DNA bending and the curious case of Fos/Jun

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DNA bending has been implicated as an important regulatory mechanism in several processes involving protein–DNA interactions. Various methods for examining intrinsic and protein-induced DNA bending may lead to different conclusions. For the Fos and Jun transcription factors, this has resulted in controversy over whether these factors significantly bend DNA at all.

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Introduction

DNA bending is emerging as a central theme in the regulation of processes such as transcription, replication and recombination in both prokaryotes and eukaryotes. In addition to sequence-based information that specifies the binding of regulatory *trans*-acting factors, structural properties of DNA have also been recognized to be important in regulation. Regulating the proximity of upstream regulatory sequences in transcription, assembly of DNA around the core nucleosome, and retroviral integration at preferred sites are a few examples of processes that may require bending of the DNA helix. The specific functional consequences of DNA bending, however, remain unknown for many protein–DNA interactions, despite a few well-studied examples.

This review describes mechanisms of intrinsic and protein-induced DNA bending, as well as some of the biological processes in which bending is thought to be important. The architectural role of DNA-bending proteins in modulating the assembly of higher order nucleoprotein complexes in eukaryotic transcription will be discussed. Finally, an overview will be presented of the techniques available for assessing protein-induced DNA bending. By way of example, these methodologies will be discussed for the curious case of the basic leucine zipper (bZIP) transcription factors Jun and Fos. The question of whether the Jun/Fos heterodimer bends DNA has sparked significant controversy.

Intrinsic and protein-induced DNA bending

DNA can either be bent by proteins or it can 'passively' assume a bent conformation caused by unique sequence elements. In both instances, a static bend requires deviations from the canonical parameters of B-form DNA. Whether intrinsic or protein-induced, DNA bending is not to be confounded with DNA flexibility which reflects the potential for DNA to be deformed in an orientation-independent fashion. In contrast, a bent region of DNA is on average both relatively inflexible and oriented in nonlinear fashion. Bent DNA behaves differently from unbent or flexible DNA in both gel-electrophoretic phasing assays and electro-optical measurements of DNA curvature.

The intrinsically bent A-tract sequences (originally identified from African trypanosome *Leishmania tarentola* kinetoplast minicircles) were the first recognized example of bent DNA sequences (reviewed in [1]). Two general models have been suggested to explain the observed bend: in the 'wedge' model, bending is progressive and occurs throughout the A-tract sequence [2], whereas in the

'junction' bending model, kinking of the DNA occurs at the transition between the As in the tract and abutting sequences [3]. Crystal structures of A-tracts have been solved and the data suggest that although the A-tract DNA itself is straight, stacking of bases at the interface between an A-tract and the surrounding sequence is disrupted [4]. An increase in roll angle at the extremities of the A-tract, as well as an increase in propeller twist throughout the A-tract sequence itself, are observed and are thought to accommodate hydrogen bonding between bases one turn apart along the helix ('inter base pair' hydrogen bonds). In contrast, the tilt, roll and propeller twist parameters of B-DNA are maintained in the sequences peripheral to the A-tract. As with regions of intrinsic curvature, protein-induced DNA bending also requires deviation from normal B-DNA parameters and is typically a result of heavy rolling and untwisting of the helix at specific dinucleotide steps [5,6].

Proteins have adopted several strategies for deforming the DNA helix depending upon which groove they interact with and the extent to which they bend the DNA. A number of co-crystals for DNA-bending proteins bound to their consensus DNA sequences have been solved and they reveal common mechanisms for distorting the helix. Intercalation of hydrophobic amino acid sidechains is one of the most common mechanisms by which proteins disrupt B-DNA and induce bending [7]. The determination of X-ray or solution structures for proteins such as TATA-binding protein (TBP), the lymphocyte enhancer factor-1 (LEF-1) and testis-determining factor (SRY) HMG (high mobility group) proteins [8,9], the purine repressor (PurR) [10], and integration host factor (IHF) [11] — all of which induce large bends — reveals striking similarities in the mechanism of DNA kinking in which disruption of stacking interactions between two consecutive base pairs and opening of the DNA towards the minor groove is compensated for by an increase in roll angle [12]. Asymmetric phosphate neutralization has also been suggested as a force responsible for DNA bending as DNA in which neutral phosphate analogs are incorporated on one face of the helix shows spontaneous bending [13]. This mechanism may also account for bending caused by interaction of the backbone with basic amino acid clusters [14]. These studies suggest that proteins with cationic surfaces may therefore bend DNA by asymmetric shielding of phosphate groups on one side of the helix.

