The endonuclease isoschizomers, *Sma*I and *Xma*I, bend DNA in opposite orientations

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ABSTRACT

The *Sma*I and *Xma*I endonucleases are imperfect isoschizomers that recognize the sequence CCCGGG. *Sma*I cleaves between the internal CpG to produce blunt end scissions whereas *Xma*I cleaves between the external cytosines to produce a four base, five prime overhang. Each of the endonucleases forms stable, specific complexes with DNA in the absence of magnesium. Circular permutation analyses of the protein-DNA complexes revealed that each of the endonucleases induces bending of the DNA. Phase sensitive detection analyses verified the existence of the *Sma*I and *Xma*I induced bends. Furthermore, bending of the helix axis by the endonucleases appeared to be directed in opposite orientations. The orientation of the *Sma*I induced bend appeared to be towards the major groove and is reminiscent of the direction of the bend induced by *EcoRV* which similarly induces blunt end scissions. Conversely, *Xma*I appeared to bend the DNA towards the minor groove.

INTRODUCTION

DNA may exhibit a bent or curved conformation derived from either sequence-specific intrinsic curvature(1) or from ligand-induced bending arising from the binding to the DNA of proteins(2) or small ligands such as spermine(3). Stable, intrinsic DNA curvature is frequently characterized by poly A-T tracts in phase with the helical repeat(4,5) and occurs in sequences such as kinetoplast DNA(6) and at the origin of replication of SV40(7) and lambda(8). GC rich sequences have also been implicated as loci of intrinsic curvature(9).

Protein-induced DNA bending is significant not only in the formation of complex macromolecular structures, such as the nucleosome(10,11) but also plays an important role in a number of cellular processes including the initiation of replication, site specific recombination and transcription(12). The functional significance of protein induced DNA bending is well illustrated by the prokaryotic catabolite gene activator protein(CAP) and the eucaryotic transcription factors Fos and Jun. The CAP protein has been shown by both crystal structure(13) and solution studies(14,15,16) to induce a bend of approximately 90° in the promoter of the lac operon. The relevance of the CAP induced

bending has been illustrated by studies in which replacement of the CAP binding site with intrinsically bent DNA is sufficient to activate transcription *in vitro*(17). The CAP-induced bending is proposed to facilitate protein-protein and/or protein-DNA interactions which are necessary for transcription but not sterically feasible with a linear promoter(17). The ability to activate transcription by inducing DNA bending has similarly been shown for other transcriptional regulatory proteins(18).

The Fos and Jun transcription factors are members of the AP1 family of regulatory proteins which recognize the consensus sequence, TGACTCA(19). The proteins contain the leucine zipper motif, which facilitates dimerization and which is adjacent to the basic DNA binding domain(20). Jun may exist as a homodimer or as a Fos/Jun heterodimer. In the regulation of the prolactin gene the homodimers and heterodimers exert opposite effects: the Jun-Jun homodimer activates expression whereas the heterodimer represses transcription(21). Protein-induced bending of DNA has been detected(22,23) for both the Jun homodimer (79°) and Fos/Jun heterodimer (94°). Significantly, the bends are directed in opposite orientations which provides a potential mechanism by which the proteins differentially influence gene expression(21).

Protein-induced DNA conformational changes have also been detected in the sequence specific interactions of the type II endonucleases. The crystal structure of the *EcoRI*-DNA complex(24-26) reveals that the protein unwinds the DNA by approximately 25° which leads to widening of both the major and the minor groove and there is altered base stacking across the center of the recognition hexanucleotide(26). The increased separation of the sugar phosphate backbone is essential for the penetration and correct orientation of the protein into the major groove of the DNA(26). Binding of *EcoRI* has been estimated to induce an approximate 50° bend in the DNA(16). Solution studies have shown that *Rsa*I, a perfect isoschizomer of *EcoRI*, similarly induces unwinding and bending of the GAATTC recognition sequence(27). The crystal structure of the *EcoRV*-DNA complex has also recently been solved(28). *EcoRV* similarly appears to unwind the DNA: however, as depicted by Winkler(28) the DNA axis bends towards and compresses the major groove.

