MOLECULAR AND CELLULAR BIOLOGY, May 1992, p. 2037–2042
0270-7306/92/052037-06$02.00/0

Vol. 12, No. 5

Binding of the Estrogen Receptor DNA-Binding Domain to the Estrogen Response Element Induces DNA Bending

ANN M. NARDULLI AND DAVID J. SHAPIRO*

Department of Biochemistry, University of Illinois, 1209 West California Street, Urbana, Illinois 61801

Received 2 December 1991/Accepted 4 February 1992

We have used circular permutation analysis to determine whether binding of purified *Xenopus laevis* estrogen receptor DNA-binding domain (DBD) to a DNA fragment containing an estrogen response element (ERE) causes the DNA to bend. Gel mobility shift assays showed that DBD-DNA complexes formed with fragments containing more centrally located EREs migrated more slowly than complexes formed with fragments containing EREs near the ends of the DNA. DNA bending standards were used to determine that the degree of bending induced by binding of the DBD to an ERE was approximately 34°. A 1.55-fold increase in the degree of bending was observed when two EREs were present in the DNA fragment. These in vitro studies suggest that interaction of nuclear receptors with their hormone response elements in vivo may result in an altered DNA conformation.

Although steroid hormone receptors constitute an intensively studied family of ligand-regulated transcription factors (reviewed in references 1 and 6), a detailed mechanism for transcription activation by nuclear receptors has not been described. It is thought that steroid hormone receptors activate transcription either directly by protein-protein contacts, which facilitate interaction between components of the basal transcription apparatus and the promoter, or indirectly by stabilizing binding of other transcription factors to their recognition sequences (33, 37, 38). It has also been suggested that steroid receptors may act by changing the organization of nucleosomes which contain transcription factor binding sites (14, 26, 29) and that DNA supercoiling may influence the interaction of steroid receptors with hormone response elements (27).

Delineation of the mechanisms of steroid hormone interaction with DNA has been hampered by difficulties involved in isolating sufficient quantities of biologically active receptor protein. However, it has been possible to express and purify large quantities of the receptor region responsible for specific interaction with DNA, the DNA-binding domain (DBD). Purified DBDs have therefore been widely used to study steroid receptor-DNA interactions. Detailed information about both DBD structure and the interaction of this region of the receptor with hormone response elements has been obtained by nuclear magnetic resonance and X-ray crystallography of the purified glucocorticoid and estrogen receptor DBDs (7, 15, 21, 34). In addition, the estrogen (25, 43), progesterone (12, 19), and glucocorticoid (5, 8, 23) receptor DBDs have been shown to retain at least some ability to activate transcription of responsive genes.

Altered electrophoretic mobility has been used in several prokaryotic systems to demonstrate that transcription activators and repressors bind to target DNA and cause it to bend (20, 45, 46). Although several eukaryotic transcription factors have also been shown to bend target DNA (13, 16, 24, 32, 36, 40, 41), other DNA-binding proteins, including the transcription factors GCN4 and NF1 and the *cro* repressor, do not induce DNA bending (9, 40, 46). It was of considerable interest to determine whether a member of the steroid receptor family bends DNA upon binding to a hormone response element. We therefore examined whether the purified, bacterially expressed *Xenopus laevis* estrogen receptor (XER) DBD is capable of bending an estrogen response element (ERE)-containing DNA fragment. We demonstrate that binding of the estrogen receptor DBD to an ERE causes the DNA to bend and that the degree of bending is greater when two EREs are present.

MATERIALS AND METHODS

Construction of ERE bending vectors. The ERE Bend I vector containing a single consensus ERE was made by using the polymerase chain reaction to amplify the ERE and the surrounding polylinker sequence present in plasmid TATA/1ERE(−74) (2) and to destroy the *Hin*dIII and *Bam*HI sites present in the polylinker and introduce new *Eco*RI and *Bam*HI sites. The 58-bp amplified product was cut with *Eco*RI and ligated to a gel-purified 377-bp *Eco*RI-*Bam*HI fragment derived from pBR322. The ligated fragments were digested with *Bam*HI, gel purified, and ligated to pBR322, which had been cut with *Bam*HI and dephosphorylated. After transformation, ERE Bend I was CsCl purified and sequenced to confirm that the ERE had been inserted correctly.

The ERE Bend II vectors contained either one or two EREs. Plasmids TATA/1ERE(−74) and TATA/2ERE (2) were digested with *Bam*HI and *Hin*dIII to yield one and two EREs, respectively. The EREs were gel purified and electroeluted. The 50-bp fragment containing a single ERE or the 71-bp fragment containing two EREs was ligated to a gel-purified 346-bp *Hin*dIII-*Bam*HI fragment derived from pBR322. After ligation, the DNA was cut with *Bam*HI and fractionated on a low-melting-temperature agarose gel, and bands containing the ERE(s) ligated to the 346-bp pBR322 fragment were isolated. The resulting fragments were ligated to pBR322 that had been cut with *Bam*HI and dephosphorylated. After transformation, the 1 ERE Bend II and 2 ERE Bend II plasmids were CsCl purified and sequenced to confirm that one and two EREs, respectively, had been correctly inserted.

