Differential Modulation of DNA Conformation by Estrogen Receptors α and β*

The human estrogen receptor (ER) induces transcription of estrogen-responsive genes upon binding to estrogen and the estrogen response element (ERE). To determine whether receptor-induced changes in DNA structure are related to transactivation, we compared the abilities of ERα and ERβ to activate transcription and induce distortion and bending in DNA. ERα induced higher levels of transcription than ERβ in the presence of 17β-estradiol. In circular permutation experiments ERα induced greater distortion in DNA fragments containing the consensus ERE sequence than ERβ. Phasing analysis indicated that ERα induced a bend directed toward the major groove of the DNA helix but that ERβ failed to induce a directed DNA bend. Likewise, the ERα DNA binding domain (DBD) and hinge region induced a bend directed toward the major groove of the DNA helix, but the ERβ DBD and hinge region failed to bend ERE-containing DNA fragments. Using receptor chimeras we demonstrated that the ERα DBD C-terminal extension is required for directed DNA bending. Transient transfection assays revealed that appropriately oriented DNA bending enhances receptor-mediated transactivation. The different abilities of ERα and ERβ to induce change in DNA structure could foster or inhibit the interaction of regulatory proteins with the receptor and other transcription factors and help to explain how estrogen-responsive genes are differentially regulated by these two receptors.

Estrogen is a hormone of critical importance in regulating the development, growth, and maintenance of reproductive tissues and also influences cardiovascular, neural, and skeletal cell function (1–6). The effects of estrogen are mediated through interaction of the estrogen receptor (ER)* with estrogen response elements (EREs) residing in target genes. Because the ER-ERE interaction plays such a crucial role in gene expression, there has been and continues to be great interest in understanding how this interaction leads to changes in transcription.

With the recent revelation that two ER subtypes exist, the previously identified ERα (7–10) and the more recently cloned ERβ (11–14), our vision of how estrogen brings about its effects in target cells must now be reevaluated and take into consideration the actions of both receptor subtypes. Although the amino acid sequence in the DNA and hormone binding domains of ERα and ERβ is highly conserved, other regions of the two receptors are more varied (15). These differences in amino acid sequence affect the affinities of ERα and ERβ for various ligands and ERE sequences, influence the association of the receptors with coactivator proteins, and thereby alter the abilities of these two receptors to modulate gene expression (13, 14, 16–25).

A number of thermodynamic and structural studies have demonstrated that specific contacts between protein and DNA are often accompanied by conformational changes in protein, DNA, or both (26–31). We and others have demonstrated that binding of ERα to ERE-containing DNA fragments induces conformational changes in DNA structure suggesting that the ability of ERα to induce changes in DNA structure may be related to its ability to activate transcription (32–40). To determine whether ERβ was capable of altering DNA conformation and whether ERα- and ERβ-induced transcription activation might be related to the abilities of these two receptors to induce conformational changes in DNA structure, we have compared the abilities of ERα and ERβ to activate transcription and induce flexibility and directed bending in ERE-containing DNA fragments and determined the effects of DNA bending on transcription activation.

EXPERIMENTAL PROCEDURES

Transient Transfections—Transient cotransfections carried out with Chinese hamster ovary (CHO) cells utilized 3 μg of the CAT reporter plasmid,ERE TATA-CAT (41), which contains an ERE in the BglII site of ATC0, 200 ng of the β-galactosidase expression vector pCH110 (Amersham Biosciences, Inc.), and 5 ng of the human ERα expression vector pCMV5 hERα (42) or the human ERβ expression vector pCMV5 hERβ (43). Transient transfections carried out in U2 osteosarcoma (U2-OS) cells utilized 25 μg of ERα or ERβ expression vector and 3 μg of a CAT reporter plasmid containing an ERE in the SalI site of ATC0 (ATC1, Ref. 44) alone or in combination with an intrinsically bent DNA sequence (ATC1-OP and ATC1-IP, previously described as 3A6/ERE3-CAT and 3A/ERE3-4-CAT, Ref. 36). Cells were transfected with Lipofectin (Life Technologies, Inc.) as described (25). CAT activity was quantitated as described (24). β-galactosidase activity was determined (45) and used to normalize for transfection efficiency.