The three endonucleases for which DNA conformational changes have been detected recognize predominantly A-T rich

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sequences. Such sequences may be expected to facilitate protein-induced DNA conformational changes. To date, there have been no other studies of the restriction enzymes to ascertain whether DNA conformational changes exhibited by these enzymes are a property of the endonucleases in general.

The endonucleases *SmaI* and *XmaI* are imperfect isoschizomers that recognize the sequence CCCGGG. *SmaI* cleaves between the internal CpG to yield a blunt end scission whereas *XmaI* cleaves between the external cytosines to yield cohesive termini. Each of the endonucleases form stable, specific complexes with DNA in the absence of magnesium. Solution studies, including circular permutation and helical phasing analyses have been carried out to assess the potential of these endonucleases to induce DNA conformational changes when specifically bound to DNA.

MATERIALS AND METHODS

Material

SmaI, *XmaI* and *EcoRI* endonucleases and T4 polynucleotide kinase were purchased from New England Biolabs. [γ - 32 P]-ATP (>3,000 Ci/mmol) and *Thermus* (HotTub) polymerase were from Amersham. Oligonucleotide primers for PCR amplification were prepared by solid phase phosphoramidate synthesis at the Macromolecular Structure Facility, Michigan State University.

Construction of DNA fragments for bending analyses

DNA fragments used for bending analyses were constructed by PCR amplification of M13mp18 using primers flanking the CCCGGG recognition sequence within the multiple cloning site of the vector. The sequences and location of the primers used to generate the 'permuted' fragments are shown in Fig. 1. PCR amplification reactions contained 300ng plasmid DNA and 450ng each primer and were carried out in 25mM Tris-Cl, pH 9.5, containing 50mM KCl, 10mM MgCl₂, 1mg/ml BSA, 200 μ M of each dNTP and 4% formamide(29). The DNA was subjected

to 35 cycles of amplification with a cycle profile of 94°C, 2min; 52°C, 3min and 72°C for 4min.

Fragments used for phasing analyses were prepared by cloning the following double stranded oligonucleotides into the *Bam*HI site of M13mp18:

- (1) GATCCCAAAAATGTGCAAAAATAGGCAAAAACG
- (2) GATCCTCAAAAATGTGCAAAAATAGGCAAAAACG
- (3) GATCCTCTAGCAAAAATGTGCAAAAATAGGCAAAAACG
- (4) GATCCTCTAGAGCAAAAATGTGCAAAAATAGGCAAAAACG
- (5) GATCCTCTAGAGTCAAAAATGTGCAAAAATAGGCAAAAACG

The *Bam*HI (GGATCC) site in M13mp18(30) overlaps the *SmaI/XmaI* site (CCCGGGGATCC). Ligation of each of the oligonucleotides into the *Bam*HI site positions the centers of the *SmaI/XmaI* site and polyA tract at a distance of 21.5, 23.5, 26.5, 28.5 and 30.5 bp for fragments 1–5, respectively. The plasmids were transfected into XL1-B(31) competent cells and the positive plaques were identified by X-gal blue/white screening. The orientation of the inserts was determined by dideoxy sequencing(32) of single stranded templates and the double stranded plasmid DNA, from those of correct polarity, was isolated by alkaline lysis. The DNA was amplified, using primer set B (Fig.1), to create a series of fragments containing approximately 235 base pairs and with the center of the intrinsic bend and the center of the endonuclease binding site phased through one helical turn.

Products from the amplification reactions were purified by electrophoresis on 1.5% low melting temperature agarose gels. Gel slices containing the DNA fragments were suspended in 2 volumes of dH₂O and incubated at 65°C for 5 min. The samples were extracted with phenol and chloroform/isoamyl alcohol and the DNA was recovered by ethanol precipitation. Fragments were subsequently 5'-end-labelled with [γ - 32 P]-ATP and the unincorporated label was removed by desalting the sample on a Bio-gel P6 column from which the DNA fragments were eluted with 20% ethanol.