Preparation of the DNA fragments and estrogen receptor DBD for gel retardation assays. DNA bending standards

* Corresponding author.

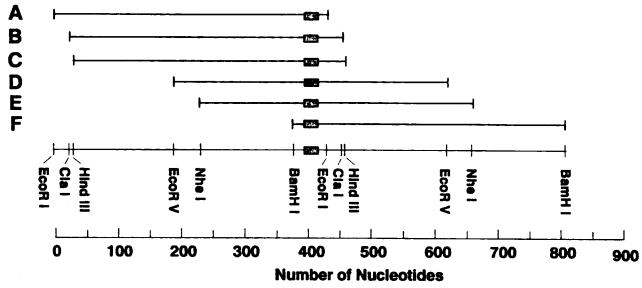

FIG. 1. ERE Bend I DNA fragments used in bending analysis. The DNA bending vector ERE Bend I was digested with either *Eco*RI, *Cla*I, *Hin*dIII, *Eco*RV, *Nhe*I, or *Bam*HI to produce 430-bp DNA fragments with a single consensus ERE at the indicated positions. The numbers of nucleotides contained in each fragment at the 5′ and 3′ ends of the ERE were 395 and 14 (*Eco*RI), 370 and 39 (*Cla*I), 364 and 45 (*Hin*dIII), 205 and 204 (*Eco*RV), 164 and 245 (*Nhe*I), and 18 and 391 (*Bam*HI). The 21-bp ERE sequence (GAT AGGTCACTGTGACCTATC) is designated by the shaded box. The restriction sites in each fragment are noted.

(pJT170-2 to pJT170-7) containing from two to seven $(dA)_6$ tracts were kindly provided by A. Landy (Brown University). They were digested with either *Bam*HI or *Nhe*I and filled in with Klenow fragment in the presence of [α-^{32}P]GTP or [α-^{32}P]CTP, respectively, fractionated on an acrylamide gel, and electroeluted.

ERE-containing DNA bending vectors (ERE Bend I, 1 ERE Bend II, and 2 ERE Bend II) were cut with appropriate enzymes (Fig. 1 and 4), dephosphorylated, labeled by incubation with polynucleotide kinase in the presence of [γ-^{32}P]ATP, and electroeluted after fractionation on acrylamide gels.

Purification of the XER DBD was carried out as we have recently described (25).

Gel retardation assays. Gel retardation assays were carried out essentially as previously described (25). Briefly, 10,000 cpm of the ^{32}P-labeled DNA fragment (150 to 250 pg) was combined with the indicated amounts of purified DBD, 1 μg of poly(dI-dC), 10% glycerol, 80 mM KCl, 15 mM Tris (pH 7.9), 0.2 mM EDTA, and 4 mM dithiothreitol in a final volume of 20 μl and incubated at room temperature for 15 min. Low-ionic-strength gels and buffers were prepared as described previously (4). To maintain a constant temperature during electrophoresis, 4°C water was recirculated through the gel apparatus.

RESULTS

Binding of the DBD to the ERE bends DNA. We have previously shown that the XER DBD binds to an ERE-containing DNA fragment with high specificity (2, 25). To determine whether the binding of the DBD could cause an ERE-containing DNA fragment to bend, a vector containing circularly permuted DNA (ERE Bend I) was constructed, exploiting the fact that DNA fragments bent in the middle migrated more slowly through an acrylamide gel matrix than did DNA fragments bent near the end (39, 45). This vector contained a single ERE with identical 5′-to-3′ sequences on either side of the ERE. By digesting ERE Bend I with different restriction enzymes, we generated a series of six 430-bp fragments containing an ERE at various positions, from the middle of the fragment to near the end of the fragment (Fig. 1). These fragments were identical in size and base composition and differed only in the location of the ERE in the fragment.

In the absence of the purified XER DBD, all of the ^{32}P-labeled DNA fragments migrated similarly, irrespective of the position of the ERE in the fragment (Fig. 2A, −DBD). However, after incubation with the purified DBD, the migration of the ^{32}P-labeled fragments was affected by the position of the ERE in the fragment (Fig. 2A, +DBD). The mobility of the DBD-DNA complex was greatest when the ERE was present near the end of the fragment (Fig. 2A, +DBD, lanes A and F), lowest when the ERE was located in the middle of the fragment (Fig. 2A, +DBD, lane D), and intermediate when the EREs were located at internal positions (Fig. 2A, +DBD, lanes B, C, and E).

