethered to the bases.<sup>92–95</sup> For example,  $\omega$ -aminoalkyl chemical groups, similar to the side chain of lysine, have been attached to the C5 position of deoxypyrimidines.<sup>92–94,96</sup> These modifications provide a more sophisticated way to model the effects of bound cationic proteins and groove-localized cations on DNA structure. Most cationic base modifications also produce axial bending in DNA, but not necessarily to the same degree as the racemic methylphosphonate substitutions.<sup>92–95</sup> For example, Maher and co-workers have recently reported that deoxyuridine bases modified with an ammonium ion attached by a rigid propynyl tether do not produce axial bends in DNA.<sup>94</sup> These investigators proposed that bending is not observed because the rigidity of the propyne moiety does

not allow optimal salt bridge formation between the tethered ammonium ion and phosphates, or that stacking of the propyne groups on neighboring bases renders the overall helix resistant to bending.<sup>94</sup> It appears that charge modifications of DNA generally cause local distortions in DNA helical structure away from the canonical B-form,<sup>95</sup> and in some cases this distortion is clearly toward the A-form.<sup>97</sup> Thus, we could also view axial bending by tethered cations, and methylphosphonate substitutions, as the result of charge-induced changes in helical structure that produce junctions with unmodified B-form DNA (Figure 4). Likewise, charge modifications that do not cause appreciable helical axis bending, such as the (*R*<sub>p</sub>)-methylphosphonate substitutions and amino groups linked by propyne tethers, may not because they do not destabilize the canonical B-form helix.

A preexisting alternative to our model, and the electrostatic collapse model, has been championed by Dickerson and co-workers. In their model for sequence-directed curvature, GC-rich sequences are bent, A-tracts are straight,<sup>78</sup> and cation localization is not involved.<sup>24</sup> This view is supported by the crystal structure of duplex d(CATGGCCATG) in which the DNA helical axis at the sequence element GGCC is bent toward the major groove.<sup>78</sup> Recently, Dickerson and co-workers have also proposed that the sequence GGGCCC is prone to undergo the B- to A-form transition due to the organization of water molecules in the major groove of this sequence.<sup>98</sup> As we have discussed above, sequence-directed curvature by G-tract sequences such as GGGCCC can be explained in terms of their cation-enhanced propensity to undergo the B- to A-form transition. Thus, the model proposed by Dickerson and co-workers for sequence-directed curvature by G-tracts is not necessarily in opposition to the cation-dependent junction model, if bending toward the major groove is also considered to be a transition toward the A-form. However, whether cation localization or water structure is to be considered the dominant factor in promoting the B- to A-form transition in G-tracts is likely to remain open to debate.

## CONCLUDING REMARKS

We observe a beautiful dichotomy in how several properties of A-tract and G-tract DNA sequences are distinct from all other DNA sequences, which we have called generic DNA. This includes the following: (1) G-tracts preferentially localize cations in the major groove, whereas A-tracts localize cations in the minor groove. (2) G-tracts are predisposed to undergo the B- to A-form transition, whereas A-tracts are

notably resistant to this transition. (3) G-tracts in the A-form helix (or a helical structure between the B- and A-forms) have a flat and wide minor groove, whereas the B\*-form helix of A-tract DNA has an extremely narrow minor groove. (4) A G-tract flanked by generic DNA sequences can cause helical axis bending toward the major groove, whereas A-tracts cause helical axis bending toward the minor groove. These four ways in which A-tracts and G-tracts are distinct from each other, and from generic DNA, can all be understood in terms of sequence-specific cation localization by the major and minor grooves. The conversion of DNA between secondary structures (i.e., from B-form to B\*-form or A-form) can be viewed as the result of a tug of war between the two grooves for cation localization, in which the groove that preferentially localizes cations narrows, and in doing so further enhances its own ability to localize cations. The adoption of the B\*-form helix by A-tracts and the A-form helix by G-tracts can be explained, at least in part, by sequence-specific cation localization and the tug of war model. The adoption of alternative helical structures by A-tracts and G-tracts produces axial bends in otherwise generic DNA polymers by way of the junction model, providing the final link between sequence-specific cation localization and sequence-directed curvature.

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