Estrogen receptor β (ERβ) activates transcription by binding to estrogen response elements (EREs) and coactivator proteins that act as bridging proteins between the receptor and the basal transcription machinery. Although the imperfect vitellogenin B1, pS2, and oxytocin (OT) EREs each differ from the consensus vitellogenin A2 ERE sequence by a single base pair, ERβ activates transcription of reporter plasmids containing A2, pS2, B1, and OT EREs to different extents. To explain how these differences in transactivation might occur, we have examined the interaction of ERβ with these EREs and monitored recruitment of the coactivators amplified in breast cancer 1 (AIB1) and transcription intermediary factor 2 (TIF2). Protease sensitivity, antibody interaction, and DNA pull-down assays demonstrated that ERβ undergoes ERE-dependent changes in conformation resulting in differential recruitment of AIB1 and TIF2 to the DNA-bound receptor. Overexpression of TIF2 or AIB1 in transient transfection assays differentially enhanced ERβ-mediated transcription of reporter plasmids containing the A2, pS2, B1, and OT EREs. Our studies demonstrate that individual ERE sequences induce changes in conformation of the DNA-bound receptor and influence coactivator recruitment. DNA-induced modulation of receptor conformation may contribute to the ability of ERβ to differentially activate transcription of genes containing divergent ERE sequences.

Transcription activation requires the coordinated interaction of multiple transacting factors with DNA recognition sites and other regulatory proteins. In response to cellular signals, transcription factors bind to specific DNA sequences residing in target genes and interact with numerous regulatory proteins to form an active transcription complex and initiate changes in gene expression. This multistep process provides a mechanism by which cells expressing different populations of proteins can differentially regulate expression of target genes.

The nuclear receptor superfamily is composed of a large number of transcription factors that bind to hormone response elements and modulate transcription. Estrogen receptors (ERs)\(^1\) α and β are members of this nuclear receptor superfam-
ily (1–5) and function as ligand-induced transcription factors that modulate expression of estrogen-responsive genes. Upon binding hormone, the ER undergoes a conformational change and binds to estrogen response elements (EREs) residing in target genes to initiate changes in transcription (6). The horm-
one-induced modulation of receptor conformation has been documented in the ligand-binding domains (LBDs) of 17β-estradiol- and raloxifene-bound ERα (7) and in genistein- and raloxifene-bound ERβ (8), with the most striking changes in conformation occurring in the positioning of helix 12 of the LBD. In addition to modulating receptor conformation, ligand binding influences the interaction of the ER with coactivator proteins such as steroid receptor coactivator 1 (SRC1) (9), transcription intermediate factor 2 (TIF2/GRIP1) (10–12), amplified in breast cancer 1 (AIB1/ACTR/RAC3) (13–15), and CREB-binding protein (CBP/p300) (16, 17). Crystal structures of the ERα LBD with the nuclear receptor interaction domain from GRIP1 (18) indicate that when the LBD is bound to an antagon-
ist, the position of helix 12 interferes with coactivator binding. Thus, ligand-induced alterations in receptor conformation may alter coactivator recruitment and ultimately influence activation of transcription by ER.

In addition to ligand-induced changes in conformation, there is a growing body of evidence to suggest that DNA sequences can modulate protein conformation. This allosteric modulation of protein conformation can dramatically alter gene expression, as has been documented with the POU domain-containing transcription factor Pit-1. Pit-1 serves as a potent activator of transcription when bound to its recognition site in the prolactin gene (19), but represses transcription when bound to its recogni-
tion sequence in the growth hormone gene. DNA-induced conformational changes can also have more subtle effects re-
sulting in alteration of the level of transcription. Allosteric modulation of nuclear receptor conformation has been impli-
cated in influencing transcription of a number of hormone-
responsive genes (19–24).

Both ERα and ERβ bind to EREs and activate transcription, but ERβ is typically a less potent activator of reporter plasmids containing the vitellogenin A2 ERE compared with ERα (25–
28). The basis for this differential activation of transcription by ERα and ERβ is unclear. The decreased affinity of ERβ for the ERE compared with ERα could impair its ability to activate...
transcription (28). Additionally, studies with ERα/ERβ chimeric proteins indicate that the amino-terminal activation function 1 (AF-1) of these receptors vary in amino acid sequence and may influence the ability of these receptors to mediate transcription activation (25).

