The EMBO Journal Vol.16 No.10 pp.2917–2925, 1997

DNA bending by Fos–Jun and the orientation of heterodimer binding depend on the sequence of the AP-1 site

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Interactions among transcription factors that bind to separate promoter elements depend on distortion of DNA structure and the appropriate orientation of transcription factor binding to allow juxtaposition of complementary structural motifs. We show that Fos and Jun induce distinct DNA bends at different binding sites, and that heterodimers bind to AP-1 sites in a preferred orientation. Sequences on each side of the consensus AP-1 recognition element have independent effects on DNA bending. A single base pair substitution outside the sequences contacted in the X-ray crystal structure alters DNA bending. Substitution of sequences flanking the AP-1 site has converse effects on DNA bending in opposite directions, suggesting that the extent of DNA bending by Fos and Jun is determined in part by the anisotropic bendability of sequences flanking the AP-1 site. DNA bending by Fos and Jun, and the orientation of heterodimer binding are interrelated. Reversal of the orientation of heterodimer binding causes a shift in the direction of DNA bending. The preferred orientation of heterodimer binding is determined both by contacts between a conserved arginine in the basic region of Fos and the central asymmetric guanine as well as the structure of sequences flanking the AP-1 site. Consequently, the structural adaptability of the Fos–Jun–AP1 complex may contribute to its functional versatility at different promoters.

Keywords: basic region/DNA curvature/leucine zipper/phasing analysis/sequence dependence

Introduction

The architecture of the promoter region is critical for the correct assembly of transcription factor complexes and their function in transcription regulation. This architecture is determined by the relative positions and orientations of binding of transcription regulatory proteins and is further elaborated through interactions among these proteins as well as protein-induced changes in DNA structure. Although many transcription regulatory proteins can function when multiple copies of their binding sites are placed upstream of a heterologous transcription initiation site, their activities at such artificial promoters frequently differ from their functions at native promoters (Thanos and Maniatis, 1995). Furthermore, the functions of promoter elements—and by inference the proteins that bind to those elements—are interdependent in transgenic animals (Robertson *et al.*, 1995). Thus, the organization of the regulatory elements in the promoter region and the correct orientation of transcription factor binding to these elements are essential for appropriate transcription regulation in the physiological context.

Fos and Jun participate in the selective activation of transcription of different genes in response to distinct extracellular signals. Structural differences between complexes formed at different binding sites, as well as interactions among transcription factors that bind to separate sequence elements, may contribute to the differences in Fos and Jun function at different promoters. Thus, determination of differences in the structures of Fos–Jun complexes at various binding sites as well as differences in their potential interactions with other transcription factors are important for understanding the specificity of their regulatory functions.

Fos and Jun regulate gene expression by binding to AP-1 sites that frequently share a conserved heptanucleotide recognition sequence. However, sequences outside of this core recognition element also influence the affinity of Fos and Jun binding (Ryseck and Bravo, 1991; Kerppola and Curran, 1994). The molecular basis of recognition of the heptanucleotide core element has been established through X-ray crystallographic analysis of the minimal leucine zipper dimerization and basic DNA-binding (bZIP) domains of Fos and Jun bound to an AP-1 site (Glover and Harrison, 1995). However, no direct contacts to nucleotides located further than four base pairs from the center of the AP-1 site are detected in the crystal. The molecular basis for the recognition of sequences outside of this core element is therefore unknown.

Fos and Jun were originally shown to induce DNA bending by phasing analysis at a consensus AP-1 site (Kerppola and Curran, 1991a,b). Both the bZIP domains as well as regions overlapping the transcription activation domains of Fos and Jun induce DNA bending (Kerppola and Curran, 1997). All of the regions in Fos and Jun that influence DNA bending contain clusters of charged amino acid residues, and DNA bending is reduced in the presence of multivalent cations, suggesting that DNA bending by Fos and Jun is caused at least in part by charge interactions. However, no DNA bending was observed in the X-ray crystal structure of the minimal bZIP regions of Fos and Jun bound to an AP-1 site (Glover and Harrison, 1995).

Studies of the sequence dependence of intrinsic DNA bending by gel electrophoresis and X-ray crystallography have also reached diametrically opposite conclusions. Whereas A tracts are the principal source of DNA bending in gel electrophoresis assays (reviewed in Haran *et al.*, 1994), A tracts are always straight in crystals, and bending is frequently observed within G:C-rich sequences

(Goodsell *et al.*, 1994 and references therein). Both crystal packing forces (DiGabriele and Steitz, 1993) as well as agents used to promote crystallization (Dlakic *et al.*, 1996) can influence the conformation of DNA in the crystal. The relationship between the variability in DNA structure observed in crystals and the conformational dynamics of DNA in solution is controversial (Goodsell *et al.*, 1994; Haran *et al.*, 1994).

The AP-1 site is asymmetric by virtue of the central C:G base pair as well as sequences flanking the heptanucleotide core. Mutational analysis of the AP-1 site as well as UV crosslinking experiments suggest that heterodimers recognize the AP-1 site in an asymmetric manner (Nakabeppu and Nathans, 1989). In contrast, in the X-ray crystal structure, the heterodimer is found to bind to the AP-1 site equally in both orientations (Glover and Harrison, 1995). Furthermore, DNA cleavage studies using Fos and Jun bZIP region peptides coupled to free radical generators at their amino-terminal ends indicated that heterodimer binding to the AP-1 site is orientation-independent (Chen *et al.*, 1995). Thus, although recognition of the core AP-1 sequence is understood on the basis of the X-ray crystal structure, the mechanism of differential recognition of the two half-sites by Fos and Jun remains unknown.

We have examined the sequence dependence of DNA bending by Fos and Jun. We have found that sequences flanking the conserved AP-1 recognition element influence DNA bending independently and that base pairs outside of the region contacted in the X-ray crystal structure affect DNA bending. Heterodimers bind to the AP-1 site in a preferred orientation, and the orientation of heterodimer binding and the direction of DNA bending are interdependent.