Figure 1. Generation of DNA fragments for use in DNA bending studies. Primers flanking the *SmaI/XmaI* site in M13mp18 and as indicated by the arrows were used in PCR reactions to generate a series of fragments containing the binding site for the endonucleases at variable distances from the end of the fragments. The fragments, A–F, represent amplification of the vector sequences from positions 6220–6417(197bp), 6170–6364(194bp), 6154–6350(196bp), 6130–6326(196bp), 6110–6306(196bp) and 6078–6275(198bp), respectively. The box indicates the relative position of the CCCGGG recognition site in each of the fragments.

Mobility shift assays

Endonuclease-DNA complexes were detected by gel retardation assays(32). *Sma*I-DNA complexes were formed by incubation of 24 units of endonuclease with 2ng DNA in 20mM HEPES, pH 7.0, 10mM KCl, 0.5mM EDTA and 1mM DTT at 25°C. *Xma*I-DNA complexes were formed by incubation of the endonuclease (2 units) with 2ng DNA in 20mM HEPES, pH 7.0, 0.5mM EDTA and 1mM DTT at 37°C. Control assays were also carried out with the *Eco*RI endonuclease. The amplified fragments described above contain an *Eco*RI site 16bp upstream of the *Sma*I/*Xma*I site. *Eco*RI (10 units) was incubated with 2ng DNA at 37°C in 50mM NaCl, 10mM Tris-Cl, pH 7.5, 1mM EDTA and 0.1mM 2-mercaptoethanol. Protein-DNA complexes were resolved on 7% non-denaturing polyacrylamide gels. Electrophoresis was carried out at a constant voltage of 200V in an electrophoresis buffer containing 50mM HEPES and 2mM EDTA, pH 7.5. Protein-DNA complexes were detected by autoradiography of the dried gel. Migration distances of the complexes and the free DNA were determined from densitometric scans of the autoradiograms. Densitometry was carried out on a BioImage laser scanning densitometer and analyzed with Visage system software (Milligen).

Retardation assays were also carried out with methylated fragments. The purified PCR fragments were methylated with *Hpa*II methylase for 1 hour at 37°C in a buffer containing 50mM Tris-Cl, pH 7.5, 10mM EDTA, 5mM 2-mercaptoethanol and 80μM S-adenosylmethionine. After purification and recovery of the DNA by phenol/chloroform/isoamyl alcohol extraction and ethanol precipitation, the samples were digested overnight with 10 units of *Hpa*II endonuclease and subsequently electrophoresed on a 7% polyacrylamide gel. The undigested fragments were isolated and used as substrates for binding assays.

Determination of the bending angle

The extent of endonuclease-induced bending of DNA was estimated by the method of Thompson and Landy(16) from both the empirical equation, $\mu M/\mu E = \cos(\alpha/2)$ (where μM and μE correspond to the electrophoretic mobility when the protein binding site occurs at the middle and end of the fragment, respectively) and also directly from the cosine curve, relating the relative electrophoretic mobility ($\mu M/\mu E$) to a known bending angle. The latter standard curve was constructed using a set of plasmid standards generously provided by Dr A. Landy. The plasmids, containing from two to five phased A:T tracts, were digested with appropriate endonucleases, to yield fragments with the intrinsic bend at either the center or the end of the DNA(16).

For phasing analyses, the relative electrophoretic mobilities were calculated as previously described by Zinkel and Crothers(34).

RESULTS

(a) Protein-induced DNA bending by the *Sma*I and *Xma*I endonucleases

The *Sma*I and *Xma*I restriction endonucleases form stable, specific complexes with DNA in the absence of magnesium (Withers and Dunbar, in preparation). It is, therefore, possible to utilize permutation analysis(33) to assay for endonuclease-induced DNA bending. The permuted fragments used in the current experiments were generated by PCR amplification of DNA using primers flanking the *Sma*I/*Xma*I recognition sequence

in the multiple cloning site of M13mp18. Incubation of each of the endonucleases with each of the PCR fragments resulted in a single retarded band. The endonuclease preparations used in

Figure 2. Electrophoretic mobility of the protein-DNA complexes as a function of the position of the endonuclease binding site. The mobility of the complexes is represented as the relative mobility (migration distance of the complex/migration distance of the free DNA) and the x-axis is plotted as the distance from the center of the recognition site to the 5' end of the fragment on the top strand (open circles) and the bottom strand (closed circles). (A) The position-dependent electrophoretic mobility *Sma*I-DNA, *Xma*I-DNA and *Eco*RI-DNA complexes. The mobilities represent the average of five independent experiments for each of the endonucleases and the standard deviation is indicated by vertical bars. The position of the recognition sequence is indicated by the heavy line. (B) Relative electrophoretic mobility of the fragments used in the bending analyses. 1-6 correspond to fragments A-F, respectively.

