The EMBO Journal Vol.16 No.10 pp.2907–2916, 1997

The transcription activation domains of Fos and Jun induce DNA bending through electrostatic interactions

Tom K.Kerppola[1] and Tom Curran[2]

Howard Hughes Medical Institute and Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI 48109-0650 and [2]Department of Developmental Neurobiology, St Jude Children's Research Hospital, Memphis, TN 38105-2794, USA

[1]Corresponding author
e-mail: kerppola@umich.edu

Transcription factor-induced DNA bending is essential for the assembly of active transcription complexes at many promoters. However, most eukaryotic transcription regulatory proteins have modular DNA-binding and activation domains, which appeared to exclude DNA bending as a mechanism of transcription activation by these proteins. We show that the transcription activation domains of Fos and Jun induce DNA bending. In chimeric proteins, the transcription activation domains induce DNA bending independent of the DNA-binding domains. DNA bending by the chimeric proteins is directed diametrically away from the transcription activation domains. Therefore, the opposite directions of DNA bending by Fos and Jun are caused, in part, by the opposite locations of the transcription activation domains relative to the DNA-binding domains in these proteins. DNA bending is reduced in the presence of multivalent cations, indicating that electrostatic interactions contribute to DNA bending by Fos and Jun. Consequently, regions outside the minimal DNA-binding domain can influence DNA structure, and may thereby contribute to the architectural reorganization of the promoter region required for gene activation.

Keywords: basic region/charge effect/DNA curvature/leucine zipper/phasing analysis

Introduction

The fundamental importance of DNA structural changes induced by transcription factor binding has been well established in prokaryotes (Hoover *et al*., 1990; Perez-Martın and Espinosa, 1993; Ansari *et al*., 1995). In eukaryotes, the LEF-1, YY1 and HMG-I/Y proteins have been shown to regulate transcription of the T-cell receptor (TCR) α, c-fos and interferon (IFN) β promoters respectively, most likely through mechanisms involving DNA bending (Natesan and Gilman, 1993; Falvo *et al*., 1995; Giese *et al*., 1995). However, the ability of regions outside the DNA-binding domains of many transcription factors to activate transcription when fused to heterologous DNA-binding domains has impeded general acceptance of this concept.

Structural studies of the basic-leucine zipper (bZIP) domain have revealed a simple and elegant mechanism for cooperative DNA recognition. The leucine zipper forms a coiled-coil dimerization interface and the basic region forms an α-helix that contacts base pairs within the major groove of the DNA recognition site (Oakley and Dervan, 1990; O'Neil *et al*., 1990; Talanian *et al*., 1990; O'Shea *et al*., 1991; Konig and Richmond, 1993; Glover and Harrison, 1995). The basic regions of Fos and Jun occupy the major groove on different sides of the DNA helix and project out from the side of the DNA helix opposite to the leucine zipper. There is no high resolution structural information available for regions outside the minimal bZIP domains. The transcription activation domains of Fos and Jun are located on opposite sides of the bZIP region in the primary structure (Abate *et al*., 1991), and it is likely that they are located on different faces of the DNA helix in the ternary complex. Fos and Jun share a low level of sequence similarity between their transcription activation domains, and chimeras containing segments from each protein display transcriptional activity (Sutherland *et al*., 1992). Multiple regions in Fos and Jun have been shown to interact with several components of the general initiation complex (Metz *et al*., 1994; Martin *et al*., 1996). The roles of these interactions in transcription activation remain to be determined.

Fos and Jun were originally shown to induce DNA bending in studies that employed phasing analysis, a method that is based on the phase-dependent interaction between two closely spaced DNA bends (Kerppola and Curran, 1991a,b). Surprisingly, Fos–Jun heterodimers and Jun homodimers were found to bend DNA in opposite directions. Furthermore, whereas the bZIP domains of Fos and Jun induced small bends, the full-length proteins induced larger bends, and each subunit in the heterodimer appeared to contribute independently to DNA bending. DNA bending by Fos and Jun has been visualized directly by atomic force and electron microscopy (Griffith *et al*., 1994; Becker *et al*., 1995). The bZIP domains induce DNA bending through charge interactions that involve amino acid residues adjacent to the basic region (Leonard *et al*., 1997). However, X-ray crystallographic analysis of proteins encompassing the minimal bZIP domains of Fos and Jun bound to the AP-1 site did not detect significant DNA bending (Glover and Harrison, 1995). X-ray crystallographic analysis and solution studies of intrinsic DNA bending have also reached diametrically opposite conclusions (Crothers *et al*., 1990; Dickerson *et al*., 1994). Thus, alternative methods for the analysis of protein induced-changes in DNA structure are required.

Results

DNA bending by the transcription activation domains

We previously reported that truncated Fos and Jun proteins encompassing the leucine zipper dimerization and basic

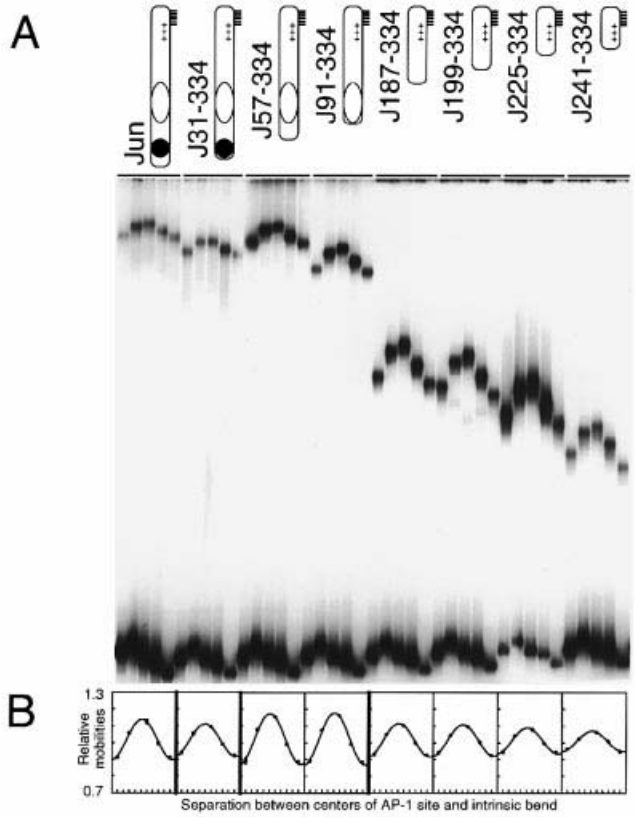


Fig. 1. DNA bending by regions of Jun outside the dimerization and DNA-binding domains. (**A**) The full-length and truncated Jun proteins indicated above the lanes were incubated with the phasing analysis probes described previously (Kerppola and Curran, 1991a,b) and the complexes were analyzed on an 8% polyacrylamide gel. The diagrams above the lanes show the bZIP (+++), activation (open oval) and repression (solid circle) domains of Jun mapped previously (Abate *et al*., 1991), with the amino-termini pointing down. Each set of lanes contained probes in which the separation between the centers of the AP-1 site and the intrinsic DNA bend was 21, 23, 26, 28 and 30 bp respectively. The differences between the mobilities of complexes bound to these probes reflect DNA bending as they result from the variation in the phasing between intrinsic and protein-induced DNA bends. The origin of electrophoresis is at the top and the free probes are at the bottom of the figure. (**B**) Phasing plots of the relative mobilities of homodimer complexes. To allow comparison between DNA bending by complexes with different absolute mobilities, the complex mobilities were normalized for differences in probe mobilities to an average mobility of 1, and these relative mobilities were plotted as a function of the separation between the centers of the AP-1 site and the intrinsic bend as described (Kerppola and Curran, 1991a). The best fit of the phasing function is superimposed on the data (Kerppola and Curran, 1991b). The degree of variation in the relative mobilities is a function of the magnitude of the DNA bend. Plots for complexes that induced significantly different bends based on statistical analysis of bending by all complexes (see Figure 3) are separated by heavy lines. The abscissa for each plot is 20–31 bp.