Construction of ERβ CD Expression Vector and Preparation of DNA Probes—To construct the CDβ expression vector, nucleotides encoding amino acids 134–260 of the ERβ DBD and hinge regions were PCR amplified using the hERβ expression vector pCMV5hERβ and primers with 5'-NheI and 3'-HindIII compatible sites (forward primer: 5'-GCT-ATGGCTACGGCCGTTTCTGGTTGCA-3' and reverse primer: 5'-GAGT-CTAAGCTTTACTAAGCAGCTGCAGCTGGCGA-3'). Oligonucleotides

* This work was supported by National Institutes of Health Grant DK 53884 (to A.M.N.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Supported by National Institutes of Health Cell and Molecular Biology Training Grant (T32 GM07283).

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† The abbreviations used are: ER, estrogen receptor; ERE, estrogen response element; CHO, Chinese hamster ovary; CD, DNA binding and hinge domains; DBD, DNA binding domain; E2, 17β-estradiol; CTE, C-terminal extension.

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Received for publication, September 4, 2001, and in revised form, December 14, 2001
Published, JBC Papers in Press, December 31, 2001, DOI 10.1074/jbc.M108491200

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 277, No. 10, Issue of March 8, pp. 8702–8707, 2002

Printed in U.S.A.
encoding the FLAG epitope (MDYKDSDK) with 5'-NdeI and 3'-NheI restriction enzyme sites (forward oligo: 5'-TATGGATCCAGGACGCCACATGACG-3' and reverse oligo: 5'-CTAGCTGGTCAGCTGTCTGGTGGATCA-3') were annealed and ligated to NheI and HindIII-digested PCR product. The resulting fragment (flag|ER|DBD) was ligated into the T7 promoter containing pET-28a(+) vector using the expression vector pET-21b(flag|ER|DBD) (39) kindly provided by David J. Shapiro (University of Illinois, Urbana, IL). CD\(\beta\)D was constructed as described above to encode the region of ER\(\beta\)N-terminal 189–219 amino acids (amino acids 134–260) corresponding to the region included in the ER\(\alpha\) construct (amino acids 166–307). 500-ml cultures of transformed BL21DE3ployS cells were induced with 1\(\mu\)M isopropyl-1-thio-\(\beta\)-galactopyranoside (IPTG) (Sigma) to a 0.5-OD, and pelleted at 4700 \(\times\) g for 5 min at 4 \(^\circ\)C. Cells were lysed by one freeze/thaw cycle. 5 ml of TEGDZ (50 mM Tris, pH 7.5, 1 mM EDTA, pH 8.0, 10\%\(\beta\)-mercaptoethanol, 50 \(\mu\)M ZnCl\(\text{2}\), 25 \(\mu\)M aprotinin, 5 \(\mu\)M phenylmethylsulfonyl fluoride, 50 \(\mu\)g/ml leupeptin, 1 \(\mu\)g/ml pepstatin) with 0.5\% Triton X-100 was added to the cell lysate and incubated with rotation for 30 min at 4 \(^\circ\)C. The lysate was centrifuged at 142,000 \(\times\) g for 30 min at 4 \(^\circ\)C, and the pellet was discarded. Polyelectrolyne was added dropwise to the supernatant to 0.1\%, incubated on ice for 10 min, and centrifuged at 142,000 \(\times\) g for 30 min at 4 \(^\circ\)C. The extract was diluted with 2 ml of TEGDZ and incubated with anti-FLAG M2 agarose (A1205, Sigma) with rotation for 4 h at 4 \(^\circ\)C. The CD-antibody complexes were washed and eluted as described for the flag|ER|DBD fragment by digestion with RasI, and the junctions of the pET-28a (+) flag|ER|DBD plasmid were verified by DNA sequencing.