(A) *Sma* I (B) *Xma* I (C) *Eco* RI

Figure 3. Mobility shift assays and phasing analyses of DNA bending for the (A) *Sma*I and (B) *Xma*I endonucleases. The electrophoretic mobility of the protein-DNA complexes (upper band in the gel retardation assays) is shown as a function of the distance (spacer length) between the center of the endonuclease binding site and the center of an intrinsic (poly A tract) bend. Fragments 1–5 correspond to spacer lengths of 21.5, 23.5, 26.5, 28.5 and 30.5, respectively. In the graphical representation the mobility of the complexes is normalized to the average mobility of all the complexes and plotted as a function of spacer length (33,34). The relative mobilities are the average of five independent experiments and the standard deviations are shown as vertical bars. (C) Graphical representation of the phasing analyses of the *Eco*RI endonuclease.

these assays contained no detectable cognate methylase activity. In addition, no complexes were detected when the fragments were specifically modified with the *Sma*I methylase (which inhibits binding by both the *Sma*I and *Xma*I endonuclease (Withers and Dunbar, in preparation). Furthermore, the addition of magnesium to assays containing the endonuclease-DNA complexes resulted

in loss of the complexes and the appearance of product bands which are of a size consistent with cleavage at the CCCGGG recognition site. These observations suggest that the retarded complexes represent the *Sma*I-DNA and *Xma*I-DNA complexes and are not derived from minor contaminants present in the endonuclease preparations.

Figure 4. Gel retardation assays of the interaction of *SmaI* endonuclease with (A) control and (B) methylated (CC⁵MeCGGG) substrates. The DNA samples were (1) fragment C and (2) fragment F which contain the recognition site at the middle and end of the fragment, respectively.

The variation of the electrophoretic mobility of the endonuclease-DNA complexes as a function of the position of the protein binding site is shown in Fig.2. The various DNA fragments used as substrates displayed minor differences in electrophoretic mobility. The differences presumably arise from sequence variations between the fragments. Small variations in the mobility of permuted fragments has been previously reported(22,27) and is taken into consideration by calculating the migration of the complexes relative to that of the free DNA for each of the fragments.

Position-dependent mobility was detected for both the *SmaI* and *XmaI* endonucleases. The lowest mobility complex for each of the endonucleases contained the CCCGGG recognition sequence near the center of the fragment. Conversely, the binding site occurred near the ends of the fragment in those complexes which displayed the highest mobility. The bending angle, calculated from the maxima and minima of the curve and using the empirical equation described by Thompson and Landy(16) was estimated at $32 \pm 3^\circ$ ($n=5$) for *SmaI* and $39 \pm 3^\circ$ ($n=5$) for *XmaI*. Estimates derived from the standard curve calibrated with poly A tract DNA yielded values of 40° and 47° for *SmaI* and *XmaI*, respectively. The permutation fragments used in these experiments also contain an *EcoRI* binding site which provides an internal control for the calculation of the bending angles for the *SmaI* and *XmaI* endonucleases. The bend angle for *EcoRI* was determined to be $42 \pm 3^\circ$ from the empirical equation and 52° calculated from the standard curve and compares favourably with the approximate 50° angle which was previously determined(16).

Orientation of endonuclease-induced DNA bending

Helical phasing analyses(33,34,35) allow discrimination between DNA bending and other DNA conformational effects, such as DNA flexure, and also enable determination of the relative orientation of the bend. Such assays were carried out in the current experiments to verify that the position dependent effects observed for the *SmaI*-DNA and *XmaI*-DNA complexes were

a reflection of DNA bending and to determine the orientation of the proposed bend.