The goal of this study was to determine whether ERβ conformation is different when bound to different EREs and, if so, to characterize the effect of conformational changes on receptor-coactivator interactions and transactivation. We find that DNA-dependent changes in receptor conformation directly translate into alterations in epitope availability and that these DNA-induced changes in receptor conformation alter interaction of ERβ with coregulatory proteins. Thus, ER-induced changes in ERβ conformation may ultimately influence the ability of ERβ to induce transcription activation.

**EXPERIMENTAL PROCEDURES**

*Cell Culture and Transfections—* U2 osteosarcoma (U2-OS) cells were maintained in Eagle’s minimal essential medium with 10 μg/ml folic acid, 25 μg/ml gentamicin, 100 units/ml penicillin, 100 μg/ml streptomycin, and 15% heat-inactivated fetal calf serum. The medium was changed to Eagle’s minimal essential medium containing phenol red with 5% charcoal dextran-treated (29) calf serum 3 days prior to transfection. Two days prior to transfections, cells were transfected for 7 h with the Lipofectin/DNA plate in maintenance in Transfection B medium for 18 h. Transfections were carried out using Lipofectin (Life Technologies, Inc.) as described (28) with 500 ng of the human ERβ expression vector CMV5-ERβ (25), 400 ng of the indicated chloramphenicol acetyltransferase (CAT) reporter vector: consERE+10-CAT, pS2ERE+10-CAT, ERE2+10-CAT (30), and oTERE+10-CAT (28), which contain a single A2, pS2, B1, or oxytocin (OT) ERE, respectively, 3.6 helical turns upstream of a TATA box. Following incubation with the Lipofectin/DNA, cells were maintained in Transfection B medium containing ethanol vehicle or 10 μm 17β-estradiol (E2) for 24 h. β-Galactosidase activity was measured as previously described (31) and used to normalize for differences in transfection efficiency. CAT assays were carried out as described (28), scanned with a Molecular Dynamics PhosphorImager, and analyzed using ImageQuant Version 5.0 software (Molecular Dynamics, Inc., Sunnyvale, CA). The coactivator expression vectors pS5G-TIF2 (12) and pcDNA3.1-AIB1 (13), kindly provided by Hinrich Gronemeyer (Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, France) and Paul Meltzer (Laboratory of Cancer Genetics, Bethesda, MD), respectively, were added as indicated.

*Partial Proteolysis and Antibody Interaction Experiments—* The vectors B3ERE, B3pS2ERE, B3ERE2, and B3OTERE (21, 30), containing the A2, pS2, B1, and OT EREs, respectively, were digested with *Staphylococcus aureus* proteinases V8 (Worthington) and proteinase K (Promega) was added as indicated, and the binding reactions were incubated at 25 °C for 10 min at 25 °C. The resulting probe (10,000 cpm) was combined with 55-base pair DNA fragments were isolated and 32P-labeled. The resulting 55-base pair DNA fragments were isolated and 32P-labeled. The resulting 55-base pair DNA fragments were isolated and 32P-labeled. The resulting 55-base pair DNA fragments were isolated and 32P-labeled.