Such departures from the normal parameters of B-form DNA are likely to be facilitated by the intrinsic flexibility or bendability of certain DNA sequences. For example, A/T-rich sequences have a tendency for a widened minor groove, whereas G/C-rich regions typically have compressed major grooves [7]. Artificially enhancing the flexibility of DNA sequences has also been shown to affect binding site selection of bending proteins [15]. The notion that the intrinsic properties of DNA sequences may influence protein binding is also implicit in the thermodynamic prediction that bending proteins should bind prebent circular sequences with higher affinity. This has been demonstrated experimentally for *Escherichia coli* catabolite activator protein (CAP) and mammalian TBP [16,17], and represents a method for assessing protein-induced DNA bending [18].

Architectural factors in regulation of gene expression

The formation of three-dimensional nucleoprotein structures is essential for the regulation of complex processes such as transcription, replication, recombination and integration. In addition to supporting the sequence-specific binding of numerous proteins onto regulatory regions such as origins, promoters or enhancers, DNA is required to modify its spatial trajectory to accommodate short range protein–protein interactions that would otherwise be energetically unfavorable. In the case of eukaryotic transcription, bending is typically induced by dedicated architectural factors that bind sequence elements located in between binding sites for promoter/enhancer regulatory sequences [19,20]. Similarly, a number of prokaryotic proteins involved in regulating transcription have been shown to bend the DNA, thereby potentially modulating both the binding affinity of other DNA-binding proteins and the overall template architecture [21].

The catabolite activator protein (CAP) was the first prokaryotic protein shown to induce bending of its target DNA sequence [22]. The functional relevance of the induced 90° bend in transcriptional regulation was demonstrated through the ability of intrinsically bent DNA sequences to replace a CAP site [23,24]. In addition, 'bend-swap' experiments were performed in which the CAP site was substituted with the target sequence of a heterologous DNA-bending protein such as RepA [25]. The remarkable 180° bend induced by IHF has also been the subject of intense investigation [12]. Although a transcriptional activator in the context of bacterial operons, IHF was originally shown to promote the assembly of the λ phage intasome [26]. As with CAP, bend-swap experiments using binding sequences for either the IHF homolog HU or eukaryotic HMG-family bending proteins like HMG-1, HMG-2 or LEF-1 demonstrate that distortion of the helix, rather than IHF binding *per se*, is essential for either phage integration [27] or transcriptional activation [28].

Bacterial RNA polymerase (RNAP) has also been shown to curve DNA [29,30] at the –35 and –10 elements and, as a result, accelerate initiation by facilitating melting of the DNA strands and driving the closed-to-open transition [21]. Intrinsically bent DNA sequences *cis* to basal promoter elements are found in numerous prokaryotic promoters [31,32], and their effect on promoter activity correlates with the orientation of the bend relative to the

RNAP-binding site [33,34]. This DNA bending in prokaryotes may affect gene expression via several mechanisms [21]: bending at a given locus influences the binding affinity of factors targeting nearby sequences, structural distortion of the double helix brings into proximity otherwise remote regulatory factors, and alteration of DNA strand melting parameters.

In the case of eukaryotic transcription, a regulatory role for DNA bending has been more complex to address, largely because *cis*-acting regulatory sequences are typically found packaged in chromatin. Intrinsically bent DNA sequences, in some instances, have been suggested to direct nucleosome positioning [35,36]. Despite the additional topological complexity inherent to chromatin templates, the assembly of higher order nucleoprotein structures probably still requires that the distance between DNA-bound factors even on nucleosomal DNA, be reduced in order for them to interact.

A number of proteins have been recognized in eukaryotes that seem to have a primary function of regulating DNA conformation. These architectural factors often lack potent transcriptional activity of their own, supporting the notion that their primary function, like IHF, may be structural. Their activity is also typically 'context dependent' in that modifying the distance or phasing of the protein's target site relative to the other regulatory sites alters promoter/enhancer architecture and abolishes their activity. As with prokaryotic systems, functional characterization of DNA bending by these proteins has been carried out by disrupting phasing between regulatory sites as well as through 'bend-swap' experiments.

The interferon-β (IFNβ) gene enhanceosome model and the 3′ distal enhancer of the T-cell receptor α (TCRα) gene are prototypical examples of transcriptional regulation via control of DNA bending [19]. In both cases, binding sites for architectural factors relative to other transcription factor consensus sites are precisely helically phased, and nucleotide insertions that disrupt this phasing can be complemented by additional insertions that restore the phasing relationship between binding sites [37,38]. In the case of the IFNβ enhancer, the HMG I(Y) minor-groove-binding protein reverses the intrinsic curvature of the DNA, and thereby facilitates the interaction of transcription factors bound on the major groove of the helix [39]. In the TCRα system, LEF-1 sits between ATF/CREB and TCF-2 consensus binding sites and induces a dramatic 130° kink in the helix, facilitating the interaction of ATF/CREB and TCF-2 [37,40]. In addition to determining the correct spatial configuration of the enhancer, LEF-1 (as well as mutants lacking all but the HMG DNA-binding domain) also enhances binding of the Ets-1/AML complex on chromatin, but not nonchromatin, templates [41]. This activity reveals an additional mechanism in which bending can influence transcription.