DNA-binding domains induce DNA bends that are smaller than those induced by the full-length proteins (Kerppola and Curran, 1991a,b). The majority of X-ray and nuclear magnetic resonance (NMR) studies of protein–DNA complexes have been performed using the minimal DNA-binding domains of the respective proteins. Thus, little is known about the effects of regions outside the minimal DNA-binding domains on the structures of protein–DNA complexes. To investigate whether the differences in DNA bending between full-length and truncated Fos and Jun were attributable to specific regions, we analyzed DNA

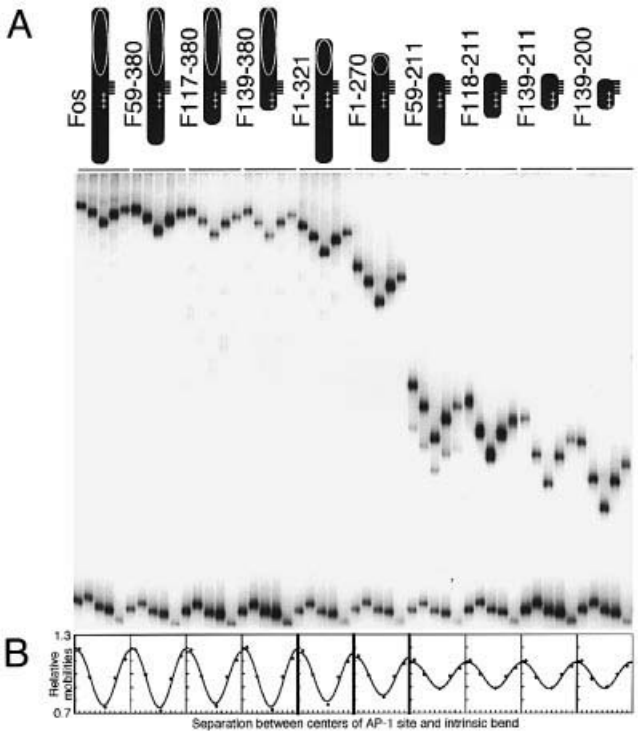


Fig. 2. DNA bending by regions of Fos outside the dimerization and DNA-binding domains. (**A**) The full-length and truncated Fos proteins indicated above the lanes were incubated with the minimal Jun bZIP domain protein (J257–318), and DNA bending by the heterodimers was analyzed as in Figure 1. The diagrams above the lanes show the bZIP (+++) and activation (oval) domains of Fos, with the amino-termini pointing down. Since J257–318 induces little DNA bending, the DNA bends induced by these complexes are due primarily to DNA bending by the respective Fos proteins and the differences in DNA bending are entirely attributable to Fos. (**B**) Phasing plots of the relative mobilities of heterodimer complexes. The relative mobilities of the complexes were plotted as a function of the separation between the centers of the AP-1 site and the intrinsic bend as in Figure 1B. Plots for complexes that induced significantly different bends based on statistical analysis of bending by all complexes (see Figure 3) are separated by heavy lines. The abscissa for each plot is 20–31 bp.

bending by successive amino- and carboxy-terminal deletion derivatives of Fos and Jun. Phasing analysis of homodimers formed by Jun deletion derivatives indicated that a region located between amino acid residues 91 and 186 increased the DNA bend angle (Figure 1). A region between residues 31 and 57 reduced the DNA bend angle, and this effect was partially reversed in full-length Jun. Intriguingly, these regions coincide with the transcription activation (91–186) and repression (31–57) domains of Jun (Abate *et al*., 1991; data not shown). The region between residues 91 and 186 also promotes T antigen-dependent DNA unwinding required for the initiation of polyoma virus DNA replication (Ito *et al*., 1996).

To examine the contributions of different regions of Fos to DNA bending, both amino- and carboxy-terminal truncations were examined (Figure 2). Since Fos does not bind DNA as a homodimer, these proteins were analyzed as heterodimers with the minimal Jun bZIP domain. Deletion of the entire region on the amino-terminal side of the Fos bZIP domain had no effect on DNA bending. Deletions from the carboxy-terminus indicated that a region on the carboxy-terminal side of residue 211 contributed to DNA bending. Partial deletions to residues 270

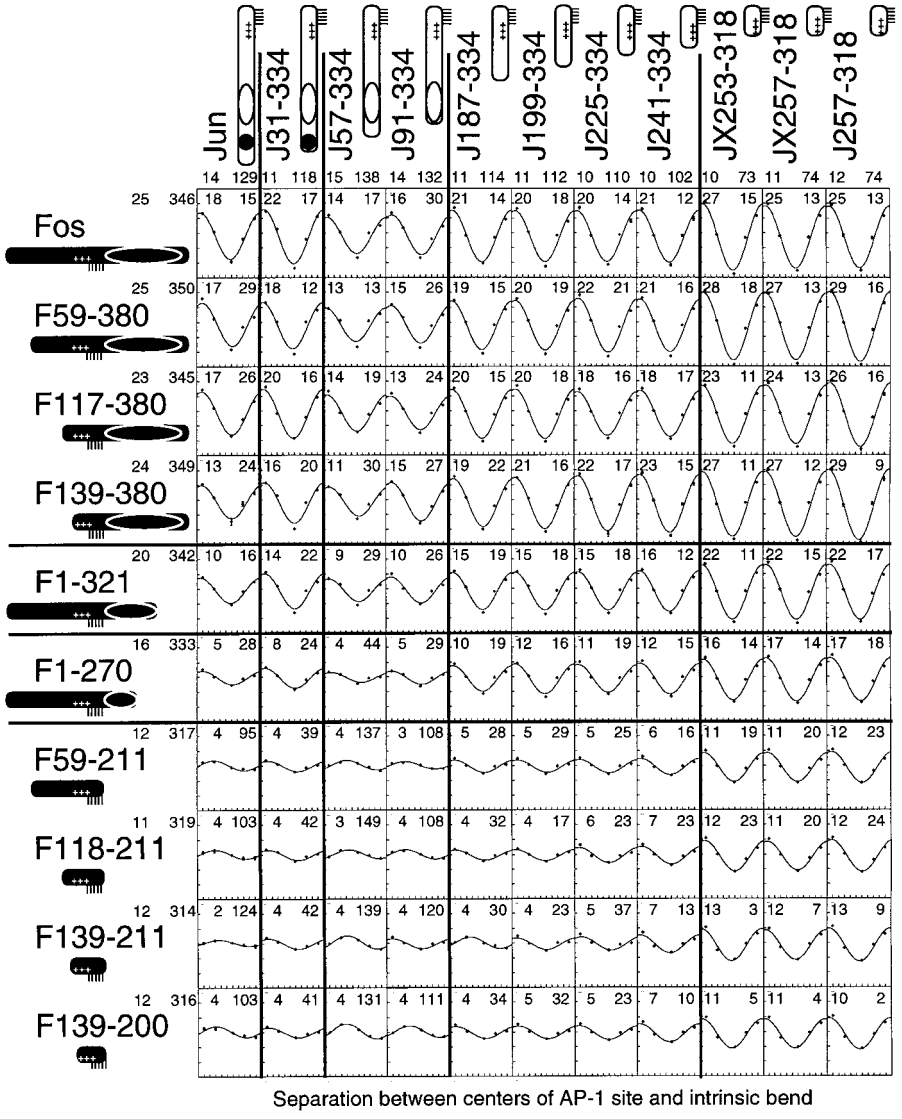


Fig. 3. Quantitation of DNA bending by heterodimers formed between all combinations of Fos and Jun deletion derivatives. The relative mobilities of complexes formed by all heterodimeric combinations between Fos and Jun deletion derivatives were plotted in the rows and columns corresponding to the subunits of each complex shown above and to the left of the matrix. The Fos and Jun deletion derivatives are shown as in Figures 1 and 2. The proteins designated JX contained a factor Xa cleavage site between the hexahistidine purification tag and the native coding region. Removal of the fusion peptide by factor Xa cleavage had no effect on DNA bending. The abscissa for each plot is 20–31 bp and the ordinate is from 0.7 to 1.3. The DNA bend angle (α_B) and direction (β_B) derived from the phasing function (see Materials and methods) are shown in the upper left and upper right corners of each plot. For 20 complexes, a sufficient number of independent experiments were performed to derive meaningful standard deviations for the relative mobilities, which are shown as vertical bars. The DNA bend induced by each monomer was calculated based on the hypothesis that each subunit induces an independent bend (Kerppola and Curran, 1991b), and are shown to the left and above the matrix of plots. These bends were calculated by least squares minimization of the error of a model in which the bend induced by each heterodimer represented the vector sum of the bends induced by its constituent subunits. The 21 calculated subunit bends predicted the bends induced by all 110 heterodimers with an error of $<3°$ in bend angle and $<15°$ in bend direction (the direction of bending was predicted for complexes that induce bend angles of $>5°$). Multivariate analysis of variance supported the validity of the model ($P < 0.001$) and pairwise T^2 tests indicated that subunits separated by heavy lines induced significantly different DNA bends.

and 321 had intermediate effects. This DNA-bending region also overlaps a transcription activation domain in Fos (Abate *et al*., 1991; Metz *et al*., 1994). Thus, in both Fos and Jun, specific regions outside the bZIP domains that coincide with transcription activation domains influence DNA bending.