The data from five independent experiments, summarized diagrammatically in Fig. 2B, support the idea that the electrophoretic mobility of DBD-DNA complexes was dependent on the position of the ERE within the DNA fragment. To ensure that the gel conditions used were not a factor in the apparent difference in mobility of the DBD-DNA complexes, these experiments were carried out with

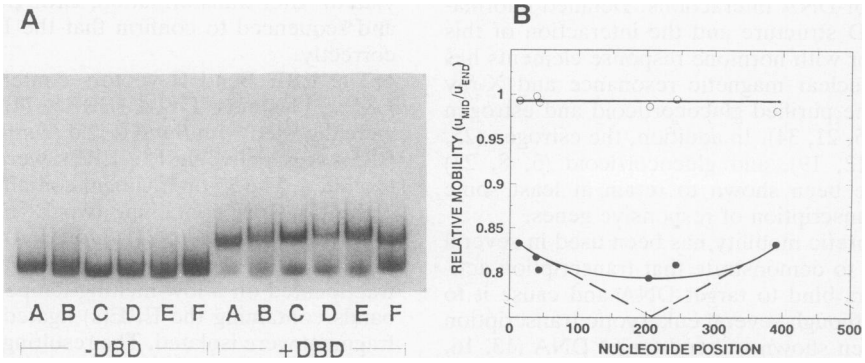

FIG. 2. Evidence that binding of the DBD to an ERE induces DNA bending. (A) ^{32}P-labeled ERE Bend I DNA fragments (see Fig. 1) were incubated in the absence (−DBD) or presence (+DBD) of 200 ng of purified estrogen receptor DBD and fractionated on an 8% acrylamide gel. The gel was dried and subjected to autoradiography. (B) The relative mobility (migration of a DNA fragment relative to the most rapidly migrating fragment) of fragments A to F is plotted as a function of the distance of the ERE from the 3′ end of the DNA. Each point represents the average of five independent experiments, with standard error of the mean of less than 8% for each point. The migration minimum (dashed lines) was determined by extrapolating from the linear ends of the curve and is near the position of the ERE. Data for uncomplexed fragments (○) and for the DBD-DNA complexes (●) are shown.

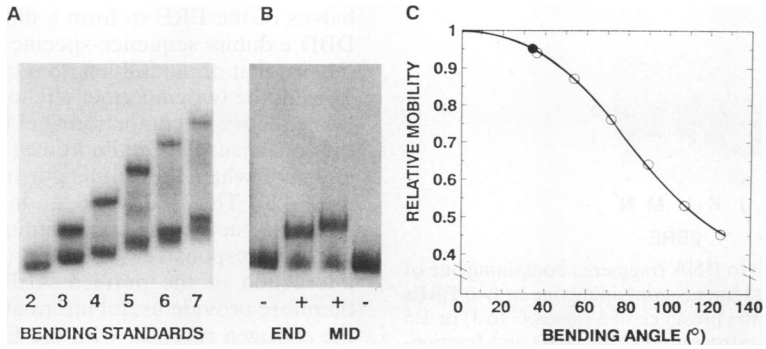

FIG. 3. Determination of the bending angle of a DBD-ERE complex. (A) ^{32}P-labeled bending standards containing two to seven $(dA)_6$ tracts in the middle (upper bands) or at the end (lower bands) of the DNA fragments were fractionated on an 8% acrylamide gel. The gel was dried and subjected to autoradiography. (B) Fragments A and D, which had been end labeled with [γ-^{32}P]ATP and contained a single ERE at either the end (END) or the middle (MID) of the DNA fragment, were incubated in the absence (−) or presence (+) of 200 ng of DBD and fractionated on an 8% acrylamide gel. The gel was dried and subjected to autoradiography. (C) The relative mobilities of the DNA bending standards (○) are plotted as a function of the bending angle. The relative mobility of the DBD-DNA complex, which has virtually the same mobility as the first bending standard, is also shown (●).

3.5, 6, 8, and 10% gels for various periods of time. In all cases, the DBD-DNA complex formed with fragment D, in which the ERE was located in the center of the DNA fragment, had the lowest mobility, complexes with fragments B, C, and E had intermediate mobility, and complexes with fragments A and F had the greatest mobility (data not shown).