Fig. 3 shows the effect on the mobility of the endonuclease-DNA complexes when an intrinsic DNA bend, generated by three phased A/T tracts, is inserted downstream of the CCCGGG recognition site. The locus of intrinsic curvature was varied from 21.5 to 30.5bp from the center of the endonuclease binding site. The mobility of both the *SmaI*-DNA and *XmaI*-DNA complexes was dependent upon the distance (spacer length) between the protein-induced and the poly A tract bend. Furthermore, there was an approximate 10bp periodicity between successive maxima or successive minima. These observations are consistent with the proposal that the position dependent effects detected in the permutation analyses arose, at least in part, from DNA bending.

The *SmaI* and *XmaI* endonucleases also appeared to bend DNA in opposite orientations. The interpretation of the bend direction relies upon the relative position of the centers of the protein and polyA tract induced bends. The center of the recognition site has been assigned as the bend locus for both the *SmaI* and *XmaI* endonucleases and is within the limits of resolution of the minima extrapolated from the circular permutation analyses. The minimum electrophoretic mobility of the *SmaI*-DNA complex occurred when the centers of the endonuclease binding site and the polyA tract DNA were separated by a distance of 26.5bp. Assuming a helical screw of 10.5bp per turn, positive co-operativity therefore occurs when the loci of curvature are separated by 2.5 helical turns and are on opposite sides of the helix. The protein-induced and sequence specific bends therefore appear to be directed in opposite orientations. Since the orientation of poly A tract DNA is reportedly towards the minor groove, *SmaI* appears to bend DNA towards the major groove. In contrast, the minimum electrophoretic mobility of the *XmaI*-DNA complex occurred when the bend centers were in phase, suggesting that the *XmaI* endonuclease bends DNA towards the minor groove. The dependence of the gel mobility on the phasing between the intrinsic bend and *EcoRI* bound to DNA is also shown Fig. 3. The *cis* isomer which represents the minimum electrophoretic mobility occurs when the centers of the *EcoRI* recognition site and the poly A tract are separated by 4.1 helical turns. These observations are consistent with *EcoRI* induced bending towards the minor groove at the center of the recognition site.

The effect of methylation on protein-induced DNA bending

The *SmaI* and *XmaI* modification enzymes are N4 cytosine methyltransferases which methylate the second cytosine (CmCCGGG) of the recognition sequence(29,36). Methylation by the cognate methylase inhibits the activity of the endonucleases by preventing protein binding to the DNA (Withers and Dunbar, in preparation). However, C5 methylation at the central cytosine has also been shown to reduce the catalytic activity of the *SmaI* endonuclease(37). Fragments D and F from the bending studies contain the *SmaI* binding site near the center and end of the fragment, respectively. The fragments were methylated with *HpaII* methyltransferase to yield the CC⁵MeCGGG derivatives for each fragment. The appearance of protein-DNA complexes was similar for both the methylated and unmethylated fragments as shown in Fig.4. C5 methylation, therefore, does not appear to inhibit binding of the endonuclease to the DNA. Furthermore, comparison of the relative mobilities of the protein-DNA complexes for the methylated D and F fragments revealed a

bending angle of $32 \pm 3^\circ$ which is not significantly different from that of the endonuclease induced bending of the control, unmethylated fragment.

DISCUSSION

The present studies provide further examples of endonuclease induced DNA bending. The approximate 40° angle estimated for the *XmaI* induced bend is comparable to that previously reported for the *EcoRI*(16) and *RsrI*(27) endonucleases. Although a consistently smaller, near 30° , bend angle was detected for the *SmaI* endonuclease, phasing analyses revealed a 10bp periodicity consistent with *SmaI*-induced bending of the DNA. Furthermore, there are now several crystal structures, including those of the 434 Cro-DNA complex(38,39) and DNaseI cocomplex(40,41) which have shown the significance of small, $20^\circ - 30^\circ$, bend angles induced by the binding of the proteins to DNA.