Previously, we proposed that the individual subunits of the Fos–Jun heterodimer induce separate DNA bends, and that the overall angle and direction of DNA bending induced by the dimeric complex represents the sum of the bends induced by the two subunits (Kerppola and Curran, 1991a,b). To determine if DNA bending by complexes formed by the various Fos and Jun deletion derivatives was consistent with this model, we quantitated the DNA bends induced by all heterodimeric combinations between these proteins (Figure 3). The same regions of Fos and Jun affected DNA bending regardless of their dimerization partner. The bends induced by the individual subunits were calculated by finding the best fit of their sums to the DNA bends induced by all heterodimeric combinations. These deduced DNA bends confirm that discrete regions of Fos and Jun influence DNA bending whereas other

regions have little or no effect. The same regions in Jun influence DNA bending in both homodimers and heterodimers. The model accurately predicted the DNA bends induced by all heterodimers and was corroborated by multivariate analysis of variance. Therefore, the individual subunits of the Fos–Jun heterodimer induce separate DNA bends.

Several of the deletions that affected DNA bending also had an effect on DNA-binding affinity. However, whereas the effects on DNA bending were observed regardless of dimerization partner or binding site, the effects on binding affinity were observed only for a subset of the complexes containing a particular protein. Thus, the region between residues 31 and 56 in Jun reduced both the DNA-binding affinity and DNA bending in the context of Jun homodimers. The same region also affected DNA bending in the context of heterodimers, but had no detectable effect on their DNA-binding affinity. Conversely, the region between residues 241 and 252 of Jun increased both DNA bending and the DNA-binding affinity of Jun homodimers at an AP-1 site. This region also affected DNA bending by heterodimers, but had no detectable effect on their DNA-binding affinity. Also, whereas this region affected DNA bending at both AP-1 and CRE sites, it had no detectable effect on the affinities of either homo- or heterodimers at the CRE site. Thus, although differences in DNA bending may influence the DNA-binding affinities of different Fos and Jun complexes, these differences in binding affinity do not influence measurement of DNA bending by these complexes using phasing analysis.

DNA bending by chimeric proteins

The observation that regions overlapping the transcription activation domains of Fos and Jun modulate DNA bending raised the possibility that DNA structural changes could contribute to transcription activation by these proteins. To determine if the transcription activation domains of Fos and Jun induced DNA bending when fused to a different DNA-binding domain, we examined DNA bending by chimeric proteins in which the transcription activation domains were fused to different bZIP regions (Figure 4). To determine the significance of the position of the transcription activation domain relative to the DNA-binding domain, these domains were fused on both sides of the respective bZIP regions. All of the chimeras induced DNA bends that were distinct from that induced by the minimal bZIP region peptides. Therefore, the transcription activation domains of Fos and Jun induced DNA bending when fused to different DNA-binding domains.

The bends induced by complexes containing the transcription activation domains fused on different sides of the bZIP region were distinct. Complexes where the transcription activation domain was fused on the carboxy-terminal side of the bZIP region induced a larger bend than the bZIP regions alone, whereas fusion of the transcription activation domains on the amino-terminal side of the bZIP region reduced DNA bending relative to the bZIP regions alone. Thus, the position of the transcription activation domain affected the direction of DNA bending. The transcription activation domains of Fos and Jun induced qualitatively similar bends when fused to the same position in the bZIP region. Thus, the opposite directions of DNA bending induced by intact Fos and Jun

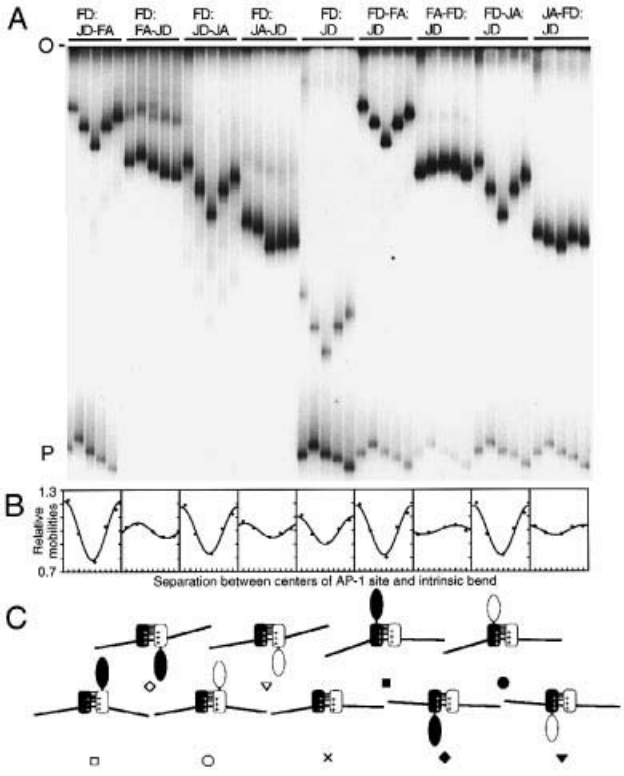


Fig. 4. DNA bending by chimeric proteins containing the Fos and Jun transcription activation domains. (**A**) Chimeric proteins consisting of all combinations between the transcription activation domains of Fos (FA) and Jun (JA) fused to the bZIP domains of Fos (FD) and Jun (JD) were incubated with the cognate bZIP domains to generate heterodimers containing the same bZIP DNA-binding motif with a single transcription activation domain fused to different positions in this motif (see diagrams in C). DNA bending by the heterodimers as well as by the minimal bZIP motif alone was analyzed as described in Figure 1. (**B**) Phasing plots of the relative mobilities of complexes formed by heterodimers containing the chimeric proteins. The relative mobilities of the complexes were plotted as a function of the separation between the centers of the AP-1 site and the intrinsic bend as in Figure 1B. The abscissa for each plot is 20–31 bp. Differences in the amplitudes of the functions reflect differences in bend angle, whereas differences in phase reflect differences in bend direction. While differences in DNA bending by deletion derivatives of Fos and Jun correlated with the absolute mobilities of the complexes (Figures 1 and 2), there was no relationship between DNA bending by the chimeric protein complexes and their absolute electrophoretic mobilities. (**C**) Schematic diagrams of DNA bending by complexes formed by the chimeric proteins. The chimeric proteins represent fusions between the transcription activation domains (ovals) of Fos (FA, solid) and Jun (JA, open) fused to the bZIP domains (rounded rectangles) of Fos (FD, solid) and Jun (JD, open), and are shown with their amino-termini pointing down. The DNA bends induced by the individual chimeras were calculated based on the model that each subunit induces an independent DNA bend (see Figure 3) and are projected onto a plane approximately parallel to the zipper axis. Each diagram is placed directly underneath the data for the complex. The symbols below the complexes are used to plot the data in Figure 5.

are not due to an intrinsic difference in the DNA-bending properties of their respective transcription activation domains. Furthermore, whereas activation domain fusions to the carboxy-terminal ends of each of the two bZIP regions resulted in very similar bends, fusions to the amino-terminal ends resulted in different directions of DNA bending. Consequently, the transcription activation domains of Fos and Jun had qualitatively similar DNA-bending properties, and differences in the positions of these

