Transcriptional Activation by the Estrogen Receptor Requires a Conformational Change in the Ligand Binding Domain

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The estrogen receptor (ER) is a strong hormone-inducible transcription factor that regulates the expression of many genes. It was shown for the human progesterone receptor that the binding of hormone causes distinct conformational changes in the ligand binding domain (LBD) and that these changes in LBD conformation are crucial for events after DNA binding. We now show that conformational changes in the LBD of the human ER are a prerequisite for *trans*-activation. Under the appropriate conditions ER binds to its response element and activates transcription only in the presence of ligand. Binding of the ligand causes changes in the conformation of the LBD. Antihormones induce distinct conformational changes, the differences between the conformations lying in the carboxy-terminal end of the receptor. Changing the experimental conditions results in a receptor that can bind to DNA and activate transcription in a ligand-independent manner. Under these conditions the LBD has a transcriptionally active conformation in the absence of ligand. Taken together, our data indicate that the conformational change induced by ligand is required for converting a receptor to the transcriptionally active form. (Molecular Endocrinology 7: 1266–1274, 1993)

INTRODUCTION

Estrogens regulate cellular events through a specific intracellular receptor which functions as a ligand-inducible transcription factor. After hormone binds to the estrogen receptor (ER), a series of events follows, which include dissociation from heat shock proteins (HSPs; 1–3), dimerization (4–6), and binding to DNA at an estrogen response element (ERE). After DNA binding the activated receptor can interact with basal transcription factors (7). This interaction is thought to stabilize preinitiation complex at the promoter, allowing RNA polymerase to initiate gene transcription (8).

It is obvious that the hormone plays the key role in initiating this cascade of events. It was suggested that dissociation of the HSPs may be sufficient to convert a steroid receptor into a DNA binding transcriptionally active form (9, 10). However, it was shown that in a yeast strain with reduced levels of HSP90, steroid receptors failed to activate transcription without hormone (11). Also, our laboratory showed that the progesterone receptor (PR) devoid of HSPs absolutely required hormone for DNA binding and transcriptional activation (12). Furthermore, the PR could be rendered to dimerize and bind to DNA *in vitro* using monoclonal antibodies, yet this DNA-bound receptor was not capable of activating transcription (13). Apparently other events distinct from HSP removal and DNA binding are necessary for receptor activation. Proteolytic analysis of the PR showed that binding of a progestin induces a marked conformational change in the LBD (14). This change precedes and is not affected by dissociation of the HSPs.

In contrast to the PR, the ER has been reported to bind to its ERE in a hormone-independent manner (15–18). In order to study the mechanism of transcriptional activation by the ER, Elliston *et al*. (8) produced mouse ER in a baculovirus expression system. This ER could both bind DNA and stimulate cell-free transcription in the absence of hormone. These *in vitro* data do not correlate with the *in vivo* situation, where ER stimulates the transcription of many genes ligand dependently and has been shown to bind to its ERE only in the presence of estrogen (19–21). Recently, Brown and Sharp (22) used recombinant human ER (hER) made in a baculovirus expression system to study the role of ligand in ERE binding *in vitro*. They were able to demonstrate that under special conditions the receptor could bind specifically to an ERE in a ligand-dependent manner.

We have used baculovirus-expressed hER to study the role of ligand in transcriptional activation by ER, using the conditions described by Brown and Sharp (22). Our results show that hormone induces ER to

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bind to its response element and to stimulate transcription in a cell-free transcription system. For the first time, these data are in agreement with the *in vivo* situation, where hormone is absolutely required for transcriptional activation by ER. Furthermore, we used these selective conditions to assess the correlation between ligand-dependent conformational changes and transcriptional activation. We show that hormone-induced conformational changes in the LBD of the receptor are a prerequisite for transcriptional activation.