There have been a limited number of comparative studies of the interaction with DNA of proteins which have the same or similar recognition sequences. Koudelka(42) has shown that the 434 Cro and 434 repressor proteins bend the same DNA substrate in a similar manner. Protein-induced bending by members of the helix-loop-helix/leucine zipper (b-HLH-ZIP) family of eucaryotic transcription factors has also recently been reported(43). Members of this family which recognize the core sequence, CACGTG, were found to induce bends of comparable size in the DNA recognition fragment. Furthermore, in the examples studied, the bends were all directed towards the minor groove. These observations suggest that proteins of similar specificity and function may induce similar types of DNA conformational changes. Additional evidence for a correlation between DNA bending and functional activity is suggested by the observation that bending by the Jun/Jun homodimer and Fos/Jun heterodimer into the minor and major grooves, respectively, appears to be related to either the activation or repression of the composite glucocorticoid response element in the promoter of the prolactin gene(22).

The *SmaI* and *XmaI* endonucleases also have identical sequence specificity. However, the apparent bending of the DNA in the opposite orientation suggests significant differences in the mechanism by which the endonucleases interact with the same DNA sequence. Since the endonucleases cleave at different positions within the recognition site it is tempting to speculate that the orientation of bending may be related to the cleavage position of the endonucleases. Consistent with this proposal is the apparent analogy between the *SmaI* and *EcoRV* restriction enzymes. The endonucleases each cleave at the center of their respective recognition sites to produce blunt end scissions. Binding by *EcoRV* has been shown to unwind DNA and to generate a large, approximate 90° bend of the helix axis towards the major groove(28). A similar orientation but much smaller bend angle is induced by the *SmaI* endonuclease: however, the CG richness of the *SmaI* recognition site may result in stacking energies which preclude gross conformational changes in the protein-DNA complexes. Nonetheless, bending into the major groove and expansion of the minor groove may be a favourable and perhaps general mechanism for blunt end scission by the typeII endonucleases.

EcoRI, in contrast to *EcoRV*, cleaves between the outer base pairs of its recognition sequence. Comparison of the crystal structures of the *EcoRI* and *EcoRV* protein-DNA complexes revealed that in both cases the DNA is underwound and that the

two central base pairs are essentially unstacked(26,28). However, the interbase pair roll angles at the central bases is of opposite sign for the two enzymes(28). Consequently, protein-induced bending at the center of the respective recognition sequences may be expected to be in opposite orientations for these two enzymes. The results of the phase sensitive detection analysis for the *EcoRI*-DNA complex, which suggest that the DNA is bent towards the minor groove at the center of the recognition site, is consistent with this proposal. *XmaI*, which is analogous to *EcoRI* in that it also cleaves the recognition sequence between the external base pairs, similarly induced bending of the DNA towards the minor groove. The specific structural changes which underlie *SmaI* and *XmaI* induced bending of DNA, however, remain to be determined.

Despite the similarities in the orientation of the *SmaI* and *EcoRV* induced DNA bending these enzymes apparently utilize distinct mechanisms of DNA recognition. In addition to the differences in the magnitude of bending, *SmaI* forms stable specific complexes with DNA in the absence of cofactor whereas *EcoRV* efficiently discriminates between specific and non-specific DNA sequences only in the presence of magnesium(44,45). The mechanism of sequence discrimination by endonucleases is generally not understood. However, the conformation of the 'naked' DNA has been suggested to play a role in specific DNA recognition(46). Crystallographic analysis has revealed kinks in the structure of the DNA adjacent to the *EcoRI* recognition site. It has been further suggested that binding of *EcoRI* may undo the kinks initially present in the flanking sequences(46). Perturbations in the DNA structure has similarly been suggested for other endonuclease binding sites (47-49) including the *BclI* (47,49) and *BamHI* (48,49) recognition sequences. The X-ray crystal structure of an octanucleotide containing the *SmaI/XmaI* recognition site has also been determined(50). Under the conditions used for crystallization the observed structure was consistent with A-form DNA. It remains to be determined, however, whether a similar conformation exists for the *SmaI/XmaI* recognition sequence in solution and in the protein-DNA complexes.