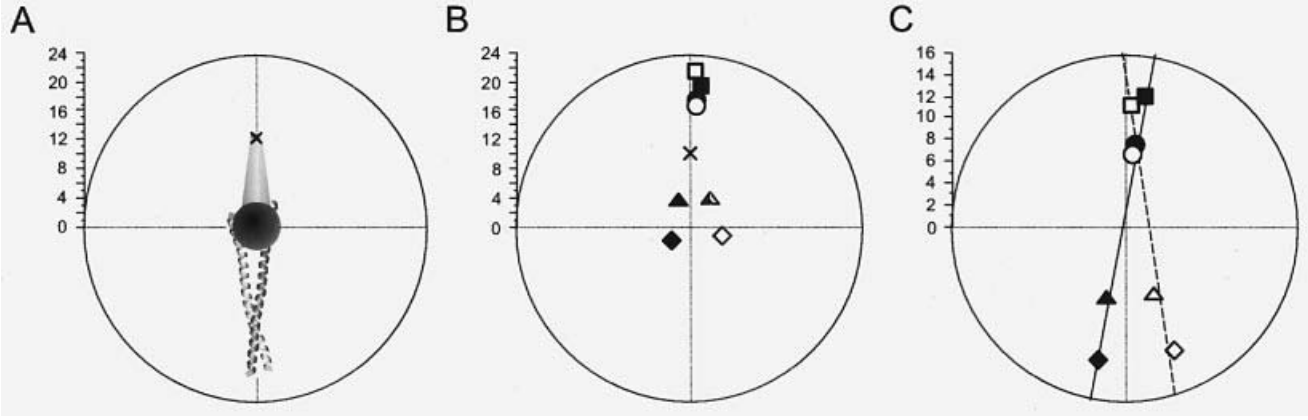


Fig. 5. The transcription activation and bZIP domains induce independent DNA bends. (**A**) Diagram of the relationship between the physical protein–DNA complex and the polar coordinate representation of DNA bending by the FD:JD complex. The direction and magnitude of the DNA bend are shown as polar coordinates, which can be considered to represent the end-on view of the DNA helix for small DNA bend angles. The direction of DNA bending is shown clockwise with the value 0 at the top. The bZIP domains of Fos and Jun are shown as helical ribbons. The DNA fragment is shown as a hollow tube with an × at the distal end, which is equivalent to the symbol (×) shown in the graph. (**B**) Polar coordinate representation of DNA bends induced by heterodimeric complexes formed by chimeric proteins and the minimal Fos and Jun bZIP domains. Chimeric proteins containing the Fos bZIP domain are shown as filled symbols, whereas chimeras containing the Jun bZIP domain are shown as open symbols (see Figure 4C). Chimeras with the Fos transcription activation domain fused on the amino- and carboxy-terminal sides of the bZIP domains are indicated by diamonds and squares respectively. Chimeras with the Jun transcription activation domain fused on the amino- and carboxy-terminal sides of the bZIP domains are indicated by triangles and circles respectively. The heterodimer consisting of the minimal Fos and Jun bZIP domains is indicated by an ×. (**C**) Polar coordinate representation of DNA bends calculated for the Fos and Jun transcription activation domains fused to different positions in the bZIP domains. The DNA bends induced by the transcription activation domains in heterodimers formed by the chimeric proteins were deduced based on the hypothesis that they contribute to DNA bending that is independent of the bZIP domains. These bends were calculated by least squares minimization of the error in a model in which the bend induced by each complex represented the vector sum of bends induced by the transcription activation and bZIP domains. This calculation was repeated for complexes formed at each of 10 separate binding sites (Rajaram and Kerppola, 1997). Multivariate analysis of variance demonstrated that the model containing 20 independent variables predicted the bends induced by 136 complexes with a high degree of significance ($P < 0.001$). The symbols used are the same as in (B), but refer to the effects of the transcription activation domains independent of the bZIP domains. Lines indicate the difference between the DNA bend directions induced by transcription activation domains in heterodimers when fused to the bZIP domains of Fos (solid line) and Jun (dashed line).

domains relative to the DNA-binding domain resulted in dramatic differences in DNA bending.

To quantitate the effects of the transcription activation domains on DNA bending, we calculated the contributions of the bZIP and transcription activation domains to DNA bending based on the hypothesis that these domains have additive effects on DNA bending (Figure 5). This hypothesis is supported by the remarkable result that the magnitudes of the DNA bends calculated based on this hypothesis were virtually identical whether the transcription activation domains were fused on the amino- or carboxy-terminal sides of the bZIP regions of Fos or Jun. In addition, multivariate analysis of variance of DNA bending by the chimeric proteins at 10 different binding sites (Rajaram and Kerppola, 1997) is consistent with independent effects of the transcription activation and DNA-binding domains on DNA bending. Thus, the transcription activation domains induce DNA bends of equal magnitude regardless of their structural context. In contrast, the direction of DNA bending was determined by the position of the transcription activation domain in the complex (Figure 5). The transcription activation domains of Fos and Jun induced bends of different magnitudes, but the directions of bending were identical for domains fused to the same position. Consequently, the extent of DNA bending induced by the transcription activation domains is independent of the bZIP domains, and the direction of bending is determined by the side of the DNA helix where the transcription activation domain is located.

The native Fos and Jun proteins induce DNA bending in opposite directions. However, when the Fos and Jun transcription activation domains were fused to the same side of the bZIP region, they induced DNA bending in the same direction (i.e. compare FD-FA:JD and FD-JA:JD, Figures 4 and 5). In contrast, when either of these transcription activation domains was fused to opposite ends of the bZIP region, it induced DNA bending in virtually opposite directions (i.e. compare FD:JD-FA and FD:FA-JD, Figures 4 and 5). The magnitudes of the DNA bends induced by the transcription activation domains when placed on either side of the bZIP region were similar to the bends induced by the same regions in the context of full-length Fos and Jun. Thus, the opposite directions of DNA bending induced by Fos and Jun are in part due to the converse arrangement of the transcription activation and DNA-binding domains in the native Fos and Jun proteins.

The DNA bends induced by Fos and Jun counteract each other, but they are not directed in diametrically opposite directions (Kerppola and Curran, 1991a,b). Similarly, the DNA bends induced by the Fos and Jun transcription activation domains fused to opposite ends of the bZIP region were oriented away from each other at an oblique angle (Figure 5C). Furthermore, the direction of DNA bending induced by the transcription activation domains when fused to the basic region of Fos was distinct from that induced when they were fused to the basic region of Jun. These DNA bends were related by a 2-fold symmetry axis, which coincided with the major groove–minor groove axis at the center of the AP-1 site. This suggests that the transcription activation domains are located in distinct, symmetrically related positions in the complex when fused

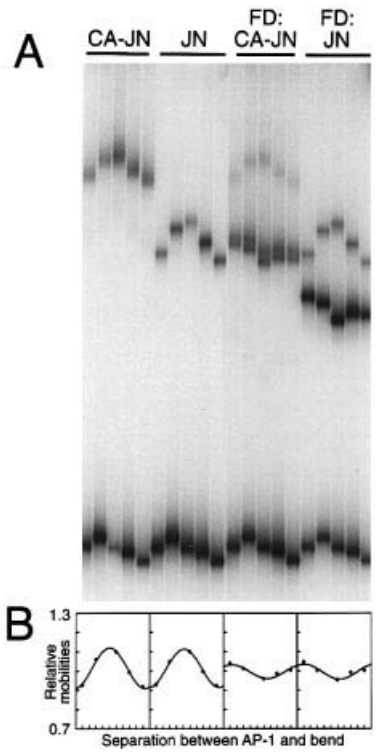


Fig. 6. The CTF-1 transcription activation domain does not induce DNA bending in chimeric proteins. (**A**) Phasing analysis of truncated Jun proteins (JN) with and without an amino-terminal fusion to the CTF-1 activation domain (CA). DNA bending by homodimers and heterodimers with the bZIP domain of Fos (FD) was analyzed as described in Figure 1. (**B**) Phasing plots of relative mobilities of complexes formed by CTF-1 fusion proteins. The relative mobilities of the complexes were plotted as a function of the separation between the centers of the AP-1 site and the intrinsic bend as in Figure 1B. The abscissa for each plot is 20–31 bp.

to the basic regions of Fos and Jun. One interpretation of this result is that the Fos–Jun heterodimer binds to the AP-1 site in a preferred orientation, placing transcription activation domains fused to the Fos and Jun basic regions in rotationally symmetrical positions (Rajaram and Kerppola, 1997). Consequently, the symmetry relations inherent in the dimeric bZIP DNA-binding motif are reflected in the relative directions of DNA bending induced by transcription activation domains fused to different positions in the bZIP regions.

To examine the specificity of the effects of the Fos and Jun transcription activation domains on DNA bending, we determined the effect of the transcription activation domain from CTF-1 on DNA bending when fused to the dimerization and DNA-binding domains of Jun (Figure 6). Phasing analysis of complexes formed by the chimeric protein demonstrated that the transcription activation domain of CTF-1 did not alter DNA bending by the Jun bZIP domain in either homodimers or heterodimers. Thus, DNA bending is a property specific for a subset of transcription activation domains.