Determination of the degree of DBD-induced DNA bending. The differences in the mobility of the ERE-containing DNA fragments appeared to be small. However, it was possible to use the method of Thompson and Landy (39) to determine the degree of DNA bending. In this method, DNA fragments containing $(dA)_6$ tracts, which contain intrinsic bends [18° per $(dA)_6$ tract], were used as bending standards. When two to seven $(dA)_6$ tracts were present in the center of the fragments, migration was less than when equivalent numbers of tracts were present near the ends of the fragments (Fig. 3A, upper and lower bands). By plotting the relative mobility of the DNA bending standards (the ratio of the migration distance of fragments with the DNA bend in the middle to the migration distance of fragments with the bend at the end), a standard curve for DNA bending was obtained. The approximate degree of bending of an unknown DNA fragment could then be determined by comparing its relative migration with the migration of known standards on the curve.

To calculate the bending angle, the relative mobilities of DBD-DNA complexes with EREs in the middle or at the ends of the DNA fragments were compared (Fig. 3B). By comparing the migration of each DBD-DNA complex with the migration of the corresponding uncomplexed DNA fragment, any slight variation in the mobility of the uncomplexed DNA fragments was factored out in the determination of the bending angle. The relative mobility of the ERE Bend I fragments from 14 separate determinations was 0.954 ± 0.004 (standard error of the mean). This was very close to the position on the standard curve of the bending standard containing two $(dA)_6$ tracts and corresponds to a bending angle of approximately 34° (Fig. 3C).

DBD binding to two EREs increased DNA bending. While these data clearly demonstrated that binding of the DBD to a single ERE induced DNA bending, many estrogen-responsive genes contain multiple EREs (17, 35, 42). We therefore examined whether DBD binding to two EREs would result in increased bending. Because we were unable to amplify the palindromic sequence containing two EREs by using the polymerase chain reaction, it was not possible to make a plasmid containing two EREs which was similar to ERE Bend I. Therefore, we constructed a different series of bending vectors, designated 1 ERE Bend II and 2 ERE Bend II, containing one and two EREs, respectively (Fig. 4). The center-to-center spacing between the two EREs was 21 nucleotides, which separated the EREs by two turns of the DNA helix.

In the presence of DBD, fragments containing one or two EREs produced different banding patterns. Binding of DBD to DNA fragments containing a single ERE produced a single DBD-DNA complex (Fig. 5, 1ERE), which we previously reported represented occupancy of both halves of the ERE palindrome by DBD monomers (25). Binding of DBD

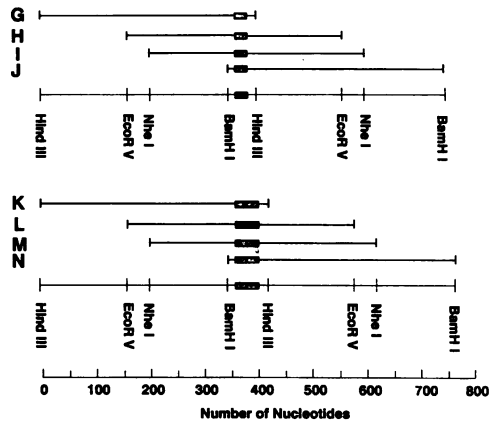

FIG. 4. ERE Bend II fragments containing one or two EREs used in bending analysis. The DNA bending vectors 1 ERE Bend II (fragments G, H, I, and J; one shaded box) and 2 ERE Bend II (fragments K, L, M, and N; two shaded boxes) were digested with either *Hin*dIII, *Eco*RV, *Nhe*I, or *Bam*HI to produce 396- and 417-bp DNA fragments, respectively. The numbers of nucleotides contained in each fragment at the 5′ and 3′ ends of the ERE were 359 and 16 (*Hin*dIII), 200 and 175 (*Eco*RV), 159 and 216 (*Nhe*I), and 13 and 362 (*Bam*HI). The restriction sites and number of nucleotides present in each fragment are shown.

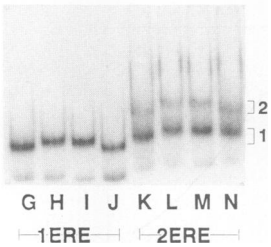

FIG. 5. Binding of the DBD to DNA fragments containing one or two EREs. ^{32}P-labeled DNA fragments containing one or two EREs (see Fig. 4) were incubated in the presence of 1 (lanes G to J) or 1.5 (lanes K to N) μg of purified estrogen receptor DBD and fractionated on an 8% acrylamide gel. The gel was dried and subjected to autoradiography. DBD concentrations used represent a several-thousand-fold molar excess over ERE binding sites. Complexes 1 and 2 represent DBD binding to one and two EREs, respectively.

to DNA fragments containing two EREs produced two DBD-DNA complexes (Fig. 5, 2ERE), with the more rapidly migrating bands representing complexes in which a single ERE was occupied by the DBD (Fig. 5, complex 1) and the more slowly migrating bands representing complexes in which both EREs were occupied by the DBD (Fig. 5, complex 2).