RESULTS

Cell-Free Synthesized ER Binds to an ERE in a Ligand-Dependent Manner

To analyze conformational changes in the ER resulting from ligand binding, the receptor was synthesized in a cell-free system. To determine whether the *in vitro* synthesized ER behaved in a manner similar to the native form, DNA binding properties were analyzed by electrophoretic mobility shift assay (EMSA). Using the conditions described by Brown and Sharp (22) it is clear that specific complexes could be observed only when the ER was incubated with estrogen (Fig. 1, E_2, lane 2), or the antiestrogens ICI 164,384 (ICI, lane 3) or nafoxidine (NAF, lane 4). Specificity was confirmed by competition with an unlabeled ERE but not by a progesterone response element (PRE), as well as by upshifting with an ER-specific antibody (data not shown). The extra complexes running just above and below the predominant specific complex are of unknown origin, but they are also specific ERE-binding complexes containing ER. The antiestrogen NAF promoted binding to the ERE that was at least as strong as that induced by estrogen, whereas ICI 164,384-bound ER showed a lower affinity for the response element.

These results show that *in vitro*-synthesized ER displays the same DNA binding properties as native ER. Only in the presence of ligand does the ER bind to specific DNA sequences. Therefore this ER appears suitable for our subsequent studies.

Ligand-Induced Conformational Changes in the C-Terminus of the ER

To study the conformations of ER in the presence or absence of ligand, we performed proteolytic analysis on this receptor. [^{35}S]Methionine-labeled ER was incubated with estrogen, ICI 164,384, or NAF and digested for a short period of time with chymotrypsin (Fig. 2, lanes 1–5). Treatment with solvent alone resulted in nearly complete digestion of the receptor under these conditions. After estrogen treatment a 32-kilodalton (kDa) fragment was more resistant to protease treatment (compare lanes 2 and 3); in contrast, a 30-kDa fragment was more resistant to protease digestion after incubation of the receptor with ICI 164,384, NAF (Fig. 2), or tamoxifen (data not shown). Note that in each

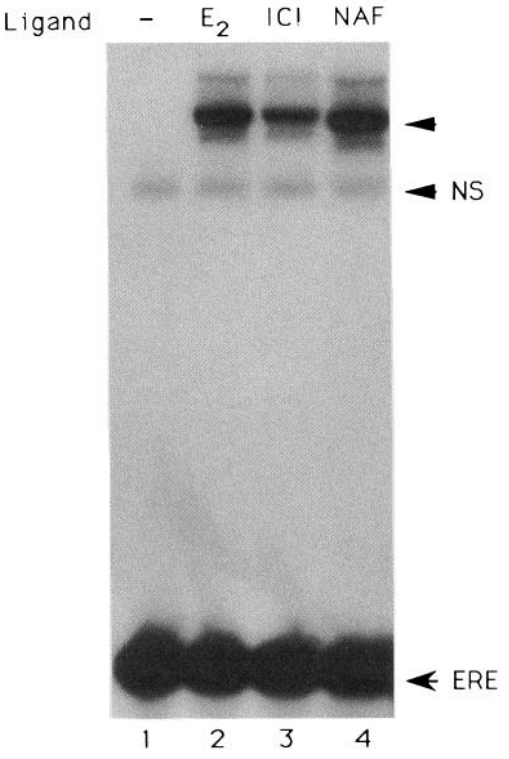

Fig. 1. *In Vitro*-Translated ER Binds to an ERE in a Ligand-Dependent Manner

Translated ER was incubated with 100 nM 17-β-estradiol (E_2, lane 2), 100 nM ICI 164,384 (lane 3), 1 μM NAF (lane 4), or an equal concentration [0.01% (vol/vol)] of ethanol (lane 1). Specific DNA binding to an ERE was analyzed by EMSA. The nonspecific band (NS) does not contain ER (data not shown).

case both resistant fragments are detectable; estradiol and the antiestrogens enhance the resistance of predominantly a 32- or 30-kDa species, respectively. This is due to the kinetic nature of the assay, which was performed at subsaturating levels of both protease and ligand.