*RESULTS* 

**Estrogen-dependent Transcription by ERβ through Four Different EREs—** ERβ induces transcription of reporter plasmids containing the consensus vitellogenin A2 ERE (25–28). In this study, we have compared the ability of ERβ to induce transcription of reporter plasmids containing the A2 ERE (GCTCANNNTGACC) (33) and ERE sequences that vary from this consensus ERE sequence. We have utilized the imperfect vitellogenin B1 (GTTAAGNTGGC) (34) and oxytocin (GGTGAAGNTGGC) (35) EREs, which differ from the A2 ERE in the 5‘-half-site, and the pS2 ERE (GCTCANNNTGACC) (36), which differs from the A2 ERE in the 3‘-half-site. Transient transfection assays were carried out in U2-OS cells to determine the ability of ERβ to activate transcription of promoters containing a TATA box and a single A2, pS2, B1, or OT ERE. U2-OS cells were transfected with an ERβ-containing CAT reporter plasmid, an ERβ expression vector, and a CMV-β-gal control plasmid and exposed to ethanol vehicle or E2. CAT assays were performed and normalized for transfection efficiency. As shown in Fig. 1, ERβ was able to significantly induce transcription through all four EREs in the presence of estrogen compared with vehicle controls (p < 0.01). However, ERβ increased transcription to the highest degree (7.3-fold) through the A2 ERE, to an intermediate degree through the OT ERE (5.0-fold), and to the lowest degree through the pS2 and B1 EREs (2.4- and 1.9-fold, respectively). These findings indicate that ERβ increases transcription of reporter plasmids contain-
ing EREs with subtle differences in nucleotide sequence to different extents. Interestingly, although similar levels of transcription were previously observed with the A2, pS2, and OT ERE-containing reporter plasmids in Chinese hamster ovary cells, transcription of the B1 ERE-containing reporter plasmid was not increased in the presence of E2 (28), suggesting that the B1 ERE may be differentially regulated by ERβ in different cell contexts.

**DNA-induced Changes in ERβ Conformation**—Studies with other nuclear receptors have suggested that subtle differences in nucleotide sequence can alter the conformation of DNA-bound receptors and thereby influence transcription activation (20–22, 24, 37, 38). To determine whether ERβ conformation was altered when bound to different ERE sequences, protease sensitivity assays were carried out. Baculovirus-expressed purified ERβ was combined with 32P-labeled DNA fragments containing the A2, pS2, B1, or OT ERE. Increasing concentrations of proteinase K (1, 1.5, 10, and 20 ng) were added to the binding reaction and incubated for 10 min. The products were loaded onto nondenaturing acrylamide gels and separated. Final digestion products are indicated by numbers (P1–P5).

**Proteinase K digestion of A2, pS2, B1, or OT ERE-bound ERβ produces distinct digestion patterns.** Baculovirus-expressed purified ERβ was incubated with 32P-labeled DNA fragments containing an A2, pS2, B1, or OT ERE. Increasing concentrations of protease V8 (250, 500, 1000, and 2500 ng) were added to the binding reaction and incubated for 10 min. The products were loaded onto nondenaturing acrylamide gels and separated. Final digestion products are indicated by numbers (V1–V5).

**Protease V8 digestion of A2, pS2, B1, or OT ERE-bound ERβ produces distinct digestion patterns.** Baculovirus-expressed purified ERβ was incubated with 32P-labeled DNA fragments containing an A2, pS2, B1, or OT ERE. Increasing concentrations of S. aureus protease V8 (250, 500, 1000, and 2500 ng) were added to the binding reaction and incubated for 10 min. The products were loaded onto nondenaturing acrylamide gels and separated. Final digestion products are indicated by numbers (V1–V5).

**Proteinase K digestion of A2, pS2, B1, or OT ERE-bound ERβ produces distinct digestion patterns.** Baculovirus-expressed purified ERβ was incubated with 32P-labeled DNA fragments containing an A2, pS2, B1, or OT ERE. Increasing concentrations of protease V8 (250, 500, 1000, and 2500 ng) were added to the binding reaction and incubated for 10 min. The products were loaded onto nondenaturing acrylamide gels and separated. Final digestion products are indicated by numbers (P1–P5).

**Protease V8 digestion of A2, pS2, B1, or OT ERE-bound ERβ produces distinct digestion patterns.** Baculovirus-expressed purified ERβ was incubated with 32P-labeled DNA fragments containing an A2, pS2, B1, or OT ERE. Increasing concentrations of protease V8 (250, 500, 1000, and 2500 ng) were added to the binding reaction and incubated for 10 min. The products were loaded onto nondenaturing acrylamide gels and separated. Final digestion products are indicated by numbers (P1–P5).
ERE was completely disrupted. These striking differences in receptor-DNA complex formation with four distinct ERE sequences, along with the differential interaction of the M2 and UICK-98 antibodies with OT ERE-bound ERβ, support the idea that ERβ epitopes were positioned differently when the receptor was bound to the A2, pS2, B1, and OT EREs.