Endonuclease activity is frequently inhibited not only by methylation of the base specifically modified by the cognate methylase but also by methylation of alternative sites within the recognition sequence(51). For the *SmaI* and *XmaI* endonucleases cognate methylation occurs at the N4 position of the second cytosine of the recognition site(36) and inhibits endonuclease binding to the DNA (Withers and Dunbar, in preparation). 5-Methyl cytosine modification of the central CpG also decreases the catalytic activity of the *SmaI* endonuclease(37). Comparison of the *SmaI*-induced bending of the modified and unmodified substrates revealed that methylation had no significant effect on the observed endonuclease induced DNA conformational changes. These results do not preclude an effect of methylation on other potential structural changes such as unwinding of the DNA.

Although only a limited number of endonucleases have been examined to date it appears that DNA bending may be a general property of these enzymes. Furthermore, a correlation may exist between the position of cleavage and the relative orientation of the protein-induced bend. The magnitude of the endonuclease induced bend appears to vary considerably, as evidenced by the *SmaI* and *EcoRV* endonucleases and presumably reflects not only the relative flexibility of the recognition sequence but also the protein structure and topology of the protein-DNA complexes.

The crystal structures of the *EcoRI* (24–26) and *EcoRV* (28) endonucleases suggest that the role of the endonuclease-induced bending and kinking is predominantly that of enhancing local protein-DNA interactions. It is also possible that the energy expended in bending may facilitate unwinding of the DNA as has been implicated for the bacterial RNA polymerase (12) and as suggested for the human and yeast TFIID transcription factor (52). Furthermore, DNA looping has been documented for the *NaeI* endonuclease and suggests that DNA bending may also facilitate long range molecular interactions by the endonucleases (53).