Expression and Purification of Wild Type and Truncated ER\(\alpha\) and ER\(\beta\) Proteins—The full-length ER\(\alpha\) and ER\(\beta\) expressed with the T7 promoter were expressed as described previously (23, 24). Protein purity was assayed by Coomassie blue-stained SDS-PAGE. ER concentration was determined using the Bio-Rad protein assay (Bio-Rad, Hercules, CA) with known bend angles as described (37). Calculations of DNA distortion angles are presented as the mean \(\pm\) S.E. from four (ER\(\alpha\)) or six (ER\(\beta\)) independent experiments.

The magnitude of the directed DNA bend was determined from phasing analysis experiments by comparing the relative mobility of each ER-DNA complex with the relative mobility of the DNA bending standards. The magnitude of the directed bend angle (\(\omega\)) was determined using the equation \(\tan (\omega) = [(\text{mobility of the fragment at the middle of the bend}) - (\text{mobility of no additional KCl (CD})]/(\text{mobility of the fragment and no additional KCl (CD})]\). The magnitude of the distortion angles induced by ER\(\alpha\) and ER\(\beta\) was also determined using the empirical equation \(\mu_\text{ER} = \cos (\omega)\), where \(\mu_\text{ER}\) is the relative mobility when the DNA bend is at the middle of the fragment and \(\mu_\text{ER}\) is the relative mobility when the bend is at the end of the fragment. A \(k\) value of 1.130 was determined by comparing the relative mobility of the DNA bending standards with the known bend angles as described (37). Calculations of DNA distortion angles are presented as the mean \(\pm\) S.E. from four (ER\(\alpha\)) or six (ER\(\beta\)) independent experiments.

RESULTS

**ER\(\alpha\) and ER\(\beta\) Activate Transcription to Different Extents**—Transient transfection assays were used to examine the ability of ER\(\alpha\) and ER\(\beta\) to activate transcription of a CAT reporter vector containing a single consensus ERE. CHO cells were transiently transfected with an ER\(\alpha\) or ER\(\beta\) expression vector, a CAT reporter plasmid containing a consensus ERE upstream of a TATA box, and a \(\beta\)-galactosidase control vector. Both receptors significantly induced transcription in the presence of 10 \(\mu\)M E\(\text{2}\) when compared with an ethanol vehicle (Fig. 1, \(p < 0.0001\)). ER\(\alpha\) and ER\(\beta\) induced 19- and 11-fold increases in transcription, respectively. Similar results were obtained in U2-OS cells.\(^2\) Thus, ER\(\alpha\) was a more potent activator of transcription than ER\(\beta\).

**ER\(\alpha\) and ER\(\beta\) Induce Distortion in ERE-containing DNA Fragments**—Previous studies from our laboratory have demonstrated that binding of ER\(\alpha\) to ERE-containing DNA fragments induces distortion and directed DNA bending (32–39). These studies and others suggest that the ability of a protein to induce conformational changes in DNA structure may be related to its ability to enhance transcription (50–52). To determine whether ER\(\beta\) induces distortion in ERE-containing DNA fragments and whether the magnitude of distortion might be related to the ability of ER\(\alpha\) and ER\(\beta\) to activate transcription, circular permutation analysis was carried out with ER\(\alpha\) and ER\(\beta\). Circular permutation assays assess the migration of pro-

\(^2\) J. R. Schultz and A. M. Nardulli, unpublished data.
Fig. 1. ERα- and ERβ-mediated transcription. CHO cells were transiently transfected with a human ERα or ERβ expression vector, the ERE-containing reporter plasmid, ERE TATA-CAT, and a β-galactosidase control vector using Lipofectin and treated with vehicle (open bars) or 10 nM E2 (closed bars). Data from three independent experiments were combined and are expressed as the mean ± S.E. Student’s t tests were used to determine whether statistical differences between ethanol and E2-treated groups existed. Asterisks (*) indicate significant differences in CAT activity in the ethanol- and E2-treated cells (p < 0.0001).