**ERE Sequence-dependent Recruitment of AIB1 and TIF2 by ERβ**—The recruitment of coactivator proteins is thought to be an important step in ER-mediated transcription activation (39, 40). It seemed possible from our protease sensitivity and antibody interaction studies that allosteric modulation of the receptor conformation by different ERE sequences might influence the recruitment of coregulatory proteins and subsequently alter transcription activation. To determine whether association of coactivator proteins with ERβ was ERE sequence-dependent, DNA pull-down experiments were carried out using U2-OS nuclear extracts. As shown in Fig. 5A, these U2-OS nuclear extracts contained substantial levels of TIF2 and AIB1, but did not contain ERβ. For the pull-down experiments, biotinylated DNA fragments containing a nonspecific sequence or the A2, pS2, B1, or OT ERE were adsorbed to streptavidin-linked magnetic beads, and ERβ was allowed to bind to the DNA. U2-OS nuclear extracts were added; the beads were washed; and the ERβ-coactivator complexes were eluted. Recruitment of the coactivator proteins TIF2 (12) and AIB1 (13) to DNA-bound ERβ was quantitated by Western analysis. When oligonucleotides contained a nonspecific DNA sequence, ERβ was not retained on the DNA, and neither AIB1 nor TIF2 was recruited (Fig. 5B, NS). However, when the DNA fragments contained the A2, pS2, B1, or OT ERE, ERβ was bound to the DNA, and AIB1 and TIF2 were recruited to the ERE-bound receptor. Interestingly, although the A2 and OT ERE-bound receptors recruited similar amounts of TIF2, the pS2 and B1 ERE-bound receptors recruited significantly less TIF2 than the A2 ERE-bound receptor (Fig. 5C). In contrast, the pS2, B1, or OT ERE-bound receptor recruited less AIB1 than the A2 ERE-bound receptor (Fig. 5D). Differences in coactivator recruitment could not be attributed to the lower affinity of ERβ for the imperfect EREs compared with the consensus sequence since the amount of coactivator recruited to ERβ was expressed as a ratio of coactivator to ERβ for each sample. Given the difference in coactivator recruitment to ERβ on the four discrete ERE sequences, our combined data from protease sensitivity, antibody interaction, and DNA pull-down studies suggest that the conformation of ERβ is different when the receptor is bound to different DNA sequences and that these changes in conformation alter coactivator recruitment.

**ERE-specific Enhancement of Transcription with AIB1 and TIF2**—A number of laboratories have demonstrated that TIF2 and AIB1 increase transcription of reporter plasmids containing the A2 ERE (11, 12, 17). However, the involvement of these proteins in transcription from promoters containing imperfect EREs is less clear. To determine whether TIF2 influences transcription of imperfect ERE-driven promoters, a CAT reporter plasmid containing a TATA box and no ERE or the A2, pS2, B1, or OT ERE was cotransfected into cells with an ERE-containing oligonucleotide (Fig. 6). Inclusion of 150 or 500 ng of the TIF2 expression vector resulted in 53 and 100% increases in transcription with the A2 ERE, 65 and 85% increases with the pS2 ERE, and 86 and 112% increases with the OT ERE, respectively, compared with no TIF2 expression vector addition. In contrast, no increases in transcription were observed when the TIF2 expression vector was included with the parental plasmid (Fig. 6). Thus, TIF2 enhanced transcription through the A2 and OT EREs to a greater extent than through the pS2 ERE, but did not affect transcription when the CAT reporter plasmid contained the B1 ERE. When similar transfection experiments were carried out with a reporter plasmid containing one of the four EREs and an AIB1 expression vector, AIB1 enhanced transcription of the A2 ERE-containing reporter plasmid to the greatest extent and enhanced transcription of the OT ERE-containing reporter plasmid to an intermediate extent, but did not affect transcription of the pS2 and B1 ERE-containing reporter plasmids or the parental plasmid (Fig. 7). Inclusion of 150 or 500 ng of the AIB1 expression vector resulted in 42 and 83% increases in transcription with the A2 ERE and 22 and 35% increases with the OT ERE, respectively, compared with no AIB1 expression vector addition. In the absence of the ER,
Differential Recruitment of Coactivators by ERE-bound ERβ