Results

To examine DNA bending by Fos and Jun complexes at different AP-1 sites, we constructed probes in which the AP-1 sites were placed adjacent to an intrinsic DNA bend, and the spacing between the AP-1 site and the reference bend was varied over one turn of the DNA helix (Figure 1). DNA bending at the different binding sites was examined by gel electrophoretic phasing analysis. When the protein-induced and intrinsic DNA bends are in phase, they cooperate to increase the overall extent of DNA bending. In contrast, when the two bends are out of phase, they counteract each other and reduce the net DNA bend. Therefore, differences in the relative mobilities of complexes in phasing analysis reflect differences in DNA bending. The DNA bend angle and orientation were calculated by comparison with intrinsic DNA bend standards (Kerppola and Curran, 1997). Since the different AP-1 sites were placed in the same helical phase relationships with the intrinsic DNA bend, comparison of DNA bending at different binding sites does not depend on quantitation of the DNA bend angles, but can be evaluated directly by examination of the relative mobilities of the protein–DNA complexes compared with the probes alone.

Sequences flanking the AP-1 site influence DNA bending

To simplify comparisons between different recognition sequences, we initiated the analysis by using a pseudo-symmetrical binding site (M) in which sequences within nine base pairs from the center of the AP-1 site were palindromic. Bending at this pseudo-symmetrical binding site was compared with bending at a site encompassing the oligonucleotide sequence used in the X-ray crystallographic analysis of DNA bending by Fos and Jun (X) (Figures 2 and 3). All of the complexes examined induced different bends at these binding sites.

To determine the effect of sequences flanking the AP-1 site on DNA bending in different directions, we examined DNA bending by chimeric proteins containing the transcription activation domains of Fos and Jun fused to the minimal bZIP domains (Kerppola and Curran, 1997) (Figures 2 and 3). Changes in the flanking sequences affected DNA bending by all of the complexes. Both homo- and heterodimers formed by chimeras that had the transcription activation domains fused on the carboxy-terminal side of the leucine zipper (i.e. JD-FA and FD-FA:JD, Figures 2 and 3) bent DNA in the same direction

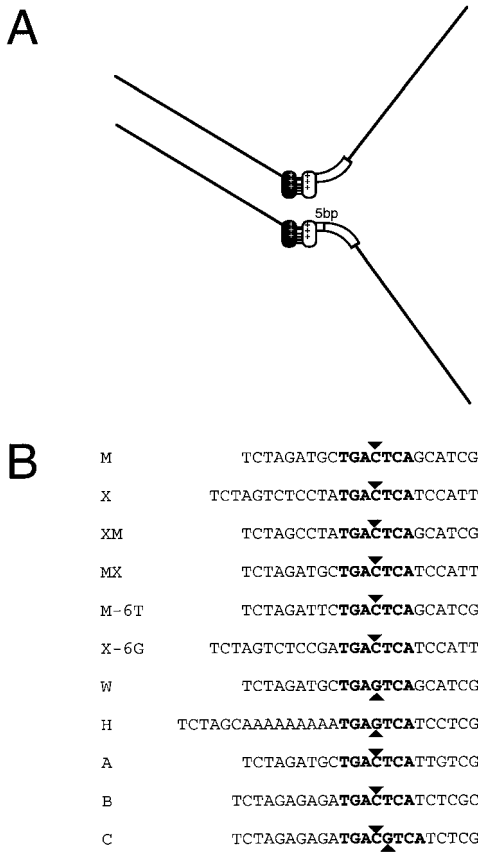

Fig. 1. Analysis of DNA bending at AP-1 sites containing different flanking sequences. (**A**) Comparison of the structures of complexes containing in phase (above) or out of phase (below) intrinsic and protein-induced DNA bends. These complexes differ by the insertion of five base pairs between the protein binding site and the intrinsic bend, resulting in a difference in net DNA bending and electrophoretic mobility. (**B**) Sequences of AP-1 sites used to examine the influence of flanking sequences on DNA bending by Fos and Jun. Flanking sequences shared with the M site are single underlined, whereas flanking sequences shared with the X site are double underlined. A central C:G base pair is indicated by a triangle above the sequence, whereas a central G:C base pair is indicated by a triangle below the sequence. Sequence A represents the consensus AP-1 site used in studies of DNA bending by Fos and Jun (Kerppola and Curran, 1991a,b). Sequences B, C and H have been used for analysis of DNA bending by GCN4 (Gartenberg *et al*., 1990; Paolella *et al*., 1994).

as Fos–Jun heterodimers and induced larger bends at the X site than at the M site. In contrast, homodimers that had the transcription activation domains fused on the amino-terminal side of the basic region (i.e. FA-JD and JA-JD, Figures 2 and 3) bent DNA in the same direction as Jun homodimers and induced larger bends at the M site than at the X site. Heterodimers that had the transcription activation domains fused on the amino-terminal side of the basic region (i.e. FA-FD:JD, Figure 3) induced smaller DNA bends at both sites, since DNA bending by the transcription activation domains was counteracted by the bZIP domain of Fos. The DNA bends induced by these complexes at the M site were directed relatively more toward the zipper than the bends induced at the X site. Thus, sequences flanking the AP-1 site influence DNA bending in a manner that depends on the bend direction.

To determine the relative contributions of base pairs on each side of the AP-1 site to the difference in DNA bending between the M and X sites, we examined bending at sites in which sequences on each side of the AP-1 site were exchanged (MX and XM, Figure 1). The DNA bends induced at the MX and XM sites were generally intermediate between those induced at the M and X sites (Figures 2 and 3). In heterodimeric complexes, DNA bending at the MX site was more similar to bending at the M site, whereas bending at the XM site was more similar to bending at the X site, indicating that sequences on the left side of the AP-1 site were the primary determinant of the difference in DNA bending between these sites by heterodimers. In homodimeric complexes, the differences in bending between the M and MX as well as X and XM sites were larger, indicating that sequences on both sides of the AP-1 site contributed to the difference in DNA bending between these sites. Thus, DNA bending by heterodimers is differentially affected by sequence changes on one side of the AP-1 site whereas DNA bending by homodimers is affected by sequence changes on both sides.