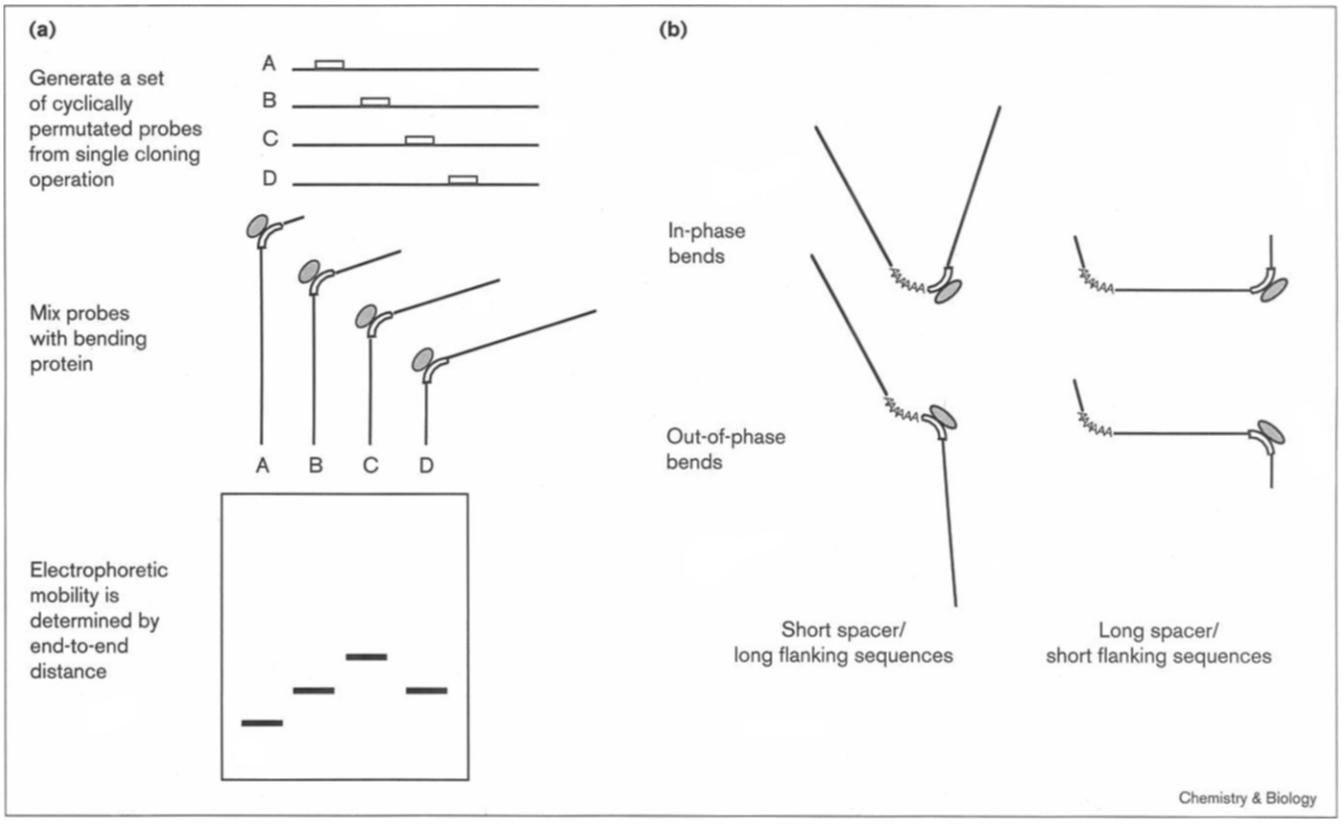
The importance of spatial disposition and phasing between transcription factor binding sites is a theme found in a growing number of enhancers/promoters. The yin/yang-1 (YY1) DNA-bending protein, for example, possesses both activator and repressor functions depending on the promoter context [42]. In the case of the *c-fos* promoter, where YY1 normally has repressor activity, YY1 can be switched into an activator by inverting the orientation of its binding sites relative to the CRE and TATA-box elements [43]. Moreover, the SRY bending protein in the case of the *c-fos* promoter [43] or intrinsically bent DNA sequences in the case of simple synthetic promoters [42] can mimic the orientation-dependent activity of YY1.