FIG. 6. Overexpression of TIF2 selectively enhances ERβ-mediated transcription through the A2, pS2, and OT EREs. An ERβ expression vector, a β-galactosidase internal control vector, and a CAT reporter vector containing a single A2, pS2, B1, or OT ERE or no ERE (−) upstream from a TATA box were transiently transfected into U2-OS cells and incubated in the presence of vehicle (white bars) or 10 nM E2 (gray bars). Increasing concentrations of TIF2 expression plasmid were added as indicated. CAT activity was compared with the amount of β-galactosidase activity (cpm/β-galactosidase (β-gal) units) to normalize for differences in transfection efficiency. Data are derived from three times in duplicate, and data are expressed as the means ± S.E.

FIG. 7. Overexpression of AIB1 selectively enhances ERβ-mediated transcription through the A2 and OT EREs. An ERβ expression vector, a β-galactosidase internal control vector, and a CAT reporter vector containing a single A2, pS2, B1, or OT ERE or no ERE (−) upstream from a TATA box were transiently transfected into U2-OS cells and incubated in the presence of vehicle (white bars) or 10 nM E2 (gray bars). Increasing concentrations of AIB1 expression plasmid were added as indicated. CAT activity was compared with the amount of β-galactosidase activity (cpm/β-galactosidase (β-gal) units) to normalize for differences in transfection efficiency. Data are derived from three independent transfection experiments and are expressed as the means ± S.E.

overexpression of TIF2 or AIB1 failed to enhance transcription of the ERE-containing reporter plasmids (data not shown). Thus, both TIF2 and AIB1 enhanced the ERβ- and E2-dependent activation through the A2 and OT EREs. Only TIF2 enhanced transcription of the pS2 ERE, and neither TIF2 nor AIB1 was capable of augmenting transcription through the B1 ERE.

DISCUSSION

We have demonstrated that four naturally occurring ERE sequences exhibit different levels of ERβ-dependent transactivation in transient cotransfection assays. Our studies provide evidence that individual EREs induce changes in ERβ conformation and that these conformational changes alter the ability of the receptor to recruit the coactivator proteins TIF2 and AIB1. The differential recruitment of coactivator proteins to the ERE-bound receptor may in turn influence transcription of estrogen-responsive genes.

Binding of ERβ to Distinct ERE Sequences Results in Allosteric Modulation of Receptor Conformation—Protease sensitivity studies with two different proteases demonstrated that there were differences in accessibility of proteinase K and protease V8 cleavage sites when ERβ was bound to four different EREs. These data indicate that binding of ERβ to different ERE sequences elicits specific changes in ERβ conformation. Interestingly, similar digestion patterns were produced when each ERβ-ERE complex was exposed to different proteases. When receptor-DNA complexes were digested with either proteinase K or protease V8, the A2 ERE-bound receptor produced lower mobility complexes; the OT ERE-bound receptor produced higher mobility complexes; and the pS2 and B1 ERE-bound receptors produced complexes with higher and lower mobilities. This similarity in digestion patterns when the receptor was bound to the same ERE but cleaved with a different protease most likely resulted from cleavage of adjacent proteinase K and protease V8 sites on exposed receptor surfaces. Similar digestion patterns were also observed for each of the EREs after chymotrypsin digestion of DNA-bound ERβ (28).

The differential interaction of antibodies with the A2, pS2, B1, or OT ERE-bound receptor provided additional evidence that individual EREs induce specific changes in ERβ conformation. Antibodies to both the amino terminus and LBD detected differences in epitope availability when ERβ was bound to the four different ERE sequences. Although no single antibody differentiated between all four conformations of ERβ, taken together, the three antibodies utilized in our studies demonstrate that each of the four EREs induces unique changes in receptor conformation. Furthermore, since each antibody recognized more than one ERβ-DNA complex, the conformation of the entire receptor protein on each ERE sequence is likely to be a composite consisting of epitopes that are common to and variant from the conformation of ERβ when bound to the other sequences.