To confirm the location of the resistant fragment, a deletion mutant was generated (ERC30) that lacks the last 30 amino acids at the C-terminus. Results of proteolytic digestion experiments with this mutant are presented in Fig. 2, lanes 7–11. The 32-kDa estrogen-induced resistant band is reduced in size to a 29-kDa band, corresponding to the loss of 30 amino acids. Now the antagonist-induced resistant bands are also reduced to a 29-kDa species. Because the deletion was made at the C-terminal end, and because the ER LBD is approximately 32 kDa in size, these data show that the resistant fragments correspond to the LBD of the receptor. They also show that antiestrogens enhance the resistance of a smaller fragment with a distinct conformation at the C-terminus of the LBD, which allows further cleavage by the protease at this position (2 kDa from the carboxy-terminal end). The antiestrogen-enhanced resistant fragment is the same size as that enhanced by estradiol with ERC30, because the C-terminal deletion has removed the protease cleavage

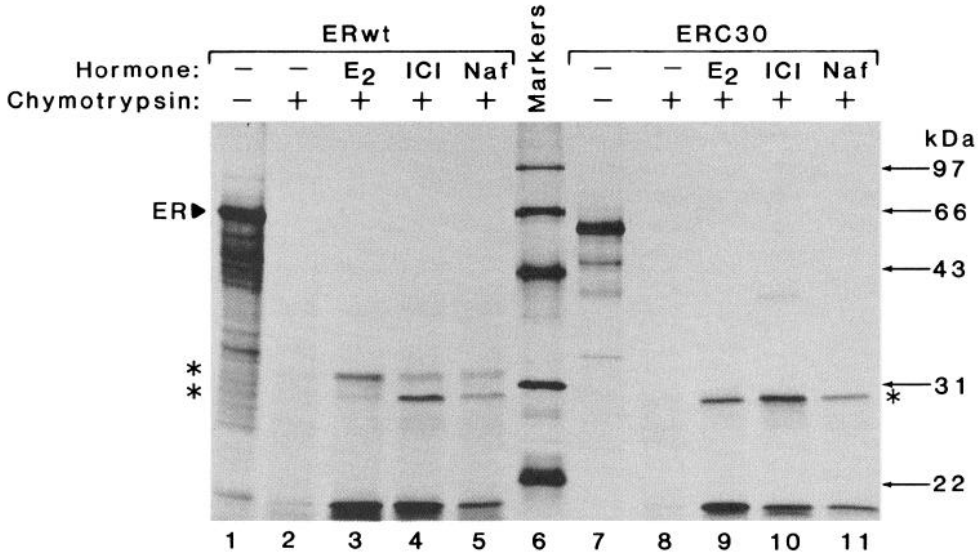

Fig. 2. Estrogen- and Antiestrogen-Specific Conformational Changes in the Ligand-Binding Domain

Wild type (ERwt) and mutant receptor (ERC30) were incubated with 100 nM 17-β-estradiol (E_2, lanes 3 and 9), 100 nM ICI 164,384 (lanes 4 and 10), 1 μM NAF (lanes 5 and 11), or 0.01% (vol/vol) ethanol (–, lanes 1, 2, 7, and 8) before digestion with 50 μg/ml chymotrypsin. An equal volume of water was added for the undigested controls (lanes 1 and 7). Digestion products were analyzed by denaturing gel electrophoresis. The sizes of the molecular weight markers (lane 6) are shown. Resistant fragments are indicated by *asterisks*.

site(s) present in the wild type receptor. Immunoprecipitation studies with a variety of monoclonal antibodies confirmed these observations (data not shown).

These data are equivalent to those obtained for the hPR (14) and imply a general model for steroid hormone receptor conformational changes induced by binding of agonists or antagonists.

Baculovirus-Expressed ER Functions in a Ligand-Dependent Manner

It was shown previously that ligand-dependent conformational changes in PR are necessary for events after DNA binding (13). To study this more extensively, ER was overexpressed in a baculovirus expression system, to produce sufficient receptor for subsequent studies. Extracts from infected Sf9 cells were analyzed by Western blotting using D75 monoclonal antibody raised against hER (23; Fig. 3A). A single band of approximately 66 kDa was observed in the recombinant extract (lane 2), corresponding to full-length ER, while no bands were visible in a lane containing a similar amount of extract from cells producing glutathione-S-transferase (lane 1).