The flanking sequences at the X and M sites differ at several base pairs, including positions –5 and +5 immediately outside the core AP-1 recognition element. To determine if single base pair substitutions in flanking sequences that are not contacted in the X-ray crystal structure could influence DNA bending, we examined the effect of exchanging a single base pair at position –6 between the M and X sites (M-6T and X-6G, Figure 1). Surprisingly, these substitutions resulted in DNA-bending properties that were more divergent than those observed at the M and X sites (Figures 2 and 3). Homo- and heterodimers formed by chimeras with the transcription activation domains fused on the carboxy-terminal side of the leucine zipper induced larger bends at the X-6 site than at the X site. Likewise, homo- and heterodimers formed by chimeras with the transcription activation domains fused on the amino-terminal side of the basic region, induced bending further toward the zipper at the M-6T site than at the M site. The substitution at the M-6T site caused a significant intrinsic DNA bend in sequences flanking the AP-1 site. The contribution of this intrinsic bend to the mobility variation of the protein–DNA complexes is accounted for by normalization to the mobilities of the free probes. Thus, the relative mobilities plotted for complexes bound to this site as well as to the other

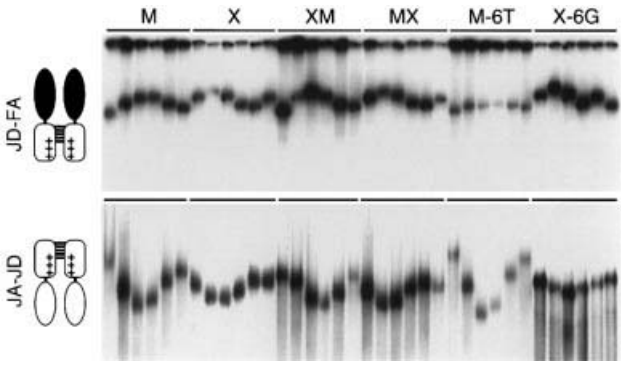

Fig. 2. The sequence of the AP-1 binding site influences DNA bending by Fos and Jun. Chimeric proteins consisting of the transcription activation domains (ovals) of Fos (FA, solid) and Jun (JA, open) fused to the bZIP domains (rounded rectangles) of Fos (FA, solid) and Jun (JD, open) (Kerppola and Curran, 1997) were incubated with phasing analysis probes containing the sites indicated above the lanes (see Figure 1 for descriptions) and the complexes were analyzed on an 8% polyacrylamide gel. Each set of lanes contained probes with 26, 28, 30, 32, 34 and 36 (M, XM, M-6T) or 28, 30, 32, 34, 36 amd 36 (X, XM and X-6G) base pair separations between the centers of the AP-1 site and the reference bend.

sites reflect the effect of protein-induced DNA bending rather than the structure of the binding site in the absence of protein. Consequently, single base pair substitutions at least five base pairs from the center of the AP-1 site influence DNA bending by Fos and Jun.

Sequences flanking the AP-1 site influence DNA bending independently

The pattern of DNA bending induced by each complex at the M, X, MX, XM, M-6T and X-6G sites displays a striking symmetry. The exchange of flanking sequences among these sites resulted in complexes with DNA-bending properties distributed around a common center of symmetry (Figure 4). Thus, the average between the bends induced at two sites that contain the same combination of flanking sequences (i.e. M and X; MX and XM; M-6T and X-6G) is identical for each complex. The only exceptions are the FD:FA–JD and FD:JA–JD complexes bound to the M-6T site, which differ in the orientation of heterodimer binding (see below), and therefore induce distinct DNA bends for reasons unrelated to the sequence dependence of DNA bending. The exchange of flanking sequences between the M and X sites to generate the MX and XM sites resulted in a difference in DNA bending between the M and MX sites that was on average equal in magnitude, but of opposite direction to the difference in bending between the X and XM sites. Likewise, exchange of a single base pair at position –6 between the X and M sites resulted in reciprocal changes in DNA bending at the two sites. Consequently, these sequences contribute independently to DNA bending, confirming and extending the model that the two sides of the AP-1 site contain separate DNA bends (Kerppola and Curran, 1991a,b).

Heterodimers bind to the AP-1 site in a preferred orientation

Our previous results indicate that the transcription activation domains of Fos and Jun induce DNA bending independent of the DNA-binding domains (Kerppola and