In summary, DNA bending in eukaryotic promoters and enhancers is likely to regulate transcription on several levels. In addition to bringing transcription factor binding sites into spatial proximity and potentially modulating the affinity of nearby factors, DNA bending may also affect the topology of DNA templates over long distances. Although in comparison to circular bacterial genomes, eukaryotic chromosomes are considered linear, the DNA sequences through which *trans*-acting factors regulate transcription are typically found in topologically closed chromatin domains, the conformation of which may be similarly subject to twist and bending variations in the helix. Interestingly, computer simulations that model the conformation of closed circular DNA reveal that the introduction of short bends as well as their relative spacing can dramatically affect template supercoiling [44]. For specific protein-dependent bends, these effects may depend on the degree of altered twist which may influence torsional strain by compensating for bending. Thus, as with prokaryotic systems, local curvature of DNA by an architectural factor may result in long-range conformational effects on the helix. Studies of the TCRα enhancer suggest that the LEF-1-assembled nucleoprotein complex activates gene expression by offsetting the topology generated by HMG-I(Y) factors, thereby derepressing the promoter [45]. Although a growing number of proteins have been shown to possess bending activity, the functional consequence of deforming the DNA helix in many cases still remains obscure.

Experimental detection of DNA bending and flexibility

Numerous biochemical and structure-based approaches have been developed to study DNA bending. X-Ray crystal structure determination of intrinsically bent DNAs, as well as cocrystals with bending proteins, has provided a wealth of information on both the angles of DNA bends and the DNA sequences and protein motifs/amino acids involved in the mechanism of bending [7,20]. Electro-optical, atomic

Figure 1



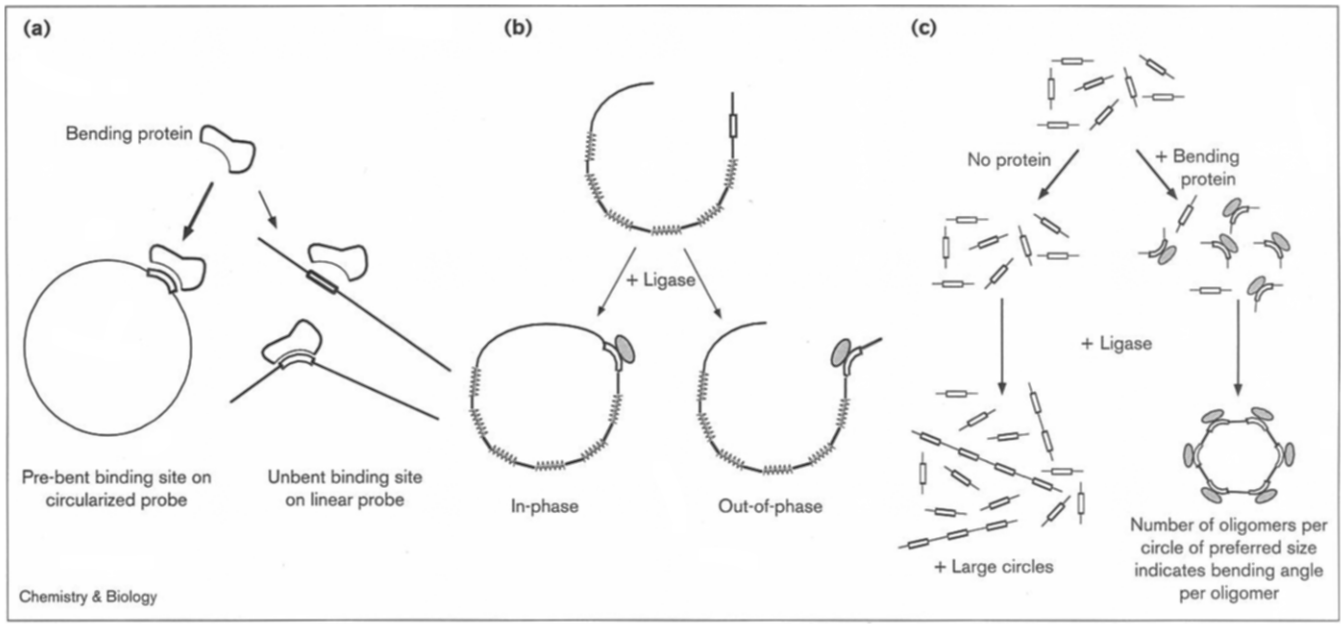
Electrophoresis-based methods to detect and measure DNA bending. **(a)** The cyclic permutation gel mobility shift assay. This assay relies on the fact that DNA probes in which the bend is closest to the center of the DNA fragment have the shortest end-to-end distance and thereby migrate more slowly than ones in which the bend is more proximal to the ends of the probe. **(b)** Phasing electrophoretic assays. In phasing assays, the helical phasing of the protein-binding site is varied relative to an intrinsically bent sequence (typically phased A-tracts). Electrophoretic mobility is determined both by the amplitude of the bend and its spatial orientation. Anomalous mobility is also influenced by the length of the spacer (the distance between A-tracts and the protein-binding site) as well as the flanking region length.

force and calorimetric methods have also been used to assess DNA curvature in solution [46,47]. The altered migratory properties of bent DNAs through gels or increased kinetics of unimolecular ligation observed for bent over linear DNA fragments, however, remain the most commonly used approaches to detect bending. Both rely on the fact that a bent molecule has a shorter end-to-end distance than its linear counterpart. In the case of gel-based methods, the position-dependent effect of a DNA bend on probe mobility is directly related to the mean square end-to-end distance (Figure 1). The shorter the end-to-end distance, the more the movement of the 'reptation' through the gel matrix is impeded and migration is slowed.