Charge interactions contribute to DNA bending by Fos and Jun

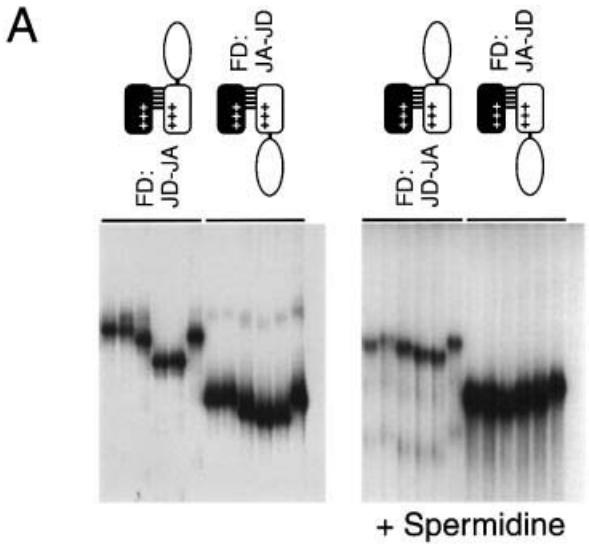
Since the Fos and Jun transcription activation domains have similar effects on DNA structure when placed in the same position relative to the bZIP domain, they are likely to rely on common molecular mechanisms of DNA bending. Truncation of Fos at residue 270 eliminated approximately half of the DNA-bending potential of this region (Figures 2 and 3). This truncation bisects a segment of limited sequence similarity between the Fos and Jun transcription activation domains that contains clusters of negatively charged amino acid residues (Abate *et al*., 1991; Sutherland *et al*., 1992). Conversely, the repressor domain of Jun that reduced DNA bending (Figures 1 and 3) has a net positive charge. Mutational analysis of residues adjacent to the bZIP domains of Fos and Jun indicates that DNA bending correlates with the net charge of residues adjoining the basic region (Leonard *et al*., 1997). The CTF-1 activation domain that did not induce DNA bending is a proline-rich transcription activation domain, and contains no clusters of charged residues. Thus, the charge of the transcription activation domains of Fos and Jun may contribute to DNA bending by these regions.

To investigate the possible role of charge interactions in DNA bending by Fos and Jun, we examined the effect of multivalent cations on DNA bending. DNA retains a shell of associated counterions in solution that partially neutralizes the charge of the phosphodiester backbone (Manning, 1978). The extent of charge neutralization varies depending on the valence of the associated counterions. Electrophoresis in the presence of 1 mM $MgCl_2$ reduced the phase-dependent variation in the mobilities of all complexes examined (Figure 7). Similar decreases in the mobility variation were observed in the presence of 1 mM spermidine and 0.1 mM hexamminecobalt. There was no significant effect of the multivalent cations on the relative electrophoretic mobilities of intrinsic DNA bend standards (Figure 7B). Thus, electrostatic interactions contribute to DNA bending by Fos and Jun.

Discussion

The paradigm of modular DNA binding and transcription activation domains has been a powerful influence in studies of the mechanisms of transcription activation. The independent functions of the two domains have lent support for models in which transcription activation domains act through mechanisms that do not involve changes in DNA structure. Here we show that regions outside the bZIP domain influence DNA bending by Fos and Jun. The localization of these DNA-bending regions within transcription regulatory domains (Abate *et al*., 1991; Metz *et al*., 1994) indicates that DNA structural changes may contribute to transcription regulation. The DNA-bending region in Jun also promotes T antigen-dependent DNA unwinding in the initiation of polyoma virus DNA replication (Ito *et al*., 1996). Activation of both transcription and replication by the same protein domain suggests that a common mechanism such as the distortion of DNA structure contributes to both processes.

Fos and Jun bend DNA in opposite directions. Nevertheless, both proteins contribute to transcription activation in cultured cells *in vitro* at most promoters that have been examined. In contrast, under physiological conditions, Fos and Jun perform distinct functions, as shown by the dissimilar phenotypes of mice in which one or the other gene has been deleted (Hilberg *et al*., 1993; Grigoriadis *et al*., 1994). Gene regulation in animals requires the



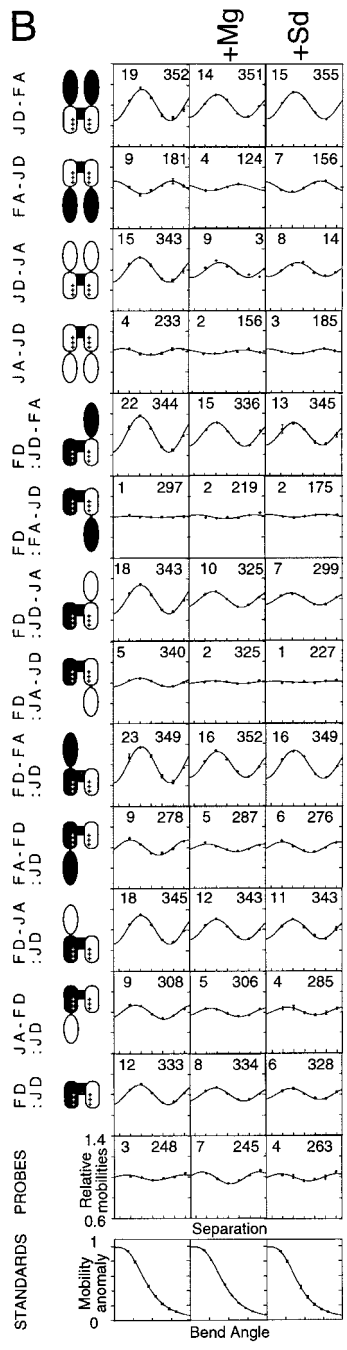


Fig. 7. Effects of multivalent cations on DNA bending by Fos and Jun bending domains. (**A**) Phasing analysis of DNA bending in the presence and absence of spermidine. The heterodimers indicated above the lanes were incubated with phasing analysis probes containing an AP-1 site (sequence X described in Rajaram and Kerppola, 1997). Each set of lanes contained probes in which the separations between the AP-1 site and the intrinsic bend were 26, 28, 30, 32, 34 and 36 bp respectively. DNA bending was examined by gel electrophoresis in the presence (right panel) and absence (left panel) of 1 mM spermidine in the gel and in the recirculated electrophoresis buffer. The time of electrophoresis was adjusted to equalize the average mobilities of the complexes in the presence and absence of spermidine. (**B**) Phasing plots of DNA bending by chimeric protein complexes in the presence of $MgCl_2$ and spermidine. The proteins indicated on the left were bound to phasing analysis probes containing the site used in the X-ray crystallographic analysis (Glover and Harrison, 1995; Rajaram and Kerppola, 1997, accompanying manuscript) and analyzed by electrophoresis in gels containing 1 mM $MgCl_2$ or 1 mM spermidine. The relative mobilities of the complexes were plotted as a function of the separation between the centers of the AP-1 site and the intrinsic DNA bend. The abscissa for each plot is 25–39 bp. Multivariate analysis of variance supported the hypothesis that the DNA bends induced in the presence of $MgCl_2$ and spermidine differed from the bends induced in the absence of multivalent counterions ($P < 0.001$). The plots at the bottom show the mobility anomalies of intrinsic DNA bend standards containing between two and nine phased A tracts prepared by *Nhe*I digestion of PCR products generated by using plasmids pJT170-2–pJT170-11 (Thompson and Landy, 1988) as templates, run on the same gels as the protein complexes. The abscissa for each plot is 0lADl–180lADl.

interdependent function of multiple regulatory elements in the promoter and can differ from that observed in cultured cells *in vitro* (Robertson *et al*., 1995). It is in the context of the topological constraints imposed by interactions among multiple proteins bound to separate promoter elements that DNA bending by the Fos and Jun transcription activation domains is likely to be most significant. Consequently, Fos and Jun regulate different target genes in the animal, and it is possible that the opposite directions of DNA bending induced by these proteins contribute to such differences in target gene selectivity.

DNA bending by Fos and Jun was reduced in the presence of multivalent cations. All of the regions of Fos and Jun that affected DNA bending contain clusters of charged residues. Thus, DNA bending by Fos and Jun is mediated at least in part by charge interactions. The converse effects of sequences flanking the AP-1 site on DNA bending in opposite directions (Rajaram and Kerppola, 1997) suggest that these charge effects include interactions with the phosphates of base pairs flanking the AP-1 site. However, a high density of charged residues is not sufficient to induce DNA bending since other charged regions in Fos and Jun have no effect on DNA bending. Thus, the structural context of the charged residues is important for their influence on DNA bending. The transcription activation domains of Fos and Jun may share a common structural fold that is required for DNA bending and transcription activation.