To confirm that our baculovirus-ER (bvER) can also form specific high affinity complexes with an ERE in a hormone-dependent fashion, an EMSA was performed under the conditions described by Brown and Sharp (22). As shown in Fig. 3B, ER-ERE complex formation is greatly enhanced by preincubation of the receptor with hormone (compare lanes 1 and 2). The addition of antibody D75 shows the presence of ER in the complex (lane 3). Specificity of binding to an ERE was shown by specific competition by excess ERE (lanes 4 and 5) but not by PRE oligonucleotides (lanes 6 and 7).

In vitro transcription experiments were performed to determine whether bvER is transcriptionally active in a hormone-dependent manner. As shown in Fig. 4, only basal transcription is observed with the control template, which only contains a TATA box (lanes 1 and 2); transcription is increased on the template with two EREs and is clearly hormone induced (lanes 3 and 4). Transcription from the internal control AdML-G-free template is not affected by the presence or absence of hormone. These results indicate that the baculovirus-expressed ER is active in both specific hormone-induced DNA binding and transcriptional activation under the conditions used.

Activation of ER Is Not Ligand Dependent at Lower Temperatures

The experiments described thus far have been carried out at 37 C and with a 10 mM concentration of Mg^{++}. These conditions promote hormone-induced DNA binding (22), hormone-induced conformational changes in the LBD of ER, and hormone-inducible transcriptional activation.

Binding of the ER to an ERE is clearly not a ligand-induced process when performed at lower temperatures (Fig. 5A). Much less ER-ERE complex was detected in the absence of ligand at 37 C (compare lanes 1 and 3). This is not due to instability of the receptor, since it was shown that wild type ER is not degraded and can still bind hormone with high affinity after incubation at 37 C (24). Activation of transcription is also