Protein-DNA complexes through a native acrylamide gel to determine the magnitude of protein-induced DNA distortion. Because the geometry of a DNA fragment affects its migration through an acrylamide gel, a DNA fragment with a distortion near the end will migrate farther through the gel matrix than a DNA fragment with a distortion in the middle.

427-bp 32P-labeled DNA fragments containing a consensus ERE near the end, in the middle, or at an intermediate position in the DNA fragment were incubated with purified, baculovirus-expressed ERα or ERβ and fractionated on a nondenaturing polyacrylamide gel. DNA bending standards, which contained 54, 72, or 90° intrinsic DNA bends (53, 54) were also included on all gels. As seen in Fig. 2A, distinct differences in the migration of the ER-bound DNA fragments were observed with both ERα and ERβ. The migration of the receptor-DNA complexes was dependent on the location of the ERE in the DNA fragment. When the ERE was near the end of the DNA fragment (R, B), the ER-DNA complex migrated farther than when the ERE was present at an intermediate position in the DNA fragment (H, V). When the ERE was at the middle of the DNA fragment (V), the ER-DNA complex migrated even more slowly indicating that both ERα and ERβ induced distortion in DNA structure. The magnitude of distortion was determined from four (ERα) or six (ERβ) independent experiments using two methods. Graphical analysis, which compares the relative mobilities of the receptor-DNA complexes with the relative mobilities of the DNA bending standards (Fig. 2B), indicated that ERα and ERβ induced distortion angles of 74.6 ± 4.1° and 63.9 ± 0.9°, respectively. Using the empirical equation \( \mu_m/\mu_v = \cos(\theta/2) \) (47), similar distortion angles were obtained for ERα (76.0 ± 4.3°) and ERβ (64.5 ± 1.0°). Thus, both ERα and ERβ induce distortion in ERE-containing DNA fragments, but ERα induces a larger distortion angle than ERβ (p < 0.05 comparing ERα to ERβ by Student’s t test).

ERα, but Not ERβ, Induces a DNA Bend toward the Major Groove of the DNA Helix—Circular permutation analysis can be used to determine the degree of distortion or flexibility induced by protein binding, but it does not provide information about the orientation of a DNA bend. To determine the magnitude and direction of receptor-induced DNA bending, phasing analysis was carried out. This assay utilizes DNA fragments with an ERE 26, 28, 30, 32, 34, or 36 bp upstream of an intrinsic DNA bend so that the orientation of the ER-induced and the intrinsic DNA bends are incrementally varied over one turn of the DNA helix. At some point the ER-induced and intrinsic DNA bends will be in phase, a larger DNA bend will be produced, and the migration of the ER-DNA complex through the gel matrix will be inhibited. Likewise, at some point the ER-induced and intrinsic DNA bends will be out of phase, the DNA fragment will have a smaller overall bend, and the ER-DNA complex will migrate more rapidly through the gel. By monitoring the migration of the ER-DNA complexes, it is possible to determine the magnitude and direction of an ER-induced DNA bend.

32P-labeled DNA fragments containing an ERE and an intrinsic DNA bend were incubated with purified, baculovirus-expressed ERα or ERβ and separated on a nondenaturing polyacrylamide gel. When the DNA fragment contained an ERE 32 bp upstream of the intrinsic DNA bend, the ERα-DNA complex migrated more rapidly (Fig. 3). This 32-bp spacing places the ERE and intrinsic DNA bend on the same side of the DNA helix. The rapid migration of the ERα-DNA complex indicates that the ERα-induced and intrinsic DNA bends must be out of phase. Since we know that the intrinsic DNA bend is oriented toward the minor groove of the DNA helix (55), the ERα-induced DNA bend must be directed toward the major groove of the DNA helix. Combined data from four independent experiments indicated that the ERα-directed DNA bend was 11.0 ± 1.6°. These findings are in good agreement with earlier work examining ERα-induced DNA bending (37). Surprisingly, when ERβ was incubated with these same DNA fragments, none of the ERβ-bound DNA fragments consistently migrated farther than the others in 10 independent experiments, demonstrating that ERβ does not induce a directed bend in ERE-containing DNA fragments. Only ERα was capable of inducing a directed bend in these ERE-containing DNA fragments and that bend was directed toward the major groove of the DNA helix. Thus, our results with ERβ are in stark contrast to our previous studies of 11 different wild type and mutant ERα proteins, each of which induced a DNA bend directed toward the major groove of the DNA helix (36, 37, 56).5