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REFERENCES

- Hagerman, P.J. (1990) *Annu. Rev. Biochem.* 59, 755–781.
- Steitz, T.A. (1990) *Q. Rev. Biophys.* 23, 205–280.
- Feuerstein, B., Pattabiraman, N. and Marton, L. (1990) *Nucl. Acids Res.* 18, 1271–1272.
- Koo, H.S., Wu, H.-M. and Crothers, D.M. (1986) *Nature* 320, 501–506.
- Koo, S.-H. and Crothers, D.M. (1988) *Proc. Natl. Acad. Sci. U.S.A.* 85, 1763–1767.
- Marin, J., Levene, S., Crothers, D.M. and Englund, P.T. (1982) *Proc. Natl. Acad. Sci. U.S.A.* 79, 7664–7668.
- Deb, S., DeLucia, A.L., Koff, A., Tsui, S. and Tegtmeyer, P. (1986) *Mol. Cell. Biol.* 6, 4568–4584.
- Zahn, K. and Blattner, F.R. (1987) *Science* 236, 416–422.
- Koepsel, R. and Khan, S.A. (1986) *Science* 233, 1316–1318.
- Travers, A. and Klug, A. (1987) *Nature* 327, 280–281.
- Hogan, M.E., Rooney, T.F. and Austin, R.H. (1987) *Nature* 328, 554–557.
- Travers, A. (1990) *Annu. Rev. Biochem.* 58, 427–452.
- Schultz, S.C., Shields, G.C. and Steitz, T.A. (1991) *Science* 253, 1001–1007.
- Wu, H.-M. and Crothers, D.M. (1984) *Nature* 308, 509–513.
- Kim, J., Zwieb, C., Wu, C. and Adhya, S. (1989) *Gene* 85, 15–23.
- Thompson, J.F. and Landy, A. (1988) *Nucl. Acids Res.* 16, 9687–9705.
- Goodman, S.D. and Nash, H.A. (1989) *Nature* 341, 251–254.
- Perez-Martin, J. and Espinosa, M. (1991) *EMBO J.* 10, 1375–1382.
- Mitchell, P. and Tjian, R. (1989) *Science* 245, 371–378.
- Kerrpola, T.K. and Curran, T. (1991) *Curr. Opin. Struct. Biol.* 1, 71–79.
- Diamond, M.I., Miner, J.N., Yoshinaga, S.K. and Yamamoto, K.R. (1990) *Science* 249, 1266–1272.
- Kerrpola, T. and Curran, T. (1991) *Cell* 66, 317–326.
- Kerrpola, T. and Curran, T. (1991) *Science* 254, 1210–1214.
- McClarín, J.A., Frederick, C.A., Wang, B.-C., Greene, P., Boyer, H.W., Grable, J. and Rosenberg, J.M. (1986) *Science* 234, 1526–1541.
- Kim, Y., Grable, J.C., Love, R., Greene, P.J. and Rosenberg, J.M. (1990) *Science* 249, 1307–1309.
- Rosenberg, J.M. (1991) *Curr. Opin. Struct. Biol.* 1, 114–122.
- Aiken, C.R., Fisher, E.W. and Gumpert, R.I. (1991) *J. Biol. Chem.* 266, 19063–19069.
- Winkler, F. (1992) *Curr. Opin. Struct. Biol.* 2, 93–99.
- Withers, B., Ambrosio, L. and Dunbar, J. (1992) *Nucl. Acids Res.* 20, 6267–6273.
- Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) *Gene* 33, 103–109.
- Bullock, W.O., Fernandez, J.M. and Short, J.M. (1987) *Biotechniques* 5, 376–378.
- Sanger, F., Nicklen, S. and Coulson, A. (1977) *Proc. Natl. Acad. Sci. U.S.A.* 74, 5463–5467.
- Crothers, D.M., Gartenberg, M.R. and Shrader, T.E. (1991) *Methods Enzymol.* 208, 103–107.
- Zinkel, S.S. and Crothers, D.M. (1987) *Nature* 328, 178–181.
- van der Vleit, P.C. and Verrijzer, C.P. (1993) *Bioessays* 15, 25–32.
- Klimasauskas, S., Steponaviciene, D., Maneliene, Z., Petrusyte, M., Butkus, V. and Janulaitis, A. (1990) *Nucl. Acids Res.* 18, 6607–6609.
- Butkus, V., Petrauskienė, L., Maneliene, Z., Klimasauskas, S., Laucys, V. and Janulaitis, A. (1987) *Nucl. Acids Res.* 15, 7091–7102.
- Mondragon, A. and Harrison, S.C. (1991) *J. Mol. Biol.* 219, 321–334.
- Brennan, R.G. (1992) *Curr. Opin. Struct. Biol.* 2, 100–108.
- Suck, D., Lahm, A. and Oefner, C. (1988) *Nature* 332, 465–468.
- Lahm, A. and Suck, D. (1991) *J. Mol. Biol.* 221, 645–667.
- Koudelka, G. (1991) *Nucl. Acids Res.* 19, 4115–4119.
- Fisher, D.E., Parent, L. and Sharp, P. (1992) *Proc. Natl. Acad. Sci.* 89, 11779–11783.
- Taylor, J.D., Badcoe, I.G., Clarke, A.R. and Halford, S.E. (1991) *Biochemistry* 30, 8737–8753.
- Theilking, V., Selent, U., Kohler, E., Landgraf, A., Wolfes, H., Alves, J. and Pingoud, A. (1992) *Biochemistry* 31, 3727–3732.
- Heitman, J. (1992) *Bioessays* 14, 445–453.
- Banks, K., Hare, D. and Reid, B. (1989) *Biochemistry* 28, 6996–7010.
- Kumar, M. R., Hosur, R.V., Roy, K.B., Miles, H.T. and Govil, G. (1985) *Biochemistry* 24, 7703–7711.
- Chuprina, V., Lipanov, A., Federoff, O., Kim, S.-G., Kintanan, A. and Reid, B. (1991) *Proc. Natl. Acad. Sci.* 88, 9087–9091.
- Kennard, O. (1986) Guschlbauer, W. and Saenger, W. (eds.) *DNA-ligand Interactions. From Drugs to Proteins.* Plenum Press, New York, N.Y. pp1–21.
- Nelson, M. and McClelland, M. (1991) *Nucl. Acids Res.* 19, 2045–2071.
- Horikoshi, M., Bertuccioli, C., Takada, R., Wang, J., Yamamoto, Y. and Roeder, R. (1992) *Proc. Natl. Acad. Sci. USA* 89, 1060–1064.
- Topal, M.D., Thresher, R.J., Conrad, M. and Griffith, J. (1991) *Biochemistry* 30, 2006–2010.