The cyclic permutation gel mobility shift assay developed by Wu and Crothers [22] remains one of the most commonly used and perhaps simplest methods to study bending. Although originally used to identify the intrinsically bent A-tract sequence of *Leishmania tarentola* kinetoplast, it is now widely used to determine protein-induced bending. The consensus binding sequence for the protein of interest is cloned into a vector from which a series of cyclically permutated DNA probes of identical length is generated [48] (Figure 1a). These probes differ primarily in the position of the consensus site relative to the ends of the fragment. If the protein bends DNA, then protein-bound complexes in which the consensus site is closest to the center of the DNA fragment will have slow (anomalous) mobilities in the gel. The magnitude of the bend might be extrapolated by comparison with mobilities of standard intrinsically bent sequences such as A-tracts. Although mobility anomalies of this sort are typically observed when bending is induced, the technique cannot be used to assess the directionality of a bend, the precise magnitude of a bend, or the possibility that other factors (such as protein structure) might also be influencing migration.

Phasing or 'helical phasing' analysis — a variation on the previous method — studies the migration of DNA in which the consensus site is separated from an intrinsic 'reference'

Figure 2



Solution-based assays to detect and measure DNA bending. **(a)** Bending proteins are predicted to have higher affinity for their binding sites when these are constrained within a circular fragment that effectively prebends their target sequence. **(b)** In the fragment cyclization assay, the phasing of the protein binding site relative to intrinsically bent A-tract sequences influences the kinetics of unimolecular ligation and is a measure of both bend orientation and magnitude. It is also predicted that bending proteins have higher affinity for their binding sites when these are constrained within a circularized fragment that effectively prebends their target sequence **(c)** In the mixed ligation method, oligomerization and circularization of small labelled oligonucleotides containing the protein-binding site is monitored in the presence and absence of protein. If the protein of interest bends its target sequence, the number of oligonucleotides required to generate a circle is decreased, and the bending angle is calculated as the number of oligonucleotides per circle of preferred size divided by 360°.

bend (typically A-tract sequences) by fractions of a helical turn [49] (Figure 1b). Unlike the cyclic permutation assay, migration of these probes is determined both by the magnitude of the bend angle and the orientation of the bend relative to the reference bend. In-phase bends result in DNA resembling the letter C, exacerbating the deviation from linearity (increasing the mobility anomaly). Out-of-phase bends resemble the letter S, partially restoring greater end-to-end distance (resulting in linearity) and diminishing the mobility anomaly. The orientation can be determined by plotting the mobility of the different gel-shifted probes as a function of spacer length (the distance between the consensus site and the reference bend). In addition to being more sensitive than the cyclic permutation assay, phasing analysis also discriminates between static DNA bends and sequences of increased DNA flexibility. In the case of a static bend, gel-migration patterns are recapitulated with a periodicity of a full helical turn (~10.5 base pairs). In contrast, if a flexible sequence is free to orient itself in two directions within a plane of curvature, it has a periodicity of half a helical turn (~5 base pairs).

In the case of protein-induced DNA bending, an independent method exists for assessing bending on the basis of the energetics required for a protein to kink a DNA sequence. It is thermodynamically predicted that proteins that bend DNA will have enhanced affinity for pre-bent as opposed to linear DNA templates. This preference can be observed in competition binding experiments, for example, in which linear or circular DNA templates of identical sequence and length are added in different orders and binding affinity is calculated from gel-shift band intensities [16,17].

DNA cyclization or 'ring-closure' methods are solution-based assays in which the efficiency of cyclization mediated by DNA ligase is predicted to increase when the ends of a DNA fragment are brought into proximity as a result of bending (Figure 2) [16]. The measure of this efficiency — the J-factor — is the ratio of equilibrium constants for unimolecular to bimolecular association. Bending of the template accelerates the kinetics of unimolecular ligation over bimolecular ligation thereby increasing the experimental J-factor. Typically, small cyclization templates with lengths close to the persistence length of DNA (~150 base pairs) are used to maximize sensitivity to protein-induced bending. The length must also be suitable for avoiding introduction of torsional strain

by incorporating integral numbers of helical DNA turns into the template. In addition, use of pre-bent templates that have several in-phase A-tract sequences allows the determination of bend orientation in a similar fashion to the phasing analysis method described above, but in a solution-based (ligation) method.