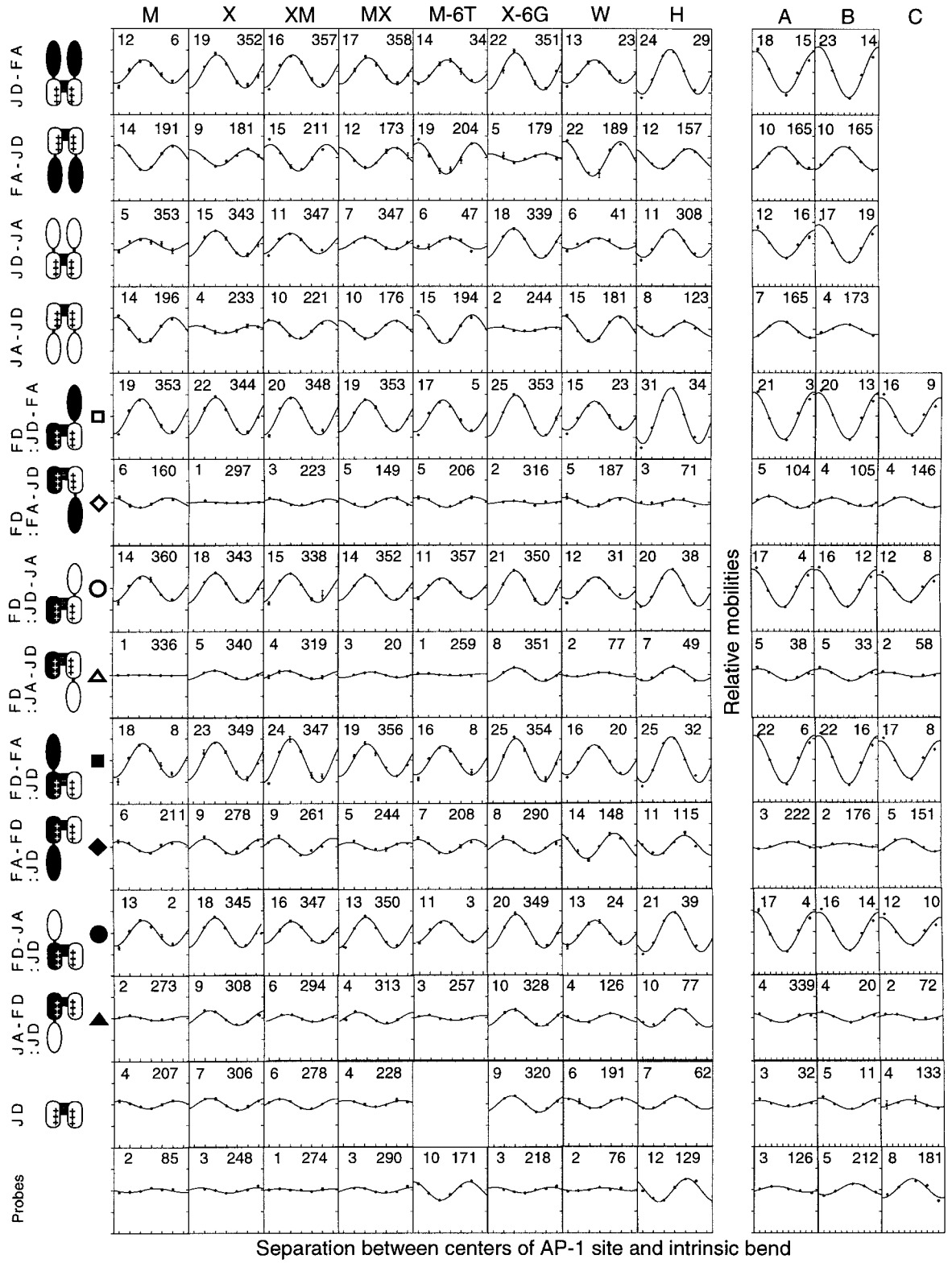

Fig. 3. Quantitation of DNA binding by chimeric Fos and Jun proteins at different binding sites. The relative mobilities of complexes formed by chimeric proteins containing different combinations of the Fos and Jun transcription activation and bZIP domains bound to the AP-1 sites shown above were plotted as a function of the separation between the centers of the AP-1 site and the intrinsic DNA bend. The diagrams on the left indicate the domain organization of the various complexes as discussed in Figure 2 and in Kerppola and Curran (1997). The abscissa is 25 to 39 base pairs for the eight plots on the left, and base pairs 20 to 31 for the three plots on the right, and the ordinate is 0.6 to 1.4. The DNA bend angle and direction are shown in the upper left and upper right corners of each plot. Standard deviations are plotted as vertical bars that are in many cases smaller than the symbols used to plot the data. Multivariate analysis of variance indicated that the differences in DNA bending between all pairs of binding sites were highly significant ($P<0.001$).

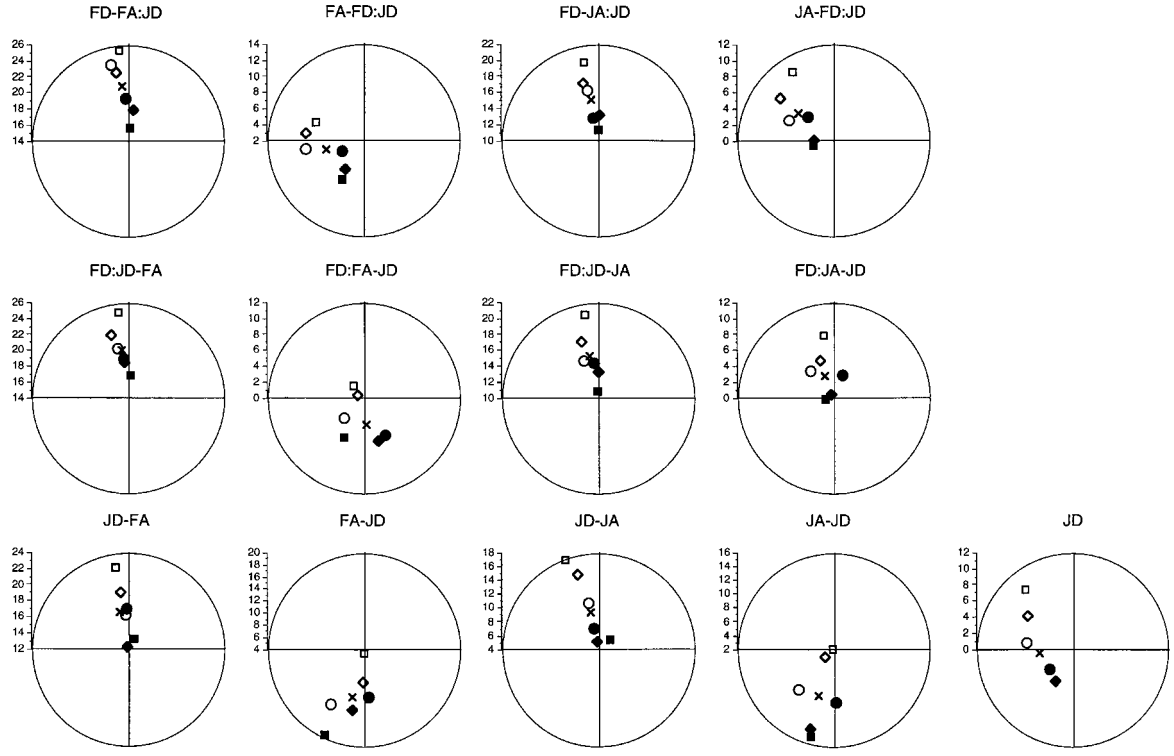

Fig. 4. Sequences flanking the AP-1 site have independent effects on DNA bending. The directions and magnitudes of the DNA bends induced at sites in which flanking sequences were exchanged are shown using polar coordinates (see Figure 5 of Kerppola and Curran, 1997). The complexes are indicated above the plots using the nomenclature described in Figure 2. Bends induced at binding sites containing the same combination of flanking sequences are indicated by open and closed symbols of the same shapes (M: ◆, X: ◇, MX: ●, XM: ○, M-6T: ■, X-6G: □). Thus, each pair of symbols of the same shape represents sites derived by reciprocal exchange of flanking sequences. The center of symmetry among DNA bends induced at these pairs of sites is indicated by an ×. The DNA bend directions and angles are plotted using polar coordinates. The direction of DNA bending is shown clockwise with the value 0 representing bending away from the zipper at the top. The magnitude of the DNA bend is indicated by the distance from the origin (DNA bends induced by different complexes are plotted on different scales to facilitate comparison of bending at different sites by complexes that induced large and small DNA bends).