The idea that DBD-DNA interaction bent the DNA was reinforced by studies with the ERE Bend II fragments. DBD-DNA complexes formed with fragments containing internal EREs (Fig. 5, lanes H, I, L, and M) showed reduced mobility compared with complexes formed with fragments containing EREs near the ends of the fragments (lanes G, J, K, and N). Since the DNA fragments formed from digestion of the ERE Bend II vectors did not contain ERE(s) in the center of the DNA fragment, the apparent degree of bending was smaller than with the ERE Bend I vector, and it was not possible to calculate a bending angle from the standard curve. It was, however, still possible to directly compare the migration of DBD-DNA complexes in which the ERE(s) was near the end of the fragment with DBD-DNA complexes in which the ERE(s) was more internal. By scanning the autoradiograms with a densitometer, it was possible to determine the precise positions of the uncomplexed and complexed DNA fragments. We then used the expression described by Thompson and Landy (39) ($\mu_M/\mu_E = \cos \alpha/2$, in which μ is the migration of the DBD-DNA complex divided by the migration of the corresponding uncomplexed DNA fragment when the ERE is near the middle [M] or the end [E] of the DNA fragment) to determine the degree of bending. In four separate experiments, the degree of bending with two EREs was 1.6-, 1.6-, 1.6-, and 1.4-fold greater than with one ERE (Fig. 5; compare complex 2 in lanes K and L and in lanes M and N with complex 1 in lanes G and H and in lanes I and J).

DISCUSSION

The estrogen receptor DBD has many of the properties of the intact XER. Although the XER DBD is a monomer in solution, both the full-length estrogen receptor and the DBD occupy both halves of the ERE palindrome (18, 22, 25, 34). This is presumably due to the presence of a conserved dimerization interface, initially identified in nuclear magnetic resonance and X-ray crystallographic studies of the estrogen and glucocorticoid receptor DBDs (15, 21, 34). The dimerization interface allows DBD monomers bound to both halves of the ERE to form a dimer on the DNA. The XER DBD exhibits sequence-specific binding to the ERE which mimics that of the full-length receptor. When the XER DBD is used, the two imperfect vitellogenin B1 EREs are fourfold less effective in competition gel mobility shift assays than is the consensus ERE palindrome (2) and three- to fourfold less effective when the full-length human estrogen receptor is used (28). The DBD, like the intact receptor, is capable of efficiently activating transcription of a vitellogenin-derived estrogen-responsive promoter (2, 3, 25). Studies of the interaction of the purified XER DBD with the ERE can therefore provide useful information about the interaction of the estrogen receptor with the ERE.

DNA bending by the XER DBD bound to the ERE. In 14 separate experiments, carried out with 3.5 to 10% acrylamide gels, we reproducibly observe that the electrophoretic mobility of the DBD-DNA fragments decreases as the distance of the ERE from the end of the fragment increases and that the fragment with the ERE in the middle shows the slowest mobility. This type of data has been widely interpreted as indicating DNA bending (39, 45).

Determination of the magnitude of DNA bending induced by DBD binding indicates that the degree of bending is not large. The degree of DNA bending (34°) could, however, still be calculated with excellent precision because it corresponds almost precisely to that seen for one of the bending standards. In addition, the same bending angle was determined by using DNA fragments from the *Xenopus* vitellogenin B1 promoter in which the less perfect ERE 1 had been deleted and ERE 2 had been mutated to form a consensus ERE (data not shown).

Although the magnitude of DNA bending observed with one ERE is small, most strongly estrogen-inducible genes including the vitellogenin B1 and B2 genes, and the avian vitellogenin II and apoVLDL II genes (17, 35, 42) appear to contain multiple EREs. To investigate the possibility that multiple EREs might act in concert to produce changes in DNA bending that are greater than those elicited by a single ERE, we examined the effect of two EREs on DNA bending. The 21-nucleotide center-to-center spacing of the two EREs that we used was similar to the 20-nucleotide spacing of the two imperfect vitellogenin B1 EREs (42). Since this spacing separates the EREs by two turns of the DNA helix, it allows bound DBD molecules to be aligned on the same side of the helix. The degree of DNA bending induced by two EREs might therefore represent the additive effect of DNA bending at each of the individual EREs. We found that when two EREs are present, the degree of DNA bending is 1.55-fold greater than is seen for a single ERE (Fig. 5). It is unclear why the increase in DNA bending induced by two EREs is less than twofold. Few studies have examined the effects of multiple DNA-binding sites on DNA bending. Two copies of the simian virus 40 large-T-antigen binding site induced increased DNA bending (31), while two heat shock transcription factor binding sites reduced DNA bending (36). Thus, it appears that multiple binding sites can have different effects on the degree of DNA bending.