The DNA Binding Domain and Hinge Region of ERα, but Not ERβ, Induce a Directed Bend in ERE-containing DNA Fragments—We have previously demonstrated that wild type, truncated, and mutant ERαs induce a DNA bend toward the major groove of the DNA helix (36–38, 56) and were quite surprised that full-length ERβ was incapable of inducing a directed DNA bend. To determine how this difference in ERα-induced DNA bending might occur, we carried out phasing analysis with the
region of the ER responsible for specific binding, the DBD. Because the DBD alone has a low affinity for the ERE (46, 57), the hinge region (domain D) was also included. The DBD and hinge region of ERα (CDα) or ERβ (CDβ) shown in Fig. 4 were expressed and purified. When the 32P-labeled DNA phasing probes were combined with purified CDα, the complex containing DNA fragments with 32 bp between the ERE and intrinsic DNA bend migrated the farthest (Fig. 5A). These findings demonstrate that CDα, like wild type, truncated, and mutant ERαs from our previous studies (36–38, 56), induced a bend directed toward the major groove of the DNA helix. Data from 11 independent experiments demonstrated that the magnitude of the CDα-directed DNA bend was 6.7 ± 0.6°. In contrast, the migration of the CDβ-DNA complexes did not differ consistently, but followed the same pattern of migration as the free DNA indicating that CDβ was incapable of inducing a directed DNA bend. These findings demonstrate that CDα could induce a bend toward the major groove of the DNA helix in ERE-containing DNA fragments, but CDβ could not.

The amino acids that comprise the ERα and ERβ DBDs are nearly identical. However, the hinge regions of these receptors vary significantly in amino acid sequence. Thus, we suspected that the hinge region of these two receptors might account for the difference in receptor-induced DNA bending we observed. Previous studies with the Type II and orphan nuclear receptors have defined a region C-terminal to the DBD, the CTE, which has been implicated in stabilizing the DBD-DNA interaction (58–60). This region has also been implicated in stabilizing ER-DNA binding (46, 57). To determine whether the CTE of ERα and ERβ played a role in DNA bending, chimeric proteins were expressed, purified, and used in phasing analysis experiments (Fig. 4). These proteins contained the ERα DBD and the ERβ CTE (CDα/β) or the ERβ DBD and the ERα CTE (CDβ/α). When these proteins were combined with the 32P-labeled DNA fragments containing an ERE and an intrinsic DNA bend, the migration of complexes containing CDβ/α chimera varied when the orientation of the CDβ/α-induced and intrinsic DNA bends was altered. The complex containing 34 bp between the ERE and intrinsic DNA bend consistently migrated more rapidly than the other CDβ/α-containing complexes (Fig. 5B). Assuming 10.5 nucleotides per DNA turn, CDβ/α, like the full-length ERα and CDα, induced a DNA bend directed toward the major groove of the DNA helix. Using data from six independent experiments, we determined that the magnitude of the CDβ/α-induced DNA bend was 5.6 ± 0.7°. In contrast, when CDα/β was utilized, the migration of protein-bound phasing probes mimicked the migration of unbound probes indicating that CDα/β was unable to alter DNA conformation. Thus, the ERα CTE plays an important role in directed DNA bending.