Curran, 1997). To examine the validity of this model, we calculated the contributions of all protein domains to DNA bending at each binding site (Figure 5). As we observed in our previous studies of DNA bending at the consensus AP-1 site (Kerppola and Curran, 1997), the direction of DNA bending induced by the transcription activation domains depended on the position in the bZIP regions where they were fused. Fusion of the transcription activation domains to opposite ends of the bZIP regions resulted in nearly opposite bend directions. Furthermore, fusion of the transcription activation domains on the amino-terminal sides of the basic regions of Fos and Jun resulted in smaller but significant differences in DNA bend direction. One interpretation of this difference between the DNA bend directions induced by transcription activation domains fused to the bZIP region of Fos versus Jun is that the heterodimer binds to the AP-1 site in a preferred orientation, causing transcription activation domains linked to the basic regions of Fos and Jun to be positioned on different sides of the DNA helix.

To test the hypothesis that the difference in the directions of DNA bending induced by transcription activation domains linked to the bZIP region of Fos versus Jun was due to a preferred orientation of heterodimer binding to the AP-1 site, we examined bending at additional binding sites. The relative directions of DNA bending induced by the transcription activation domains fused to the Fos versus Jun bZIP domains at the M, X, MX, XM and X6G sites were similar (Figure 5), suggesting that Fos–Jun heterodimers bind to these sites in the same preferred orientation. These AP-1 sites share an asymmetric central C:G base pair. To examine the influence of this central base pair on the orientation of heterodimer binding and to explore the relationship between binding orientation and DNA bending, we examined DNA bending at two sites that contained a central G:C base pair. One site (W) is identical to the M site with the exception of transversion of the central C:G base pair to a G:C base pair. The second site (H) is derived from the yeast his3-189 gene (Gartenberg and Crothers, 1990), and contains a central G:C base pair in the context of different flanking sequences.

The relative directions of DNA bending induced by the transcription activation domains fused to the Fos and Jun bZIP domains at the W and H sites were opposite to those observed at sites containing a central C:G base pair (Figure 5). This suggests that heterodimers bind to the W and H sites in the opposite orientation. These results demonstrate that the central base pair in the AP-1 site can influence the direction of DNA bending by transcription activation domains fused to the Fos and Jun bZIP domains, and suggest that the difference in DNA bend direction is caused by reversal of the preferred orientation of Fos–Jun heterodimer binding to the inverted AP-1 site.

All AP-1 sites contain a central asymmetric base pair.

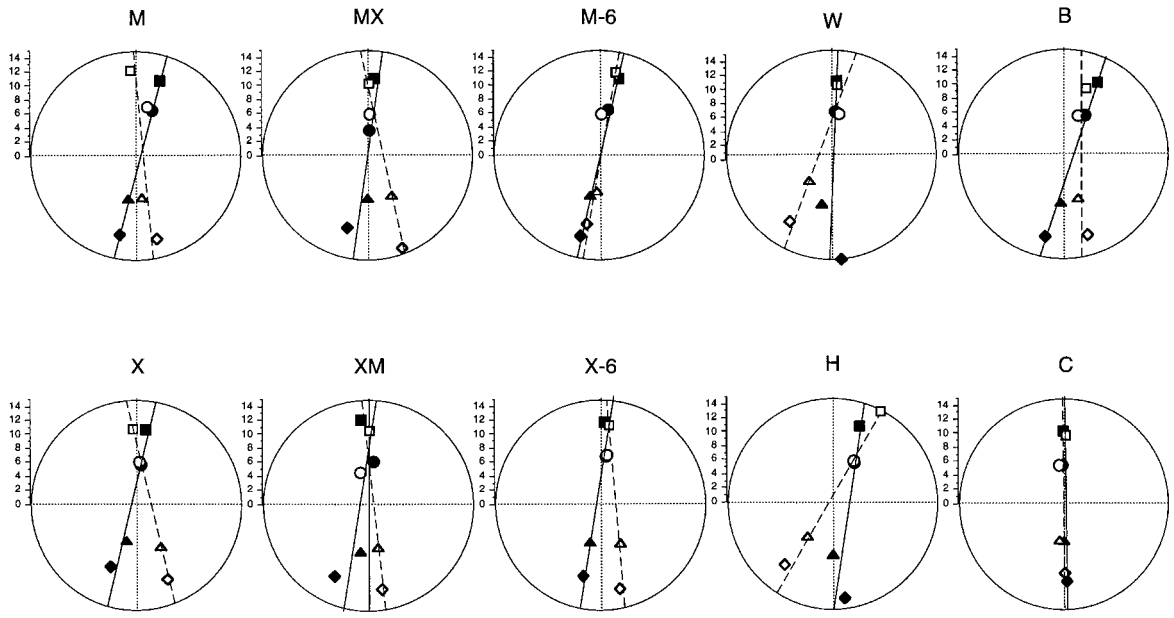

Fig. 5. The transcription activation domains induce different directions of DNA bending when fused to the bZIP domains of Fos and Jun. The DNA bends induced by the Fos and Jun transcription activation domains at different binding sites were calculated based on the model in which DNA bending by each complex represents the sum of independent bends induced by the DNA binding and transcription activation domains, and are shown using polar coordinates as described in Figure 5 of Kerppola and Curran (1997). The binding sites are indicated above the plots. DNA bends induced by transcription activation domains fused to the Fos and Jun bZIP domains are shown by filled and open symbols respectively (diamonds and squares, Fos transcription activation domain fused on the amino- and carboxy-terminal sides of the bZIP domain; triangles and circles, Jun transcription activation domain fused on the amino- and carboxy-terminal sides of the bZIP domain). Lines indicate the difference between the directions of DNA bending induced by transcription activation domains fused to the bZIP region of Fos (solid line) and Jun (dashed line), but are not intended to imply that the DNA bend directions of different complexes are necessarily linearly related.