Although our demonstration of DNA bending employs the XER DBD, the data may be extrapolated to other systems. The two zinc fingers in the DBDs of the *Xenopus* and human estrogen receptors differ by only one amino acid (10, 11, 44), and the two proteins bind to and protect similar regions of the ERE palindrome (18, 22). The overall structures of the DBDs of the glucocorticoid and estrogen receptors are quite similar (7, 15, 21, 34), suggesting that they interact with DNA through similar mechanisms. However, an X-ray crys-

tallographic study of the complex between the glucocorticoid receptor DBD and the glucocorticoid response element complex did not detect DNA bending (21). This may reflect a genuine difference between the action of the estrogen and glucocorticoid receptors but is more likely due to alterations in DNA contacts caused by the use of a mutant glucocorticoid response element with an increase in spacing between the two halves of the palindrome. Since there have been no other reports of DNA bending by members of the steroid/nuclear receptor gene superfamily, we cannot state with absolute certainty that DNA bending will be a general consequence of binding of steroid receptors to their hormone response elements.

Role of DNA bending in transcription activation. Several transcription regulatory proteins in prokaryotes (20, 45, 46), *Saccharomyces cerevisiae* (41), *Drosophila melanogaster* (36), and vertebrates (13, 16, 24, 32, 40) appear to bend DNA upon binding to their recognition sequences in cell-free systems. These in vitro studies suggest that interaction between many transcription factors and their recognition sequences may result in changes in DNA structure in vivo. A role for DNA bending in transcription activation in vivo has only been inferred, not directly demonstrated (13, 16, 20, 36, 41, 46). A direct role for DNA bending in transcription repression has been suggested by a recent functional study of the phage ϕ29 p4 protein. This regulatory protein bends DNA and represses transcription of the P_{A2b} promoter. Introduction of an intrinsically bent DNA sequence effectively mimics both p4-induced DNA bending and in vitro repression of P_{A2b} transcription (30).

DNA bending induced by binding of the XER DBD to the ERE could serve any of several functions. Bending of the DNA may be necessary to form an effective dimerization interface on the ERE and thus stabilize the interaction of the estrogen receptor with the ERE. Bending of the DNA could serve to facilitate interactions between transcription factors or between transcription factors and components of the basal transcription apparatus. Although it is not possible in this study to determine whether the DNA bend is within or adjacent to the ERE, DNA bending could facilitate recognition of the DNA by other proteins. The energy input required to bend DNA might also kink the DNA in the vicinity of the bend and alter protein binding.

Studies of the mechanism of glucocorticoid induction of transcription of the mouse mammary tumor virus promoter have lent support to the hypothesis that binding of glucocorticoid receptor to one or more glucocorticoid receptor response elements in the 5′-flanking region of this gene shifts the location of a nucleosome and makes an NF1 site accessible (14, 26, 29). It is possible that steroid receptor-induced DNA bending plays a role in nucleosome displacement. In future investigations, it may be possible to delineate the role, if any, of estrogen receptor-induced DNA bending in the overall process of transcription activation.

ACKNOWLEDGMENTS

We are grateful to A. Landy for the gift of the DNA bending standards, to C. Aiken and R. Gumport for useful advice, to M. McCormack for help in preparation of the manuscript, and to S. Teeple for artwork.

This work was supported by grant HD-16720 from the National Institute of Child Health and Human Development and by an American Cancer Society postdoctoral fellowship to A.M.N.