The 'mixed ligation' method [50] (as modified by Lyubchenko and colleagues [51]) is also used as an alternative solution-based approach to calculate the bend angle induced by a DNA-bending protein (Figure 2c). Double-stranded oligonucleotides that contain the protein consensus sequence are incubated with protein, ligated and separated in two dimensions (eg. 4% acrylamide/10% acrylamide). Following autoradiography to determine the size distribution of minicircles, individual spots are excised from the gel for radioactive quantitation and to confirm circularity by exonuclease resistance. The distribution of minicircle products of different sizes indicates the preferred size species generated by ligation, from which can be deduced the bending angle per oligomer (360°/number of oligomers in the most abundant minicircle species). This method has been shown to yield bend angles consistent with those obtained from several other methods as well as crystallographic data.

Finally, it should be noted that each of the above-mentioned methods is subject to its own set of theoretical pitfalls. The electrophoresis-based methods, for example, measure the migratory anomalies of bent DNAs but they can also be subject to protein-dependent anomalies [18] and may, in some cases, appear to exaggerate bending or yield false-positive results. Studies that measure rates of cyclization must also take into account a number of important steric factors which, independent of bending, can lead to misalignment or nonplanarity of template ends and inhibition of template ligation. Even crystallographic data should be interpreted with caution as nucleoprotein complexes are invariably stabilized by crystal packing interactions which may restrain the spatial conformation of the DNA. Consequently, it is prudent to employ novel methods that provide independent complementary means of assessing DNA bending. The Fos/Jun proteins have provided a 'case in point' of how different approaches to studying DNA bending may yield potentially contradictory results. A number of studies have addressed DNA bending by these two proteins and produced a substantial controversy over whether these proteins bend DNA at all.

Fos/Jun and DNA bending

Fos and Jun are bZip (basic leucine zipper) eukaryotic transcription factors that bind DNA as homodimers or heterodimers [52,53]. Proteins from this family bind to the activator protein-1 (AP-1; as well as TRE and CRE) consensus site found upstream of many cellular and viral genes [54,55]. The varied possibilities for heterodimerization with other family members and ensuing competition for target site occupation probably create an intricate combinatorial web of regulated gene expression. The leucine zipper motif of Fos and Jun mediates homodimerization and heterodimerization, whereas the basic region is responsible for binding to the major groove of DNA. Circular dichroism studies have revealed that, as with a number of other bZip or bHLHZip factors (HLH, helix–loop–helix), the basic region is disordered in solution and assumes an α-helical structure upon binding to the major groove of DNA [56,57]. Although DNA binding by bending proteins is often thought to occur via an 'induced fit' mechanism [7], Kerppola and Curran [58] hypothesized that the Fos/Jun proteins may induce a complementary conformational change in the DNA to which they bind.

Using the electrophoretic methodologies to study DNA bending, Kerppola and Curran [58] obtained evidence consistent with the possibility that Fos and Jun proteins are capable of bending DNA. Circular permutation analysis showed significant electrophoretic mobility anomalies for both Fos/Jun and Jun/Jun dimers. When compared with previously described mobility anomalies of A-tract DNA or protein–DNA complexes, the authors suggested DNA curvatures of approximately 94° and 79° for Fos/Jun and Jun/Jun respectively. In a separate study [59], the authors further defined the contributions of different regions of Fos and Jun proteins for the mobility anomaly and modeled a structure of Fos/Jun and Jun/Jun dimers onto bent DNA. Electrophoretic phasing studies showed that Fos/Jun–DNA complexes are dramatically different from Jun/Jun–DNA complexes and that these different dimers might bend the DNA in the opposite directions. Correlating with *in vivo* results demonstrating that Fos and Jun have opposite effects on dexamethasone-stimulated prolactin gene transcription [60], this observation suggested that differential DNA bending might provide a structural key to understanding transcriptional regulation by these proteins. An investigation of the DNA-bending properties of a variety of bZip proteins that bind to the AP-1 site suggested that bending is a common, although not universal, characteristic within this family transcription factors [61]. At least one other bZip protein, GCN4, was found in both electrophoretic phasing [62] and crystallographic [63] studies to produce little if any DNA bending, despite the presence of mobility anomalies in simple circular permutation studies. The magnitude of the Fos and Jun effects, however, were potentially larger than the GCN4 effect and they were also observed in phasing electrophoretic experiments [58].

Surprisingly, however, the X-ray crystal structure of Fos and Jun complexed with AP-1 DNA [64] did not reveal any significant bending of the helix. In a commentary accompanying this structure, Kerppola and Curran [65] proposed three possible explanations to explain the path

of the DNA in the crystal. First, regions outside those used for the crystals could play an important role in bending the DNA; second, the crystallization process requires high ionic concentrations which may have shielded regions of the proteins required for induction of bending; and finally, end-to-end crystal packing forces may have stabilized the helix into a straight rod.