However, the core of the closely related cyclic AMP response element (CRE) is symmetric by virtue of substitution of the central base pair by a CG dinucleotide. Because of this symmetry, the previous results predicted that the complexes would bind to these sites in both orientations. Analysis of DNA bending at a CRE site (C) and an AP-1 site with identical flanking sequences (B) confirmed that transcription activation domains fused to the Fos and Jun basic regions induce DNA bending in the same direction at the CRE site (Figure 5). Moreover, the direction of bending induced by the transcription activation domains at the CRE site represented the average of the DNA bend directions induced by the transcription activation domains fused to the Fos and Jun basic regions at AP-1 sites. Therefore, either Fos–Jun heterodimers bind to the C site in a manner that places the transcription activation domains fused to the Fos and Jun basic regions on the same side of the DNA helix, or more likely, the heterodimer has no orientation preference for binding to the C site, and the two orientations of binding alternate during gel electrophoresis, resulting in a mobility reflecting the average conformation.

In contrast to the different DNA bends induced by transcription activation domains fused to the Fos and Jun basic regions at other AP-1 sites, at the M-6T site the DNA bends induced by the transcription activation domains fused to the Jun bZIP domain were virtually identical to those induced by the same domains fused to the Fos bZIP domain (Figure 5). However, in contrast to the situation at the CRE site, where the direction of bending induced by transcription activation domains fused to the Fos or Jun basic region was intermediate between those observed at AP-1 sites, at the M-6T site the direction of DNA bending induced by transcription activation domains fused to the basic region of Fos or Jun was similar to the direction of bending induced by transcription activation domains fused to the basic region of Fos at other AP-1 sites containing a central C:G base pair. We therefore favor the hypothesis that heterodimers bind to the M-6T site in a preferred orientation, but that the preferred orientation of binding is determined by the position of the transcription activation domain in the heterodimer. Thus, fusion of the transcription activation domain to the basic region of Fos may cause the heterodimer to bind preferentially to the M-6T site in one orientation, whereas fusion to the basic region of Jun may cause the heterodimer to bind in the opposite orientation. The single base pair difference between the M and M-6T sites causes an intrinsic DNA bend on the left side of the AP-1 site. We hypothesize that the preferred orientation of heterodimer binding at the M-6T site is determined by alignment of this intrinsic bend with the direction of protein-induced bending, which is dictated by the position of the transcription activation domain in the complex.

Since DNA bending by the transcription activation domains is directed away from the side of the DNA helix where they are located (Kerppola and Curran, 1997), their rotational positions relative to the DNA helix can be inferred from the DNA bend directions (Figure 6). Furthermore, since the architecture of the bZIP domain is known (Glover and Harrison, 1995), the rotational position of the transcription activation domain allows determination of the side of the AP-1 site where it is located. Thus, activation domains fused to the amino-terminal end of the basic region of Fos (Figure 5, ◆ and ▲) are preferentially

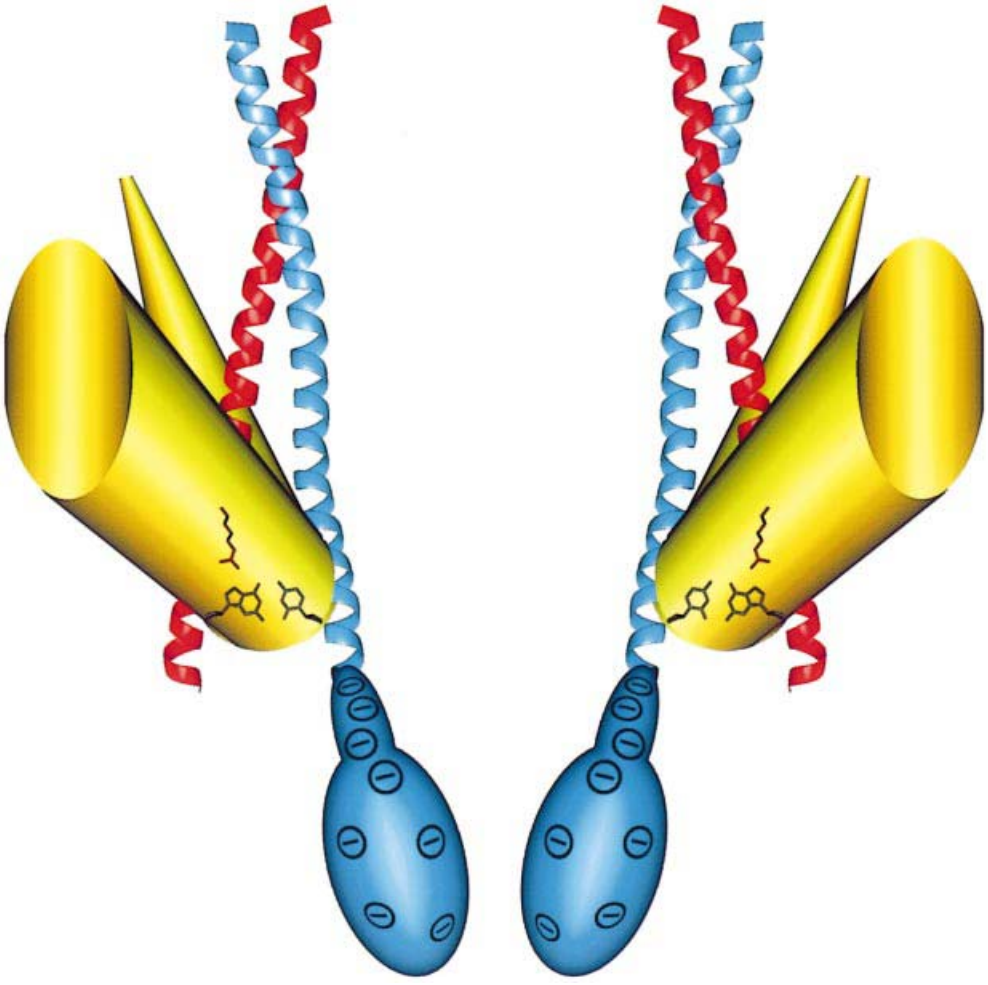

Fig. 6. The orientation of heterodimer binding and the direction of DNA bending are interrelated. Heterodimers are shown bound to AP-1 sites that differ in the central asymmetric base pair. The α-helical bZIP domains of Fos (red) and Jun (blue) and the contact between the conserved arginine in Fos and the central asymmetric base pair in the AP-1 site (gray) are shown based on the X-ray crystal structure coordinates. The difference between the directions of DNA bending induced by the Jun activation domain (blue spheroid) when the complex binds to AP-1 sites in opposite orientations is shown schematically.

located on the left side of AP-1 sites containing a central C:G base pair (excepting the M-6T site) whereas activation domains fused to the amino-terminal end of the basic region of Jun (Figure 5, ◇ and △) are located on the right side. In contrast, activation domains fused to the carboxy-terminal end of the leucine zipper of Fos (Figure 5, ● and ■) are preferentially located on the right side, whereas activation domains fused to the carboxy-terminal end of the leucine zipper of Jun (Figure 5, ○ and □) are located on the left side of recognition sites containing a central C:G base pair. The reciprocal situation applies at the W and H sites. This converse relationship between the positions of the activation domains fused to the amino- and carboxy-termini of the bZIP domain is consistent with the opposite sides of the AP-1 site occupied by the two ends of the bZIP domain as a result of the superhelical intertwining of the coiled coil (see Figure 8 of Kerppola and Curran, 1997).