REFERENCES

1. **Beato, M.** 1989. Gene regulation by steroid hormones. Cell **56**:335–344.
2. **Chang, T.-C., A. M. Nardulli, D. Lew, and D. J. Shapiro.** The role of estrogen response elements in expression of the *Xenopus laevis* vitellogenin B1 gene. Mol. Endocrinol., in press.
3. **Chang, T.-C., and D. J. Shapiro.** 1990. An Nf1-related vitellogenin activator element mediates transcription from the estrogen regulated *Xenopus laevis* vitellogenin promoter. J. Biol. Chem. **265**:8176–8182.
4. **Chodosh, L. A.** 1989. Mobility shift DNA-binding assay using gel electrophoresis, p. 12.2.1-12.2.10, *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. D. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. Greene Publishing Associates and Wiley Interscience, New York.
5. **Debs, R. J., L. P. Freedman, S. Edmunds, K. L. Gaensler, N. Düzgünes, and K. R. Yamamoto.** 1990. Regulation of gene expression *in vivo* by liposome-mediated delivery of a purified transcription factor. J. Biol. Chem. **265**:10189–10192.
6. **Evans, R. M.** 1988. The steroid and thyroid hormone receptor superfamily. Science **240**:889–895.
7. **Freedman, L. P., B. F. Luisi, Z. R. Korszun, R. Basavappa, P. B. Sigler, and K. R. Yamamoto.** 1988. The function and structure of the metal coordination sites within the glucocorticoid receptor DNA binding domain. Nature (London) **334**:543–546.
8. **Freedman, L. P., S. K. Yoshinaga, J. N. Vanderbilt, and K. R. Yamamoto.** 1989. *In vitro* transcription enhancement by purified derivatives of the glucocorticoid receptor. Science **245**:298–301.
9. **Gartenberg, M. R., C. Ampe, T. A. Steitz, and D. M. Crothers.** 1990. Molecular characterization of the GCN4-DNA complex. Proc. Natl. Acad. Sci. USA **87**:6034–6038.
10. **Green, S., P. Walter, V. Kumar, A. Krust, J.-M. Bornert, P. Argos, and P. Chambon.** 1986. Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. Nature (London) **320**:134–139.
11. **Greene, G. L., P. Gilna, M. Waterfield, A. Baker, Y. Hort, and J. Shine.** 1986. Sequence and expression of human estrogen receptor complementary DNA. Science **231**:1150–1154.
12. **Guiochon-Mantel, A., H. Loosfelt, T. Ragot, A. Bailly, M. Atger, M. Misrahi, M. Perricaudet, and E. Milgrom.** 1988. Receptors bound to antiprogestin form abortive complexes with hormone responsive elements. Nature (London) **336**:695–698.
13. **Gustafson, T. A., A. Taylor, and L. Kedes.** 1989. DNA bending is induced by a transcription factor that interacts with the human c-Fos and α-actin promoters. Proc. Natl. Acad. Sci. USA **86**:2162–2166.
14. **Hager, G. L., and T. K. Archer.** 1991. The interaction of steroid receptors with chromatin, p. 217–234. *In* M. G. Parker (ed.), Nuclear hormone receptors: molecular mechanism, cellular functions, and clinical abnormalities. Academic Press, San Diego, Calif.
15. **Härd, T., E. Kellenbach, R. Boelens, B. A. Maler, K. Dahlman, L. P. Freedman, J. Carlstedt-Duke, K. R. Yamamoto, J.-A. Gustafsson, and R. Kaptein.** 1990. Solution structure of the glucocorticoid receptor DNA-binding domain. Science **249**:157–160.
16. **Kerppola, T. K., and T. Curran.** 1991. Fos-jun heterodimers and jun homodimers bend DNA in opposite orientations: implications for transcription factor cooperativity. Cell **66**:317–326.
17. **Klein-Hitpass, L., M. Schorpp, U. Wagner, and G. U. Ryffel.** 1986. An estrogen-responsive element derived from the 5′ flanking region of the *Xenopus* vitellogenin A2 gene functions in transfected human cells. Cell **46**:1053–1061.
18. **Klein-Hitpass, L., S. Y. Tsai, G. L. Greene, J. H. Clark, M.-J. Tsai, and B. W. O'Malley.** 1989. Specific binding of estrogen receptor to the estrogen response element. Mol. Cell. Biol. **9**:43–49.
19. **Klein-Hitpass, L., S. Y. Tsai, N. L. Weigel, G. F. Allan, D. Riley, R. Rodriguez, W. T. Schrader, M.-J. Tsai, and B. W. O'Malley.** 1990. The progesterone receptor stimulates cell-free transcription by enhancing the formation of a stable preinitiation complex. Cell **60**:247–257.
20. **Kuhnke, G., C. Theres, H.-J. Fritz, and R. Ehring.** 1989. RNA polymerase and gal repressor bind simultaneously and with DNA bending to the control region of the *Escherichia coli*