In agreement with the X-ray structure data, however, subsequent studies from the Crothers laboratory [66] using both solution-based DNA cyclization assays and the phase-sensitive electrophoretic method provided strong evidence that Fos and Jun do not significantly bend the DNA. Both of these approaches were different from those used in the original Kerppola experiments [58,59]. Although a solution-based assay for bending (cyclization) had not yet been tried in the case of Fos and Jun, the phase-sensitive method originally developed in the Crothers laboratory [49] when applied to Fos/Jun did not suggest significant bending, and differed slightly from Kerppola's method. As pointed out by the authors [66] a larger spacer between the A-tracts and the Fos/Jun site than in the experiments by Kerppola may have played a role in the observation of insignificant DNA bending, in agreement with the X-ray structure and cyclization predictions. As noted and experimentally tested by Kerppola [67], there are two key differences between these methods: first, the length of the spacer which separates the intrinsic reference bend and the AP-1 site, and second, the length of the sequences flanking these two sites.

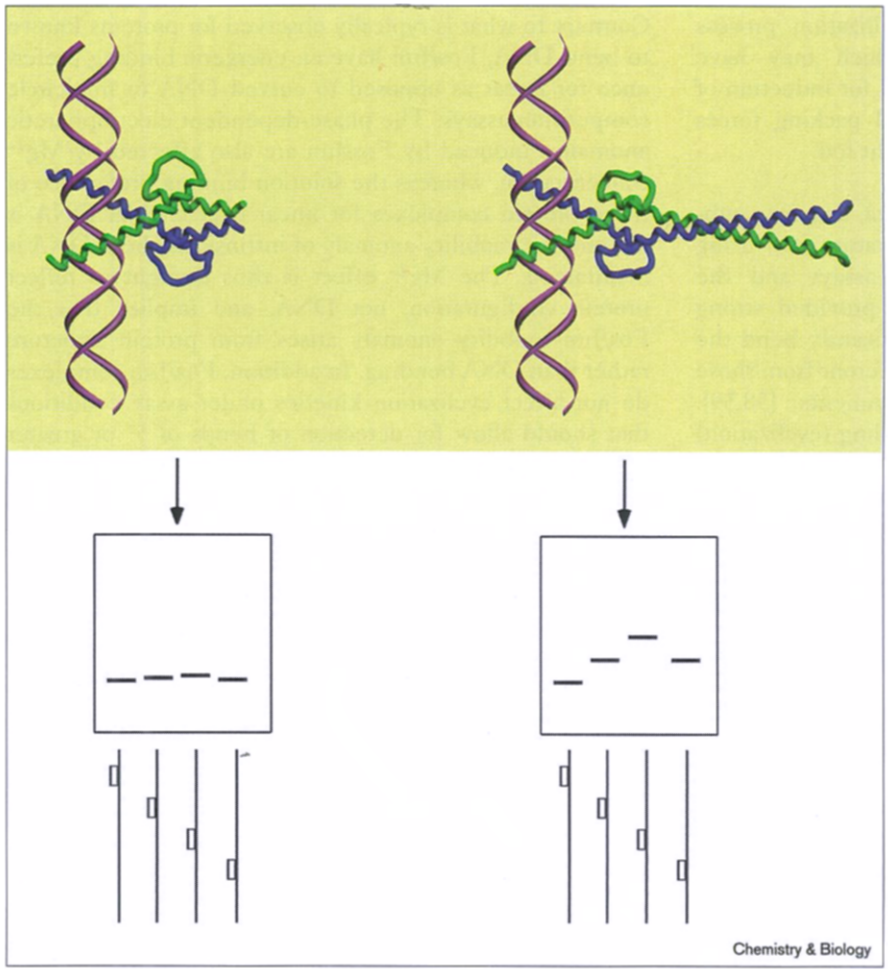
In a series of carefully formulated studies, Kerppola [67] demonstrates that increasing spacer length between two intrinsically bent sequences reduces phase-dependent mobility changes, and that reducing the length of flanking sequences diminishes phase-sensitive detection of bends. These studies help to define the limits of detection for phase-dependent electrophoretic assays and thus provide very useful information for the DNA-bending field, although the precise ability to predict the behavior of protein-induced bends from the behavior of A-tracts remains to be verified. Kerppola also examined ligation behavior and showed a propensity of Fos/Jun to stimulate multimerization instead of cyclization regardless of the phasing or presence of intrinsic DNA bends. Although Kerppola suggests that this behavior results from an uncharacterized property of these proteins, the same results might be obtained if the proteins stiffen the DNA in unbent configuration. A clear conclusion of these data is that phasing probes with larger spacer lengths (e.g. three helical DNA turns) represent a more stringent test of bending, particularly for larger bends.