The X-ray crystallographic analysis of the minimal

bZIP regions of Fos and Jun was performed in the presence of high concentrations of salt, including multivalent cations (Glover and Harrison, 1995). Thus, the charge interactions that induce DNA bending may have been partially shielded in the crystal. Multivalent cations do not completely neutralize the charge of the phosphodiester backbone, nor do they eliminate DNA bending by Fos and Jun. Analysis of DNA bending at different $MgCl_2$ concentrations indicated no concentration dependence of DNA bending, consistent with the near-constant charge of DNA under these conditions predicted by polyelectrolyte theory (Manning, 1978). Thus, moderate concentrations of multivalent cations, such as those encountered in the cell, do not preclude DNA bending by charge interactions with the phosphodiester backbone. Additional factors including crystal packing forces (DiGabriele *et al*., 1989) and agents used to promote crystallization (Sprous *et al*., 1995) may influence the conformation of DNA in the crystal.

A simplified Coulombic analysis indicated that the electrostatic potential energies of the transcription activation domains exceed the energy required for DNA bending. Long range electrostatic interactions have been reported previously between the active sites on proteins and distant charged residues (Thomas *et al*., 1995; Gao *et al*., 1996). More detailed calculation of the electrostatic interactions between the transcription activation domains and DNA is not possible because of the lack of structural information about the Fos and Jun transcription activation domains. However, studies of other structurally defined complexes using linearized Poisson–Bolzmann equations predict free energies for long-range charge interactions that are of magnitudes comparable with that required for DNA bending (Zacharias *et al*., 1992; Misra *et al*., 1994; Phillips and Phillips, 1994).

Four different mechanisms of protein-induced DNA bending have been proposed. The mechanism observed most commonly in X-ray crystal and NMR structures is mediated by side chain intercalation between the base pairs in the DNA (J.L.Kim *et al*., 1993; Y.Kim *et al*., 1993; Love *et al*., 1995). A second mechanism is mediated by the arrangement of DNA contact residues on a curved surface, generally in a dimeric protein complex (Brennan *et al*., 1990; Schultz *et al*., 1991; Jin *et al*., 1995; Li *et al*., 1995). DNA bending can also be induced indirectly through interactions between proteins that bind to separate sites on DNA and thereby constrain the intervening DNA in a loop (Lobell and Schleif, 1990; Mandal *et al*., 1990). Finally, it has been shown that neutralization of phosphates on one side of the DNA helix by chemical modification can result in DNA bending (Strauss and Maher, 1994; Strauss *et al*., 1996). Such asymmetric charge neutralization has been proposed to mediate wrapping of DNA around the nucleosome core (Mirzabekov and Rich, 1979).

Fos and Jun induce DNA bending through a mechanism that also involves asymmetric charge interactions. However, in the case of DNA bending by the Fos and Jun transcription activation domains, this asymmetric charge interaction does not alter the local charge distribution of the DNA helix, but imposes a directional electrostatic force on DNA (Figure 8). Mutational analysis of the bZIP domains indicates that DNA bending is directly proportional to the charge of amino acid residues adjoining the basic region, demonstrating that DNA bending by the bZIP domains is also caused by electrostatic interactions (Leonard *et al*., 1997). Such electrostatic forces provide a potentially general mechanism for DNA bending by charged domains that does not require direct contact between the bending domain and DNA.

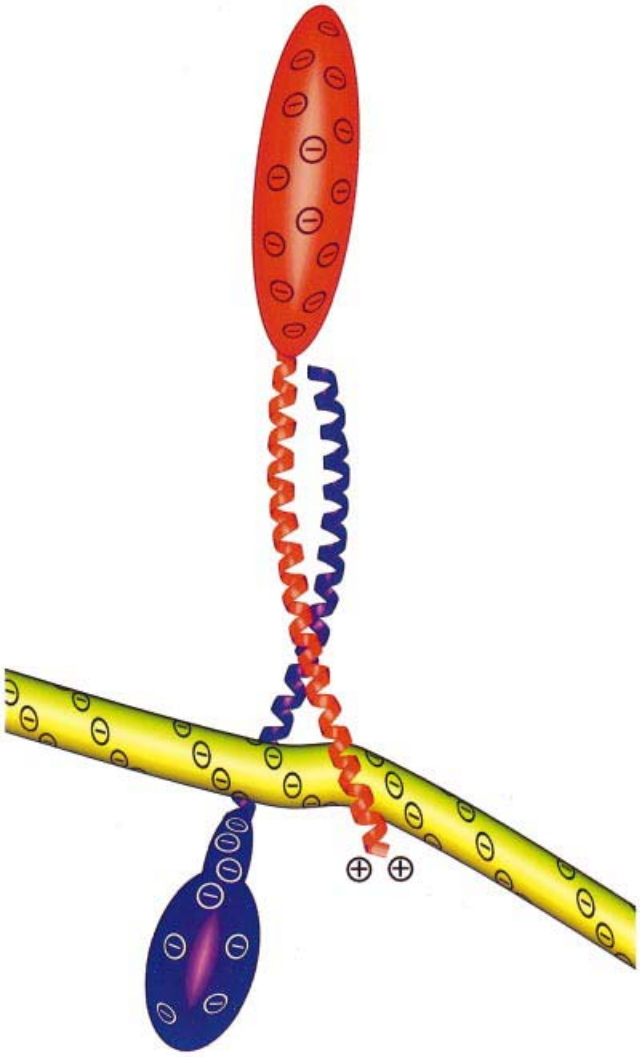


Fig. 8. Fos and Jun induce DNA bending through electrostatic interactions. The relative positions and charges of regions that influence DNA bending by Fos and Jun are indicated schematically. Regions that have no effect on DNA bending are omitted. The bZIP region is shown as α-helical ribbons based on coordinates from the X-ray crystal structure. The transcription activation domains are shown as spheroids linked to the bZIP regions. Charges in both the proteins and DNA are indicated. The path of the DNA helix through the complex is approximated based on the calculated DNA bend angles and directions together with the positions of base pair substitutions that influence DNA bending by Fos and Jun (Rajaram and Kerppola, 1997).

The observation that charge interactions between transcription activation domains and the phosphodiester backbone can induce DNA bending independently of the DNA-binding domain has important implications for eukaryotic transcription regulation. A large number of transcription activation domains contain a high proportion of negatively charged residues. These domains may, in addition to contacting several components of the transcription initiation machinery (reviewed in Goodrich *et al*., 1996), also alter DNA structure. Many transcription fac-

tors, including TATA box-binding protein, have been shown to bind preferentially to distorted DNA structures (Kahn and Crothers, 1992; Pil *et al*., 1993). In addition, DNA bending can promote looping to allow interactions between transcription factors bound to separate recognition elements (Lobell and Schleif, 1990; Mandal *et al*., 1990; Natesan and Gilman, 1993; Falvo *et al*., 1995; Giese *et al*., 1995). Thus, the possibility that many transcription activation domains induce DNA bending indicates that DNA structural changes may be a general mechanism of transcription regulation. The relationship between transcription regulatory domains and DNA bending supports the model that protein-induced changes in DNA structure can control the three-dimensional architecture and functional activity of the transcription complex.

Materials and methods

Plasmid construction and protein purification.

The phasing analysis plasmids pTK401-21, -23, -26, -28 and -30 have been described (Kerppola and Curran, 1991a). Phasing analysis plasmids pNR421–28, -30, -32, -34, -36 and -38, containing the sequence used for X-ray crystallography, were constructed as described (Rajaram and Kerppola, 1997).