galactose operon. EMBO J. **8:**1247–1255.
21. **Luisi, B. F., W. X. Xu, Z. Otwinowski, L. P. Freedman, K. R. Yamamoto, and P. B. Sigler.** 1991. Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. Nature (London) **352:**497–505.
22. **Martinez, E., and W. Wahli.** 1989. Cooperative binding of estrogen receptor to imperfect estrogen-responsive DNA elements correlates with their synergistic hormone-dependent enhancer activity. EMBO J. **8:**3781–3791.
23. **Miesfeld, R., P. J. Godowski, B. A. Maler, and K. R. Yamamoto.** 1987. Glucocorticoid receptor mutants that define a small region sufficient for enhancer activation. Science **236:**423–427.
24. **Moskaluk, C., and D. Bastia.** 1988. DNA bending is induced in an enhancer by the DNA-binding domain of the bovine papillomavirus E2 protein. Proc. Natl. Acad. Sci. USA **85:**1826–1830.
25. **Nardulli, A. M., D. Lew, L. Erijman, and D. J. Shapiro.** 1991. Purified estrogen receptor DNA binding domain expressed in *Escherichia coli* activates transcription of an estrogen-responsive promoter in cultured cells. J. Biol. Chem. **266:**24070–24076.
26. **Piña, B., U. Brüggemeier, and M. Beato.** 1990. Nucleosome positioning modulates accessibility of regulatory proteins to the mouse mammary tumor virus promoter. Cell **60:**719–731.
27. **Piña, B., R. J. G. Haché, J. Arnemann, G. Chalepakis, E. P. Slater, and M. Beato.** 1990. Hormonal induction of transfected genes depends on DNA topology. Mol. Cell. Biol. **10:**625–633.
28. **Ponglikitmongkol, M., J. H. White, and P. Chambon.** 1990. Synergistic activation of transcription by the human estrogen receptor bound to tandem responsive elements. EMBO J. **9:**2221–2231.
29. **Reik, A., G. Schütz, and A. F. Stewart.** 1991. Glucocorticoids are required for establishment and maintenance of an alteration in chromatin structure: induction leads to a reversible disruption of nucleosomes over an enhancer. EMBO J. **10:**2569–2576.
30. **Rojo, F., and M. Salas.** 1991. A DNA curvature can substitute phage φ29 regulatory protein p4 when acting as a transcriptional repressor. EMBO J. **10:**3429–3438.
31. **Ryder, K., S. Silver, A. L. DeLucia, E. Fanning, and P. Tegtmeyer.** 1986. An altered DNA conformation in origin region 1 is a determinant for the binding of SV40 large T antigen. Cell **44:**719–725.
32. **Schreck, R., H. Zorbas, E.-L. Winnacker, and P. A. Baeuerle.** 1990. The NF-κB transcription factor induces DNA bending which is modulated by its 65-κD subunit. Nucleic Acids Res. **18:**6497–6502.
33. **Schule, R., M. Muller, C. Kattschmidt, and R. Renkawitz.** 1988. Many transcription factors interact synergistically with steroid receptors. Science **242:**1418–1420.
34. **Schwabe, J. W. R., D. Neuhaus, and D. Rhodes.** 1990. Solution structure of the DNA-binding domain of the oestrogen receptor. Nature (London) **348:**458–461.
35. **Seiler-Tuyns, A., P. Walker, E. Martinez, A. M. Mérillat, F. Givel, and W. Wahli.** 1986. Identification of estrogen-responsive DNA sequences by transient expression experiments in a human breast cancer cell line. Nucleic Acids Res. **14:**8755–8770.
36. **Shuey, D. J., and C. S. Parker.** 1986. Bending of promoter DNA on binding of heat shock transcription factor. Nature (London) **323:**459–461.
37. **Strähle, U., W. Schmid, and G. Schütz.** 1988. Synergistic action of the glucocorticoid receptor with transcription factors. EMBO J. **7:**3389–3395.
38. **Tasset, D., L. Tora, C. Fromental, E. Scheer, and P. Chambon.** 1990. Distinct classes of transcriptional activating domains function by different mechanisms. Cell **62:**1177–1187.
39. **Thompson, J. F., and A. Landy.** 1988. Empirical estimation of protein-induced DNA bending angles: applications to λ site-specific recombination complexes. Nucleic Acids Res. **16:**9687–9705.
40. **Verrijzer, C. P., J. A. W. M. van Oosterhout, W. W. van Weperen, and P. C. van der Vliet.** 1991. Pou proteins bend DNA via the Pou-specific domain. EMBO J. **10:**3007–3014.
41. **Vignais, M.-L., and A. Sentenac.** 1989. Asymmetric DNA bending induced by the yeast multifunctional factor TUF. J. Biol. Chem. **264:**8463–8466.
42. **Walker, P., J. E. Germond, M. Brown-Luedi, F. Givel, and W. Wahli.** 1984. Sequence homologies in the region preceding the transcription initiation site of the liver estrogen-responsive vitellogenin and apo-VLDL II genes. Nucleic Acids Res. **12:**8611–8625.
43. **Waterman, M. L., S. Adler, C. Nelson, G. L. Greene, R. M. Evans, and M. G. Rosenfeld.** 1988. A single domain of the estrogen receptor confers deoxyribonucleic acid binding and transcriptional activation of the rat prolactin gene. Mol. Endocrinol. **2:**14–21.
44. **Weiler, I. J., D. Lew, and D. J. Shapiro.** 1987. The *Xenopus laevis* estrogen receptor: sequence homology with human and avian receptors and identification of multiple messenger ribonucleic acids. Mol. Endocrinol. **10:**355–362.
45. **Wu, H.-M., and D. M. Crothers.** 1984. The locus of sequence-directed and protein-induced DNA bending. Nature (London) **308:**509–513.
46. **Zwieb, C., J. Kim, and S. Adhya.** 1989. DNA bending by negative regulatory proteins: gal and lac repressors. Genes Dev. **3:**606–611.