Despite only 58% conservation of overall amino acid sequence between human ERα and ERβ in the LBD (3), the crystal structures of the two ER LBDs are quite similar (8, 18). X-ray crystallographic and mutation analyses of both the thyroid hormone receptor and ERs LBDs have been used to identify a coactivator interaction site for the LXXLL motif found in a number of coactivator proteins (18, 41, 42). ERα amino acids Leu354–Lys362 of helix 3, Phe367–Val368 of helix 4, Leu370 from the turn between helices 4 and 5, and Glu375–Glu380 of helix 5 form a shallow nonpolar groove with charged ends that coordinate the LXXLL motif of GRIP1 (18). In the ERβ crystal structure (8), these key amino acids are similarly positioned, suggesting that the coactivator interaction surface is conserved in ERα and ERβ. The CWK-F12 antibody used in our studies recognizes ERβ amino acids Leu273–Arg285, which map to the carboxyl terminus of helix 2 and the region between helices 2 and 3 (8, 32). This region borders the amino acids in ERα and the corresponding amino acids in the thyroid hormone receptor that interact with coactivators in crystal structure studies. Significantly, the interaction of CWK-F12 with ERβ-ERE complexes was strikingly different, suggesting that ERβ conformation in the region bordering the coactivator interaction site was influenced by ERE sequence. CWK-F12 blocked or severely reduced the interaction of the receptor with the pS2, B1, and OT EREs. In contrast, when the receptor was bound to the A2 ERE, the formation of the receptor-DNA complex was minimally affected by addition of CWK-F12, suggesting that the antibody interaction site was occluded or placed in a conformation that was not recognized. As Feng et al. (41) point out, the coactivator-binding surface in nuclear receptors is small (300 Å). The size of the interaction surface coupled with allosteric
modulation of a nearby epitope in the LBD of ERβ when the receptor is bound to the A2, pS2, B1, or OT ERE could alter the ability of the receptor to interact with coregulatory proteins. Differences in coactivator recruitment in the pull-down experiments reported here illustrate the functional consequences of the altered ERβ conformation.

**Allosteric Modulation of ERβ Conformation Influences Recruitment of Coactivator Proteins and Transcription Activation**—Coactivator and corepressor proteins bind to agonist- and antagonist-occupied ERs in vitro and play a critical role in transcription activation and repression (9–14, 16, 17, 43, 44). Interaction of coregulatory proteins with ERs also occurs in vivo. For example, immunoprecipitation assays showed ligand-dependent interaction of AIB1 with endogenous ER in MCF-7 breast cancer cells (45). In the DNA pull-down experiments presented here, different levels of the coactivator proteins TIF2 and AIB1 were recruited to the A2, pS2, B1, and OT ERE-bound receptors. Both the pS2 and B1 ERE-bound receptors recruited significantly less TIF2 and AIB1 than the A2 ERE-bound receptor. The OT ERE-bound receptor recruited significantly less AIB1, but similar levels of TIF2 compared with the A2 ERE-bound receptor. Interestingly, the ability of ERE-bound ERβ to recruit AIB1 and TIF2 was related to the ability of the receptor to activate transcription. The pS2 and B1 EREs, which were associated with significantly lower levels of ERβ-bound coactivator proteins, were the least effective transcriptional enhancers. The A2 ERE, which was associated with the highest levels of ERβ-bound coactivator proteins, was the most potent transcriptional enhancer. The OT ERE, which was associated with high levels of ERβ-bound TIF2 (but not AIB1), had an intermediate ability to function as a transcriptional enhancer.