The positions of the transcription activation domains in the chimeric proteins indicate that the basic region of Fos binds to the left half-site of elements containing a central C:G base pair, and the right half site of elements containing a central G:C base pair. This binding orientation is also consistent with the larger effect of sequence substitutions on the left side of AP-1 sites containing a central C:G base pair on DNA bending by heterodimers (Figures 3 and 4). Based on the contacts observed in the X-ray crystal structure (Glover and Harrison, 1995), this suggests that the guanine nucleotide in the central base pair is preferentially contacted by a conserved arginine residue in Fos, providing a molecular mechanism for the preferred orientation of heterodimer binding to the AP-1 site. Independent evidence in favor of this hypothesis is provided by a shift in the direction of DNA bending caused by substitution of the conserved arginine in the bZIP region of Fos by an isoleucine (Leonard *et al*., 1997). This shift in DNA bend direction is consistent with reversal of the binding orientation of heterodimers containing the mutant protein.

At the M-6T site, the direction of DNA bending indicates that heterodimers bind to the site in an orientation that places the transcription activation domain on the same side of the AP-1 site as the intrinsic DNA bend. Thus, this orientation of binding is consistent with the hypothesis that the binding orientation at the M-6T site is determined

by the optimal alignment of intrinsic and protein-induced DNA bends. Consequently, the orientation of heterodimer binding and the structure of the AP-1 binding site are interrelated.

Discussion

The regulatory elements that mediate transcription factor binding to promoter regions frequently differ from the optimal recognition sequence for those factors. The functional significance of the variation in the recognition sequences at different promoters is for the most part unknown, although differences in binding affinity may contribute to the differential sensitivity of various genes to the concentration of a transcriptional regulator. Different recognition sequences may also confer distinct regulatory properties to transcription factors. Many proteins have distinct functions at different promoters, and in some cases their functional properties can be altered by mutations in their DNA-binding domains (Starr *et al.*, 1996). Thus, differences in the structures of complexes formed by transcription regulatory proteins at different binding sites may contribute to the functional specificities of such complexes.

The structure of DNA in the Fos–Jun–AP-1 complex predicted based on gel electrophoretic phasing analysis (Kerppola and Curran, 1991a,b) does not agree with the structure observed by X-ray crystallographic analysis (Glover and Harrison, 1995).This difference is, in part, explained by the effects of regions outside of the minimal bZIP domains on DNA bending (Kerppola and Curran, 1997). However, the minimal bZIP domains also induce DNA bending detectable by phasing analysis (Kerppola, 1996; Kerppola and Curran, 1997). Additionally, although the sequence of the AP-1 site affects DNA bending by Fos and Jun, the sequence used for crystallization is bent by Fos and Jun in phasing analysis. It has been suggested that protein structure may influence the relative mobilities of complexes in phasing analysis (Sitlani and Crothers, 1996). However, since the sequence of the AP-1 binding site influences the extent of DNA bending by Fos and Jun, the differences in complex mobilities are unlikely to be caused by differences in protein structure. Furthermore, since complexes that bend DNA in opposite directions display the converse sequence dependence of DNA bending, the differences in complex mobilities must involve a directed change in DNA structure such as DNA bending or anisotropic DNA flexibility. Although we cannot distinguish between these two possibilities based on our data, we refer to the phenomenon as DNA bending since anisotropic DNA flexibility induced by protein binding would result in an average DNA conformation that is bent.

The effect of sequences flanking the AP-1 site on DNA bending by Fos and Jun is consistent with the hypothesis that DNA bending is mediated by charge interactions (Kerppola and Curran, 1997). No direct contacts were reported between the minimal bZIP regions of Fos and Jun and nucleotides located further than four base pairs from the center of the AP-1 site based on X-ray crystallographic analysis (Glover and Harrison, 1995). Flanking sequences that promoted DNA bending in one direction impeded bending in the opposite direction, indicating that the variation in DNA bending at different binding sites was caused by sequence-dependent differences in DNA bendability rather than altered contacts with the nucleotide bases. These results imply a mechanism of DNA bending, such as charge interactions with the phosphodiester backbone, that does not rely on sequence-specific DNA contacts.

Previous studies of the sequence dependence of DNA bending by CAP and the sequence periodicities of nucleosome binding sites have yielded similar rankings of dinucleotide bending preferences (Satchwell *et al.*, 1986; Gartenberg and Crothers, 1988). In these rankings, dinucleotides containing only A:T base pairs generally favor bending toward the minor groove, whereas dinucleotides composed of G:C base pairs favor bending toward the major groove. Mixed dinucleotides generally have intermediate properties, although some display stronger preferences. Among AP-1 sites containing different flanking sequences, the order of preference for bending by complexes that induce bending in the same direction as Fos–Jun heterodimers was X-6G $>$ X $>$ XM $>$ MX $>$ M $>$ M-6T. This hierarchy was identical to the ranking of the binding sites based on the G:C content of base pairs flanking the AP-1 site at positions ± 6, ± 7 and ± 8, and was reversed for complexes that bend DNA in the opposite direction. Since Fos–Jun heterodimers are predicted to bend these sequences toward the major groove, this ranking is consistent with the sequence dependence of DNA bending by CAP and nucleosomes (Satchwell *et al.*, 1986; Gartenberg and Crothers, 1988). However, since the base pair at position –6 apparently affected DNA bending independent of other flanking sequences, further studies will be necessary to determine whether the effects of individual base pairs on DNA bending by Fos and Jun are affected by the identities of neighboring base pairs.