A more recent study from the Crothers laboratory [68] addresses the suggestions of Kerppola that phasing linker length, nature of AP-1 site flanking sequences, and buffer ionic strength may affect either the ability of Fos/Jun to bend DNA or the experimental detection of such a bend. Contrary to what is typically observed for proteins known to bend DNA, Fos/Jun have an energetic binding preference for linear as opposed to curved DNA in minicircle competition assays. The phase-dependent electrophoretic anomalies induced by Fos/Jun are also affected by Mg^{2+} concentration, whereas the solution binding preference of these protein complexes for linear over circular DNA is not and the mobility anomaly of intrinsically bent DNA is maintained. The Mg^{2+} effect is thus thought to reflect protein configuration, not DNA, and implies that the Fos/Jun mobility anomaly arises from protein structure rather than DNA bending. In addition, Fos/Jun complexes do not affect cyclization kinetics under assay conditions that should allow for detection of bends of 5° or greater (using probes in which the AP-1 site is replaced with an A-tract). Taken together, these results support the notion that Fos/Jun dimers do not bend the AP-1 site significantly greater than 5°, and the phase-dependent gel-mobility variations observed with these proteins are probably due to factors other than DNA bending.

What else might account for probe-specific mobility anomalies if not DNA bending? One possibility is protein structure, as previously suggested [62] and demonstrated in the case of DNA bending by Myc/Max. These proteins contain the b-HLH-Zip motif and they were found to produce mobility anomalies consistent with DNA bending in circular permutation and phasing electrophoresis studies [69,70]. In fact, Max/Max dimers were suggested to bend DNA in the opposite orientation to Myc/Myc homodimers [70], a feature reminiscent of Fos/Jun and Jun/Jun orientation predictions. Still more similar is the feature that Myc/Myc homodimers are very unstable and they are often difficult to observe in DNA-bound complexes. Myc binds DNA more easily in heterodimers with Max, analogously to Fos/Jun which is more stable than Jun/Jun [71]. Finally, like Fos/Jun, the crystal structure of Max/Max–DNA showed no significant DNA bend [72], despite the electrophoretic mobility anomalies.

For the b-HLH-Zip family, however, significant insight was gained when another b-HLH-Zip protein, Microphthalmia, was found to retain DNA binding but lose anomalous electrophoretic mobility upon truncation of the leucine zipper [18]. Progressive truncation of the leucine-zipper motif of this protein abolished the gel-mobility differences, suggesting that the shape of the protein due to ordered secondary structure motifs (in particular the extended leucine zipper) may effect probe migration through gels irrespective of DNA bending (Figure 3). This peptide motif was thus linked to protein-specific electrophoretic migratory effects. As the leucine zipper is a nonglobular coiled-coil motif, the vector of which is perpendicular to the palindromic DNA-binding site, this

Figure 3



The leucine zipper may induce electrophoretic mobility anomalies without DNA bending. A Max/Max homodimer [72] is modeled on a linear DNA fragment to demonstrate the effect of progressive truncation of the leucine-zipper region (left, truncated; right, control) on the mobility of the protein-bound probes in a cyclic permutation gel mobility shift assay.

strongly suggested that the protein may retard electrophoretic mobility like a spoke sticking off the DNA in a position-specific fashion. In addition, the intact b-HLH-Zip of Max was shown to bind linear DNA with higher affinity than circular 'pre-bent' DNA, and circularization was inhibited (in a sequence-specific fashion), all consistent with the b-HLH-Zip stabilizing DNA in a linear, unbent configuration as seen in the crystal structure.

The altered mobility of various Fos/Jun truncated proteins, which has been ascribed to the ability of these factors' activation domains to bend the DNA [73], may, in fact, reflect protein-dominated mobility effects rather than altered DNA bending. It is also plausible that the altered 'direction of bend' predictions for less stable homodimers (Jun/Jun and Myc/Myc) could reflect unusual protein/zipper configurations. Nevertheless, modest deviations from linear DNA may well occur and, although not of the magnitude predicted by early electrophoretic studies [58], could still carry functional significance in principle. Additional direct structural (e.g. crystallographic) studies, including larger protein regions of Jun/Jun homodimers may also provide more information.

Future directions

As the complexities surrounding DNA transcription and replication in both prokaryotes and eukaryotes are being revealed, DNA bending is increasingly recognized as a potential regulatory mechanism. Although many proteins are now known to bend DNA, their precise functions in the context of natural promoters remain largely unknown aside from a few well-studied examples such as the IFNβ enhanceosome and the TCRα enhancer.

Despite the numerous *in vitro* methods available to study protein-induced DNA bending, many of the variables being measured in these approaches remain incompletely understood. The use of gel-mobility assays to study DNA bending by proteins that contain a leucine zipper, for example, must be carefully tempered with caution about the molecular source of anomalous mobilities. Similarly, the Fos/Jun studies have demonstrated

that the choice of methodology — whether gel- or solution-based — is important because different methods may yield opposite results. Although it is beneficial to learn from the experimental limits of these approaches, it is also essential that combinations of these methods that are both technically feasible and meaningfully predictive are used. With these, and perhaps newer methods as well, it is hoped that fundamental biological processes will emerge from the buried mysteries of chromatin.

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