We believe that transcription of estrogen-responsive genes is subject to the cooperative interaction of numerous coregulatory proteins with the ER. We have demonstrated that two well characterized coactivators, TIF2 and AIB1, may play a role in selectively altering transcription of promoters containing discrete ERE sequences. The decreased recruitment of TIF2 and AIB1 to the pS2 and B1 ERE-bound receptors, the modest ability of ERβ to activate transcription through the pS2 and B1 EREs in transient transfection assays, and the decreased ability of overexpressed TIF2 and AIB1 to augment transcription via the pS2 and B1 ERE-containing reporter plasmids compared with the A2 ERE suggest that TIF2 and AIB1 may be less important in E2-induced transcription through the pS2 and B1 EREs than through the A2 ERE. The ability of the OT ERE-bound receptor to recruit TIF2 (but not AIB1) in our in vitro assays, the intermediate ability of the OT ERE to function as a transcriptional enhancer, the potent transcriptional enhancement with overexpression of TIF2, and the moderate transcriptional enhancement with AIB1 suggest that TIF2 may play a more important role in regulating transcription of OT ERE-containing promoters than AIB1. We propose that differential recruitment of TIF2, AIB1, and other coregulatory proteins by the ERE-bound receptor plays an important role in regulating transcription of estrogen-responsive genes.

**Modulation of Protein Conformation Provides a Mechanism for Differential Regulation of Gene Expression**—A recent study demonstrated that the POU domain-containing transcription factor Pit-1 undergoes a conformational change in response to binding to its recognition site in the growth hormone gene, resulting in recruitment of cofactors that mediate transcriptional repression (19). In contrast, Pit-1 activates transcription when bound to its recognition site in the prolactin gene. Crystal structure analysis of Pit-1 bound to the prolactin or growth hormone gene recognition sequences showed dramatic alteration in the domain spacing of the Pit-1 protein. When Pit-1 was bound to its recognition sequence in the growth hormone gene, nuclear corepressor (NCoR) was recruited, and transcription was repressed. Scully et al. (19) propose that Pit-1-mediated repression of the growth hormone gene in certain cell types is mediated by conformational changes induced by the Pit-1-binding site and the resulting recruitment of coexpressors.

Experiments carried out with thyroid receptor (TR) / retinoid X receptor (RXR) heterodimers support the idea that DNA-induced changes in receptor conformation can alter association of coactivator proteins. TR/RXR heterodimers are more resistant to trypsin digestion when bound to transcriptionally active thyroid response elements (TREs) than when bound to transcriptionally inactive TREs (22), suggesting that there are also differences in the conformation of the TR/RXR heterodimer when it is bound to different TRE sequences. Furthermore, fragments of steroid receptor coactivator 1 associated with TR/RXR heterodimers in the presence of a transcriptionally active TRE, but failed to associate with TR/RXR heterodimers in the presence of DNA containing a transcriptionally inactive TRE (23).

Combined with our previous studies of ERα (21), we have now demonstrated that the conformation of ERα and ERβ is modulated by ERE sequence and that these DNA-induced changes in receptor conformation alter recruitment of coactivator proteins. Interestingly, our earlier studies with purified ERs and HeLa nuclear extracts showed that A2, pS2, and OT ERE-bound ERα receptors recruited similar amounts of TIF2; that B1 ERE-bound ERα recruited significantly less TIF2; and that the level of AIB1 recruitment by A2, pS2, B1, or OT ERE-bound ERα did not vary. The decreased ability of ERβ to recruit TIF2 and AIB1 when bound to these same EREs may help to explain the decreased ability of ERβ to enhance transcription of reporter plasmids containing these ERE sequences compared with ERα (28).

Few studies have examined the role of DNA sequence in modulating recruitment of coregulatory proteins to DNA-bound nuclear receptors. Here we have shown that ERβ undergoes discrete changes in conformation when bound to EREs with slight variations in nucleotide sequence. These studies document the functional consequences of DNA-induced changes in receptor conformation and highlight the importance of each individual ERE sequence in regulating transcription of estrogen-responsive genes. We propose that ER sequence can alter the conformation of the DNA-bound receptor and influence recruitment of regulatory proteins. Furthermore, the effect of DNA sequence on receptor conformation and subsequent coactivator recruitment is probably not limited to ERs, but likely plays a role in differential expression of other hormone-responsive genes.

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