**DNA Bending Influences ER-mediated Transactivation—**

Our *in vitro* binding assays demonstrated that ERα, CDα, and CDβ/α induced DNA bending directed toward the major groove of the DNA helix, but that ERβ, CDβ, and CDα/β failed to induce a directed DNA bend. To determine whether receptor-induced DNA bending might play a role in E2-mediated transactivation, another set of transfection assays was carried out. Reporter plasmids that contained an ERE alone or in combination with an intrinsic DNA bend directed toward the minor groove of the DNA helix were tested for their abilities to enhance ER-mediated transcription activation. When the reporter plasmid contained a single ERE (Fig. 6, ATC1), ERα significantly increased CAT activity in the presence of E2. The magnitude of the increase was less than observed in Fig. 1, however, because the ATC1 reporter plasmid contained a less potent promoter than ERERE TATA-CAT used in those experiments. When the reporter plasmid contained a promoter with the centers of the intrinsic and ERα-induced DNA bends on the same sides of the DNA helix so that the intrinsic and induced DNA bends were out of phase (ATC1-OP), the level of E2-induced CAT activity was similar to the level observed with ATC1. When the reporter plasmid contained a promoter with the centers of the intrinsic and ERα-induced DNA bends on opposite sides of the DNA helix so that the intrinsic and induced DNA bends were in phase (ATC1-IP), the level of E2-induced CAT activity was greater than the level observed with ATC1 or ATC1-OP. These data suggest that orienting an intrinsic DNA bend so that it complements an ERα-induced DNA bend enhances ERα-mediated transactivation.

When the ERβ expression plasmid was utilized in transfection assays, no increase in CAT activity was observed with ATC1, again reflecting the decreased potency of the ATC1 promoter compared with the ERERE TATA-CAT promoter (Fig. 1). When ATC1-OP was utilized, ERβ was still transcriptionally inert. However, when ATC1-IP was utilized, ERβ was capable of substantially increasing CAT activity in the presence of E2. These combined experiments are consistent with previous studies carried out with ERα in CHO cells (36) and highlight the importance of DNA bending and the orientation of that bend in E2-mediated transactivation.

**DISCUSSION**

We have demonstrated that ERα and ERβ have different abilities to activate transcription and alter DNA structure. Although both ERα and ERβ can distort DNA structure, only...
ERα can induce a directed DNA bend. Taken together with earlier studies, we have now demonstrated that wild type ERα and 10 different ERα mutants induce directed DNA bending (36, 37, 56). These 11 ERα proteins all contained the CTE, and all induced a bend directed toward the major groove of the DNA helix. Even the smallest ERα construct utilized, which included the ERα DBD and hinge region (CDα), was capable of inducing a bend directed toward the major groove of the DNA helix. However, when the ERα DBD was combined with the ERβ CTE (CDβ/α), the chimeric protein failed to bend ERE-containing DNA fragments. Neither full-length ERβ nor the DBD and hinge region of ERβ (CDβ) was capable of inducing a directed DNA bend. However, if the ERβ DBD was combined with the ERα CTE (CDβ/α), this chimeric protein gained the ability to bend ERE-containing DNA fragments. Thus, replacing the ERβ CTE with the ERα CTE resulted in a gain of function and replacing the ERα CTE with the ERβ CTE resulted in a loss of function. Taken together, these combined experiments highlight the importance of the ERα CTE in receptor-induced DNA bending.

The amino acids that comprise the human ERα and ERβ DBDs are 96% conserved and differ by only two amino acids (12, 61). However, the CTE adjacent to the DBD varies substantially in amino acid sequence. Our experiments demonstrate that the ERα CTE plays a critical role in receptor-induced DNA bending. It is unclear at this point whether the entire ERα CTE or a specific domain within the ERα CTE is required for inducing these structural changes in ERE-containing DNA fragments. Interestingly, the type II thyroid hormone and retinoid X receptors and the orphan receptor RevErb contain ordered structures in the CTE that interact with the minor groove of the DNA helix and stabilize the protein-DNA interaction (58–60). Of particular interest is the Grip-box in the RevErb CTE, which forms direct contacts with bases in the minor groove. In contrast to these nuclear receptors, the ERα CTE is unstructured. However, it is possible that a region in the ERα CTE plays a similar role in stabilizing the receptor-DNA interaction and fostering structural changes in ERE-containing DNA fragments.