Plasmid vectors for expression of chimeric Fos and Jun fusion proteins were constructed in two steps. First, plasmids encoding the minimal bZIP regions of Fos (residues 139–200, FD) and Jun (residues 257–318, JD) containing a *Sal*I site (encoding AST) at the amino-terminus, a *Bgl*II site (encoding RS) at the carboxy-terminus, and an amino-terminal hexahistidine purification tag were constructed by PCR amplification of the respective fragments from expression vectors for the full-length proteins (Abate *et al*., 1991). Likewise, plasmids encoding the transcription activation domains of Fos (residues 206–320, FA) and Jun (residues 100–198, JA) containing a *Bgl*II site at the amino-terminus and a *Sal*I site at the carboxy-terminus were constructed using the same strategy. Plasmids encoding the chimeric proteins were then constructed by ligation of fragments generated by *Sph*I and *Bgl*II digestion of plasmids containing the bZIP domains to plasmids containing the activation domains digested with the same enzymes. Plasmids encoding chimeric proteins containing the domains in the converse order were constructed by ligation of fragments generated by *Sal*I and *Hin*dIII digestion of plasmids containing the bZIP domains to plasmids containing the activation domains digested with the same enzymes. The plasmid encoding the CTF-1 fusion protein was constructed by amplifying the sequence encoding the activation domain of CTF-1 (residues 400–486, CA) from the CTF-1 cDNA clone, and inserting it between the initiation codon and residue 186 in Jun. The sequences of all subcloned fragments and oligonucleotides were confirmed by DNA sequencing. All proteins were purified by nickel chelate affinity chromatography as described previously (Kerppola and Curran, 1991a).

Phasing analysis

Probes were prepared for phasing analysis by amplification of fragments between 349 and 366 bp in length containing the AP-1 site and the intrinsic bend at the center. The separation between the centers of the AP-1 site and the intrinsic DNA bend varied between 21 and 38 bp among the probes, and all probes contained 150 bp flanking the AP-1 site and the intrinsic DNA bend. Intrinsic DNA bend standards containing different numbers of phased A tracts at the middle or at the end of probes were prepared by *Nhe*I and *Bam*HI cleavage of PCR products generated by using plasmids pJT170-2–pJT170-11 (Thompson and Landy, 1988) as templates.

Heterodimers were prepared by incubation of a 2-fold molar excess of the protein containing the Fos bZIP domain with the protein containing the Jun bZIP domain to ensure quantitative heterodimerization. Homo- and heterodimers were incubated with the probes under the conditions described previously (Kerppola and Curran, 1991a). The complexes were analyzed by electrophoresis in 8% polyacrylamide (29:1 acrylamide: bisacrylamide) gels in 25 mM Tris, 195 mM glycine buffer with a field strength of 10 V/cm at 4°C for 22 h. Gels were pre-run for 1 h prior to loading. Where indicated, 1 mM $MgCl_2$, 1 mM spermidine trihydrochloride or 0.1 mM $Co(NH_3)_6Cl_3$ was added to both the gel mix and the running buffer, the running time was extended to compensate for the lower mobility of the complexes under these conditions, and the buffer was recirculated at a rate of one tank volume each hour.

Data analysis

The mobilities of the complexes and the free probes were measured either manually or by automatic band recognition of phosphorimager data. The complex mobilities were normalized for differences in probe mobilities to an average relative mobility of 1. The reproducibility of the relative mobilities was high, with an average relative standard deviation of $<1\%$. To calculate the DNA bend angles and directions, the best fit of the phasing function (Kerppola and Curran, 1991b) to the data was determined. All complexes yielded a good fit to this function (average $r^2 = 0.96$). The direction of protein-induced DNA bending was determined by comparison of the phase of the mobility variation caused by protein binding with that observed for DNA fragments containing two intrinsic bends (Kerppola, 1996). The absolute direction of bending was calculated based on the assumption that A tracts bend DNA toward the minor groove at a position 0.5 bp toward the 3′ end of the center of the A tract (Zinkel and Crothers, 1987; Crothers and Drak, 1992). The DNA bend direction was defined at the center of the AP-1 site such that bending away from the leucine zipper (toward the minor groove) was assigned the value 0°.

The DNA bend angle was calculated from the amplitude of the phasing function. Since the intrinsic and protein-induced DNA bends were closely apposed, the overall shapes of probes containing in-phase and out-of-phase DNA bends were similar to those of probes containing single DNA bends with magnitudes that represent the sum and difference of the two bends. The electrophoretic mobilities of probes containing closely apposed in-phase and out-of-phase bends are comparable with those of probes containing contiguous DNA bends that represent the sum and difference of the individual bends (Kerppola, 1996). Thus, the protein-induced DNA bend angle was calculated by finding the angle which, when added to and subtracted from the reference DNA bend, resulted in DNA bend angles with a mobility difference predicted by the intrinsic bend calibration curve (Figure 8B) that was equivalent to the observed amplitude of the phasing function. The DNA bend angle was expressed in degrees based on the consensus estimate of 18° per A tract (Crothers and Drak, 1992). This purely empirical approach is different from and, based on the better fit of the calibration function, more accurate than the approach used previously (Kerppola and Curran, 1991b), which was based on the presumed dependence of electrophoretic mobility on the end to end distance of a DNA fragment (Thompson and Landy, 1988). Protein-induced DNA bending is dynamic, and the DNA bend angles were calculated by comparison with intrinsic DNA standards that may differ in flexibility. The calculated DNA bend angles are therefore intended primarily for comparison of bending among related protein–DNA complexes.

Acknowledgements

We thank Nirmala Rajaram for construction of phasing analysis plasmids, and Ronald Diebold, David Engelke, David Leonard, Neelam Taneja and Dennis Thiele for critical comments on the manuscript. T.C. received support from the NIH Cancer Center Support CORE grant P30 CA21765 and from ALSAC.

References

Abate,C., Luk,D. and Curran,T. (1991) Transcriptional regulation by Fos and Jun *in vitro*: interaction among multiple activator and regulatory domains. *Mol. Cell. Biol.*, **11**, 3624–3632.

Ansari,A.Z., Bradner,J.E. and O'Halloran,T.V. (1995) DNA-bend modulation in a repressor-to-activator switching mechanism. *Nature*, **374**, 371–375.

Becker,J.C., Nikroo,A., Brabletz,T. and Reisfeld,R.A. (1995) DNA loops induced by cooperative binding of transcriptional activator proteins and preinitiation complexes. *Proc. Natl Acad. Sci. USA*, **92**, 9727–9731.

Brennan,R.G., Roderick,S.L., Takeda,Y. and Matthews,B.W. (1990) Protein–DNA conformational changes in the crystal structure of a lambda Cro–operator complex. *Proc. Natl Acad. Sci. USA*, **87**, 8165–8169.

Crothers,D.M. and Drak,J. (1992) Global features of DNA structure by comparative gel electrophoresis. *Methods Enzymol*., **212**, 46–71.

Crothers,D.M., Haran,T.E. and Nadeau,J.G. (1990) Intrinsically bent DNA. *J. Biol. Chem.*, **265**, 7093–7096.

Dickerson,R.E., Goodsell,D.S. and Neidle,S. (1994) '...the tyranny of the lattice...'. *Proc. Natl Acad. Sci. USA*, **91**, 3579–3583.

DiGabriele,A.D., Sanderson,M.R. and Steitz,T.A. (1989) Crystal lattice packing is important in determining the bend of a DNA dodecamer containing an adenine tract. *Proc. Natl Acad. Sci. USA*, **86**, 1816–1820.

Falvo,J.V., Thanos,D. and Maniatis,T. (1995) Reversal of intrinsic DNA bends in the IFNβ gene enhancer by transcription factors and the architectural protein HMG I(Y). *Cell*, **83**, 1101–1111.

Gao,J., Mammen,M. and Whitesides,G.M. (1996) Evaluating electrostatic contributions to binding with the use of protein charge ladders. *Science*, **272**, 535–537.

Giese,K., Kingsley,C., Kirshner,J.R. and Grosschedl,R. (1995) Assembly and function of a TCR alpha enhancer complex is dependent on LEF-1-induced DNA bending and multiple protein–protein interactions. *Genes Dev.*, **9**, 995–1008.

Glover,J.N. and Harrison,S.C. (1995) Crystal structure of the heterodimeric bZIP transcription factor c-Fos–c-Jun bound to DNA. *Nature*, **373**, 257–261.

Goodrich,J.A., Cutler,G. and Tjian,R. (1996) Contacts in context: promoter specificity and macromolecular interactions in transcription. *Cell*, **84**, 825–830.

Griffith,J.D., Wang,Y.H., Kerppola,T., Curran,T., Zawel,L. and Reinberg,D. (1994) Electron microscopic visualization of active transcription complexes. *J. Cell Biochem.*, **S18C**, 12.

Grigoriadis,A.E., Wang,Z.Q., Cecchini,M.G., Hofstetter,W., Felix,R., Fleisch,H.A. and Wagner,E.F. (1994) c-Fos: a key regulator of osteoclast–macrophage lineage determination and bone remodeling. *Science*, **266**, 443–448.