We have found that Fos and Jun binding to different AP-1 sites results in distinct DNA bends. Moreover, Fos–Jun heterodimers bind to AP-1 sites in a preferred orientation that is determined by contacts to the asymmetric central base pair and by the structure of sequences flanking the AP-1 site. Thus, one function of DNA bending by Fos and Jun may be to control the orientation of heterodimer binding at different regulatory elements both based on the sequence of the recognition site as well as in response to DNA bending by other proteins. It is interesting that Fos–Jun heterodimers can bind to an AP-1 site adjacent to an NFAT site bound by NFATp with a stronger orientation preference than to the AP-1 site in the absence of NFATp (Chen *et al.*, 1995). Therefore, protein-induced changes in DNA structure may mediate both the cooperative binding of transcription factors to overlapping or adjacent binding sites and folding of the promoter region into a conformation compatible with interactions among multiple regulatory proteins and transcription activation.

Materials and methods

Plasmid construction and protein purification

Phasing analysis plasmids pNR412-26..-36, pNR421-28..-38, pNR413-28..-38, pNR431-26..-38, pNR414-26..36, pNR441-28..-38, pNR502-26..-36 and pNR512-26..-36 (where .. indicates a series spaced by two base pairs) containing sites M, X, MX, XM, M-6T, X-6G, H and W

respectively were constructed by cloning the oligonucleotides shown in Figure 1 between the *Xba*I and *Sal*I sites of plasmids pTK401-26 and pTK401-28 (Kerppola and Curran, 1991a). To generate plasmids with different separations between the centers of the AP-1 sites and the intrinsic DNA bends, oligonucleotides of different lengths containing an additional TGAC or TGACTGAC sequence inserted between the AP-1 site and the intrinsic bend were used. The phasing analysis plasmids pTK401-21..-30, pDP-AP1-21..30 and pDP-CRE-21..30 containing sites A, B and C respectively have been described (Kerppola and Curran, 1991a; Paolella *et al*., 1994). Plasmid vectors for expression of truncated and chimeric Fos and Jun fusion proteins have been described (Kerppola and Curran, 1997).

Probes for phasing analysis were prepared by PCR amplification of fragments between 349 and 366 base pairs in size from the various plasmids and phasing analysis of DNA bending by complexes formed by various Fos and Jun complexes was performed as described (Kerppola and Curran, 1997). The DNA bend angle was calculated from the amplitude of the phasing function by using the relative mobilities of DNA fragments containing intrinsic DNA bends as a calibration curve. The direction of DNA bending was calculated from the minima of the phasing function based on the known orientation of DNA bending by A:T tracts.

Acknowledgements

We thank David Paolella and Alanna Schepartz for providing plasmids pDP-AP1-21..30 and pDP-CRE-21..30, David Leonard, Ronald Diebold and Neelam Taneja for critical comments on the manuscript.

References

Chen,L., Oakley,M.G., Glover,J.N.M., Jain,J., Dervan,P., Hogan,P., Rao,A. and Verdiner,G. (1995) Only one of the two DNA-bound orientations of AP-1 found in solution cooperates with NFATp. *Curr. Biol.*, **5**, 882–889.

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

DiGabriele,A.D. and Steitz,T.A. (1993) A DNA dodecamer containing an adenine tract crystallizes in a unique lattice and exhibits a new bend. *J. Mol. Biol.*, **231**, 1024–1039.

Dlakic,M., Park,K., Griffith,J.D., Harvey,S.C. and Harrington,R.E. (1996) The organic crystallizing agent 2-methyl-2,4-pentanediol reduces DNA curvature by means of structural changes in A-tracts. *J. Biol. Chem.*, **271**, 17911–17919.

Gartenberg,M.R. and Crothers,D.M. (1988) DNA sequence determinants of CAP-induced bending and protein binding affinity. *Nature*, **333**, 824–829.

Gartenberg,M.R., Ampe,C., Steitz,T.A. and Crothers,D.M. (1990) Molecular characterization of the GCN4-DNA complex. *Proc. Natl Acad. Sci. USA*, **87**, 6034–6038.

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.

Goodsell,D.S., Kaczor,G.M. and Dickerson,R.E. (1994) The crystal structure of C-C-A-T-T-A-A-T-G-G. Implications for bending of B-DNA at T-A steps. *J. Mol. Biol.*, **239**, 79–96.

Haran,T.E., Kahn,J.D. and Crothers,D.M. (1994) Sequence elements responsible for DNA curvature. *J. Mol. Biol.*, **244**, 135–143.

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) DNA bending by Fos and Jun: The flexible hinge model. *Science*, **254**, 1210–1214.

Kerppola,T.K. and Curran,T. (1994) A conserved region adjacent to the basic domain is required for recognition of an extended DNA binding site by Maf/Nrl family proteins. *Oncogene*, **9**, 3149–3158.

Kerppola,T.K. and Curran,T. (1997) The transcription activation of Fos and Jun induce DNA bending through electrostatic interactions. *EMBO J.*, **16**, 2917–2926.

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.

Nakabeppu,Y. and Nathans,D. (1989) The basic region of Fos mediates specific DNA binding. *EMBO J.*, **8**, 3833–3841.

Paolella,D.N., Palmer,C.R. and Schepartz,A. (1994) DNA targets for certain bZIP proteins distinguished by an intrinsic bend. *Science*, **264**, 1130–1133.

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.

Ryseck,R.-P. and Bravo,R. (1991) c-Jun, Jun B, and Jun D differ in their binding affinities to AP-1 and CRE consensus sequences: effect of Fos proteins. *Oncogene*, **6**, 533–542.

Satchwell,S.C., Drew,H.R. and Travers,A.A. (1986) Sequence periodicities in chicken nucleosome core DNA. *J. Mol. Biol.*, **191**, 659–675.

Sitlani,A. and Crothers,D.M. (1996) Fos and Jun do not bend the AP-1 recognition site. *Proc. Natl Acad. Sci. USA*, **93**, 3248–3252.

Starr,D.B., Matsui,W., Thomas,J.R. and Yamamoto,K. (1996) Intracellular receptors use a common mechanism to interpret signaling information at response elements. *Genes Dev.*, **10**, 1271–1283.

Thanos,D. and Maniatis,T. (1995) Virus induction of human IFN beta gene expression requires the assembly of an enhanceosome. *Cell*, **83**, 1091–1100.

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