The ability of a protein to induce conformational changes in DNA structure is not limited to ERα and β. A number of nuclear receptors induce DNA bending upon binding to their cognate response elements including the retinoid X receptor, thyroid hormone receptor, glucocorticoid, and progesterone receptors (62–66). Proteins involved in the formation of transcription complexes also induce DNA bending. Crystal structure analysis has revealed that T7 RNA polymerase induces a 63° bend (67). This polymerase-induced DNA bending has been implicated in lowering the DNA melting point to allow for separation of the DNA strands and initiation of transcription. Fos/Jun heterodimers and Jun/Jun homodimers induce bends oriented in opposite directions (54), which may influence their abilities to recruit and interact with different transcription factors. LEF1- and SRY-induced DNA bending appears to play a role in formation of higher order transcription complexes (68–70). The C-terminal zinc finger of the GATA-1 transcription factor induces a DNA bend that allows for homodimerization and stable DNA binding (71). Prokaryotic transcription factors, including the Borrelia burgdorferi Hbb protein, which is thought to be important in bacterial DNA replication (72), numerous cytosine-5-methyltransferases (73), and the E. coli catabolite activator protein CAP (74) cause conformational changes in their cognate DNA recognition sequences. Thus, the modification of DNA structure by proteins involved in DNA
ERα- and ERβ-induced DNA Bending

replication, modification, and transcription activation is widely used in both prokaryotic and eukaryotic systems.

Our laboratory and others have documented the enhanced ability of ERα to activate transcription of reporter plasmids in transient transfection experiments compared with ERβ (11, 12, 20, 23, 43, 75). We have now demonstrated that full-length ERα, CDα, and a CDβ/α chimera induce directed DNA bending but that full-length ERβ, CDβ, and a CDβ/α chimera cannot.

The ability of an appropriately aligned DNA bend to enhance E2-mediated transcription provides evidence that changes in DNA structure can influence ERα-mediated transactivation. Furthermore, the fact that an appropriately positioned intrinsic bend is able to help establish ERβ-mediated estrogen responsiveness substantiates the role of DNA bending in ERβ-mediated transactivation. It seems possible that the decreased ability of ERβ to activate transcription may in part be due to its limited ability to induce changes in DNA flexibility and directed bending. ERα-induced changes in DNA architecture could provide structural motifs required for recruitment of transcription factors or the scaffolding required for assembly of a basal transcription complex. Alternatively, DNA bending could help stabilize DNA loop formation and foster binding and interaction of multiple DNA-bound transcription factors. Our combined studies suggest that DNA bending influences the ability of ERα and ERβ to activate transcription and that intrinsic and protein-induced changes in DNA structure could play an important role in regulating expression of estrogen-responsive genes in target cells.

Acknowledgements—We thank James Kadonaga and Lee Krauss for viral stock used in producing ERα, Arthur Landy for DNA bending standards, Sietse Misselman for the ERβ expression vector, David Shapiro for the pET-21b (+) flagEraDBB expression plasmid, and Jennifer Wood and Lawrence Petz for providing valuable insight and advice.

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Figure 1: The ability of DNA bending to enhance ERα- and ERβ-induced transcription.

The figure shows the ability of DNA bending to enhance ERα- and ERβ-induced transcription. The figure includes a graph that illustrates the increased transcription levels with DNA bending compared to non-bending conditions. The x-axis represents the concentration of ERα or ERβ, while the y-axis represents the level of transcription. The graph shows a significant increase in transcription levels with DNA bending, indicating the importance of DNA bending in ERα- and ERβ-induced transcription.

The text provides additional details on the experimental design, including the use of reporters such as luciferase and gamma-glutamyl transpeptidase, as well as the use of transient transfection assays to measure transcriptional activity. The study also highlights the role of DNA bending in the regulation of estrogen-responsive genes in target cells.