Hilberg,F., Aguzzi,A., Howells,N. and Wagner,E.F. (1993) c-jun is essential for normal mouse development and hepatogenesis. *Nature*, **365**, 179–181.

Hoover,T.R., Santero,E., Porter,S. and Kustu,S. (1990) The integration host factor stimulates interaction of RNA polymerase with NIFA, the transcriptional activator for nitrogen fixation operons. *Cell*, **63**, 11–22.

Ito,K. *et al*. (1996) c-Jun stimulates origin-dependent DNA unwinding by polyomavirus large T antigen. *EMBO J.*, **15**, 5636–5646.

Jin,Y., Mead,J., Li,T., Wolberger,C. and Vershon,A.K. (1995) Altered DNA recognition and bending by insertions in the α2 tail of the yeast a1/α2 homeodomain heterodimer. *Science*, **270**, 290–293.

Kahn,J.D. and Crothers,D.M. (1992) Protein-induced bending and DNA cyclization. *Proc. Natl. Acad. Sci. USA*, **89**, 6343–6347.

Kerppola,T.K. (1996) Fos and Jun bend the AP-1 site: effects of probe geometry on the detection of protein induced DNA bending. *Proc. Natl Acad. Sci. USA*, **93**, 10117–10122.

Kerppola,T.K. and Curran,T. (1991a) Fos–Jun heterodimers and Jun homodimers bend DNA in opposite orientations: implications for transcription factor cooperativity. *Cell*, **66**, 317–326.

Kerppola,T.K. and Curran,T. (1991b) GDNA bending by Fos and Jun: the flexible hinge model. *Science*, **254**, 1210–1214.

Kim,J.L., Nikolov,D.B. and Burley,S.K. (1993a) Co-crystal structure of TBP recognizing the minor groove of a TATA element. *Nature*, **365**, 520–527.

Kim,Y., Geiger,J.H., Hahn,S. and Sigler,P.B. (1993b) Crystal structure of a yeast TBP/TATA-box complex. *Nature*, **365**, 512–520.

Konig,P. and Richmond,T.J. (1993) The X-ray structure of the GCN4-bZIP bound to ATF/CREB site DNA shows the complex depends on DNA flexibility. *J. Mol. Biol.*, **233**, 139–154.

Leonard,D.A., Rajaram,N. and Kerppola,T.K. (1997) Structural basis of DNA bending and oriented heterodimer binding by the bZIP domains of Fos and Jun. *Proc. Natl Acad. Sci. USA*, **94**, 4913–4918.

Li,T., Stark,M.R., Johnson,A.D. and Wolberger,C. (1995) Crystal structure of the MATa1/MAT alpha 2 homeodomain heterodimer bound to DNA. *Science*, **270**, 262–269.

Lobell,R.B. and Schleif,R.F. (1990) DNA looping and unlooping by AraC protein. *Science*, **250**, 528–532.

Love,J.J., Li,X., Case,D.A., Giese,K., Grosschedl,R. and Wright,P.E. (1995) Structural basis for DNA bending by the architectural transcription factor LEF-1. *Nature*, **376**, 791–795.

Mandal,N., Su,W., Haber,R., Adhya,S. and Echols,H. (1990) DNA looping in cellular repression of transcription of the galactose operon. *Genes Dev.*, **4**, 410–418.

Manning,G.S. (1978) The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. *Q. Rev. Biophys.*, **11**, 179–246.

Martin,M.L., Lieberman,P.M. and Curran,T. (1996) Fos–Jun dimerization promotes interaction of the basic region with TFIIE-34 and TFIIF. *Mol. Cell. Biol.*, **16**, 2110–2118.

Metz,R., Bannister,A.J., Sutherland,J.A., Hagemeier,C., O'Rourke,E.C., Cook,A., Bravo,R. and Kouzarides,T. (1994) c-Fos-induced activation of a TATA-box-containing promoter involves direct contact with TATA-box-binding protein. *Mol. Cell. Biol.*, **14**, 6021–6029.

Mirzabekov,A.D. and Rich,A. (1979) Asymmetric lateral distribution of unshielded phosphate groups in nucleosomal DNA and its role in DNA bending. *Proc. Natl Acad. Sci. USA*, **76**, 1118–1121.

Misra,V.K., Hecht,J.L., Sharp,K.A., Friedman,R.A. and Honig,B. (1994) Salt effects on protein–DNA interactions. The lambda cI repressor and *Eco*RI endonuclease. *J. Mol. Biol.*, **238**, 264–280.

Natesan,S. and Gilman,M.Z. (1993) DNA bending and orientation-dependent function of YY1 in the c-*fos* promoter. *Genes Dev.*, **7**, 2497–2509.

O'Neil,K.T., Hoess,R.H. and DeGrado,W.F. (1990) Design of DNA-binding peptides based on the leucine zipper motif. *Science*, **249**, 774–778.

O'Shea,E.K., Klemm,J.D., Kim,P.S. and Alber,T. (1991) X-ray structure of the GCN4 leucine zipper, a two-stranded, parallel coiled coil. *Science*, **254**, 539–544.

Oakley,M.G. and Dervan,P.B. (1990) Structural motif of the GCN4 DNA binding domain characterized by affinity cleaving. *Science*, **248**, ,847–850.

Perez-Mart'n,J. and Espinosa,M. (1993) Protein-induced bending as a transcriptional switch. *Science*, **260**, 805–807.

Phillips,K. and Phillips,S.E.V. (1994) Electrostatic activation of *Escherichia coli* methionine repressor. *Structure*, **2**, 309–316.

Pil,P.M., Chow,C.S. and Lippard,S.J. (1993) High-mobility-group 1 protein mediates DNA bending as determined by ring closures. *Proc. Natl Acad. Sci. USA*, **90**, 9465–9469.

Rajaram,N. and Kerppola,T.K. (1997) DNA bending by Fos–Jun and the orientation of heterodimer binding depend on the sequence of the AP-1 site. *EMBO J.*, **16**, 2907–2916.

Robertson,L.M., Kerppola,T.K., Vendrell,M., Luk,D., Smeyne,R.J., Bocchiaro,C., Morgan,J.I. and Curran,T. (1995) Regulation of c-fos expression in transgenic mice requires multiple interdependent transcription control elements. *Neuron*, **14**, 241–252.

Schultz,S.C., Shields,G.C. and Steitz,T.A. (1991) Crystal structure of a CAP–DNA complex: the DNA is bent by 90 degrees. *Science*, **253**, 1001–1007.

Sprous,D., Zacharias,W., Wood,Z.A. and Harvey,S.C. (1995) Dehydrating agents sharply reduce curvature in DNAs containing A tracts. *Nucleic Acids Res.*, **23**, 1816–1821.

Strauss,J.K. and Maher,L.J.,III (1994) DNA bending by asymmetric phosphate neutralization. *Science*, **266**, 1829–1834.

Strauss,J.K., Roberts,C., Nelson,M.G., Switzer,C. and Maher,L.J.,III (1996) DNA bending by hexamethylene-tethered ammonium ions. *Proc. Natl Acad. Sci. USA*, **93**, 9515–9520.

Sutherland,J.A., Cook,A., Bannister,A.J. and Kouzarides,T. (1992) Conserved motifs in Fos and Jun define a new class of activation domain. *Genes Dev.*, **6**, 1810–1819.

Talanian,R.V., McKnight,C.J. and Kim,P.S. (1990) Sequence-specific DNA binding by a short peptide dimer. *Science*, **249**, 769–771.

Thomas,P.G., Russell,A.J. and Fersht,A.R. (1985) Tailoring the pH dependence of enzyme catalysis using protein engineering. *Nature*, **318**, 375–376.

Thompson,J.F. and Landy,A. (1988) Empirical estimation of protein-induced DNA bending angles: applications to lambda site-specific recombination complexes. *Nucleic Acids Res.*, **16**, 9687–9705.

Zacharias,M., Luty,B.A., Davis,M.E. and McCammon,J.A. (1992) Poisson–Boltzmann analysis of the lambda repressor–operator interaction. *Biophys J.*, **63**, 1280–1285.

Zinkel,S.S. and Crothers,D.M. (1987) DNA bend direction by phase sensitive detection. *Nature*, **328**, 178–181.

Received on October 18, 1996; revised on January 27, 1997