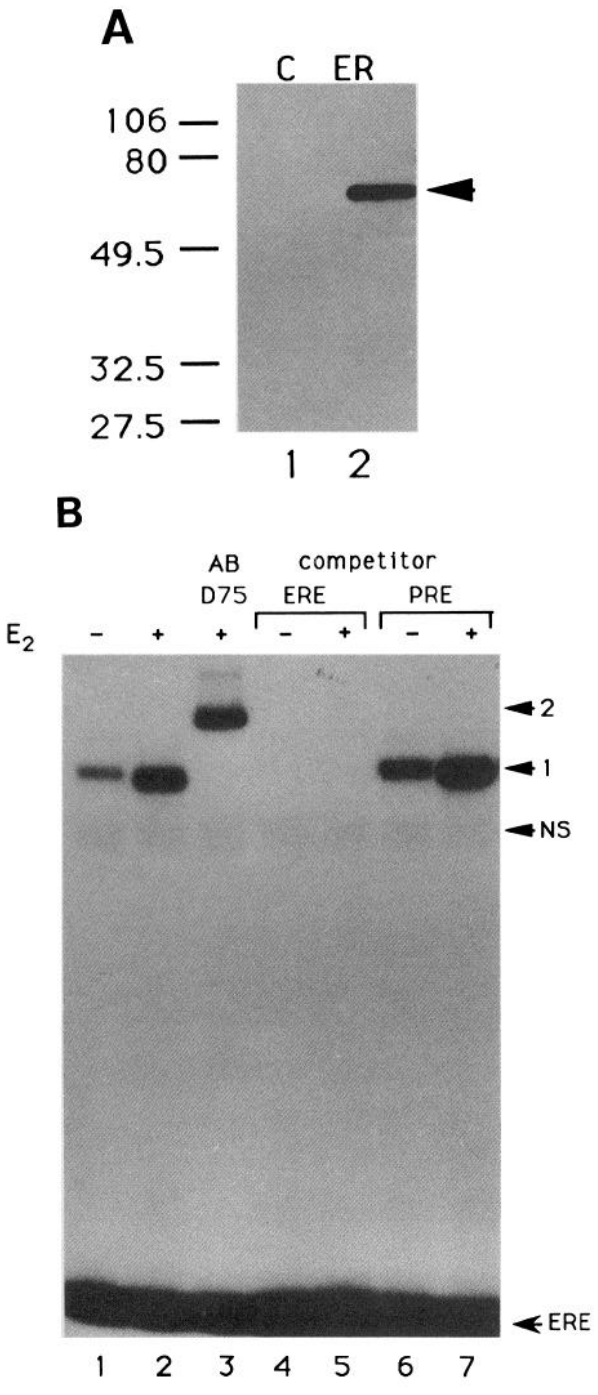

Fig. 3. ER Expressed in a Baculovirus System Binds to an ERE in an Estrogen-Inducible Manner

A, Whole cell extracts from recombinant hER virus-infected cells (ER, lane 2) or from recombinant glutathione-S-transferase-virus-infected cells (lane 1) were analyzed by Western blot analysis using the monoclonal antibody D75 (lane 3). The *arrowhead* denotes the specific 66-kDa ER band. Molecular weights (in kDa) correspond to the positions of prestained standards. B, Whole cell extracts containing hER were incubated with 100 nM 17-β-estradiol (E_2 +, lanes 2, 3, 5, and 7) or 0.015% (vol/vol) ethanol (−, lanes 1, 4, and 6) and with a concentration of 10 mM $MgCl_2$ at 37 C. To the indicated samples a 100-fold molar excess of unlabeled ERE (lane 4 and 5) or PRE (lanes 6 and 7) oligonucleotides, or 0.1 μg anti-ER monoclonal antibody D75 were added. Specific DNA binding was analyzed by EMSA. The nonspecific (NS) band does not contain ER.

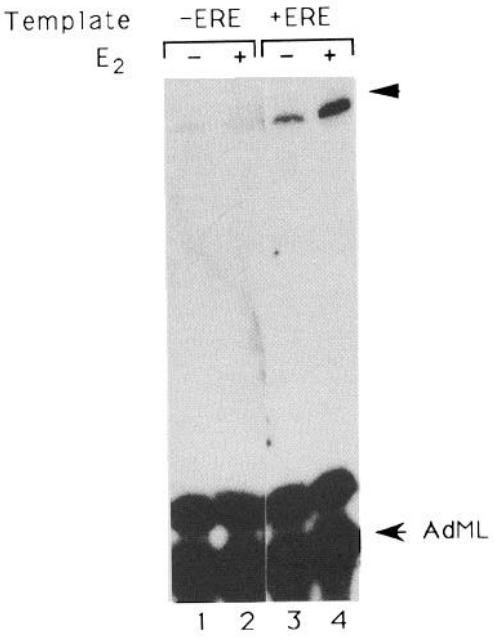

Fig. 4. Hormone- and ERE-Dependent Induction of *in Vitro* Transcription by Baculovirus-Expressed ER

Whole cell extracts were incubated in the absence (−, lanes 1 and 3) or presence (+, lanes 2 and 4) of 100 nM 17-β-estradiol and with a concentration of 10 mM Mg^{++} at 37 C. The *arrowheads* indicate correctly initiated transcripts from the test templates pLovTATA (−ERE, lanes 1 and 2) and $pERE_2LovTATA$ (+ERE, lanes 3 and 4) and from the internal control (AdML).

not ligand dependent at a lower temperature. Figure 5B shows the results of an *in vitro* transcription assay performed with bvER preincubated at 25 C or 37 C with a 10 mM Mg^{++} concentration. At 25 C the receptor does not need ligand to activate transcription from an ERE-driven template (lanes 1 and 2). Preincubation of the receptor at 37 C clearly makes it dependent on ligand to induce similar levels of transcription (lanes 3 and 4).

Thus, under different conditions ER can bind to DNA either ligand dependently or independently. Under the conditions that ER binds to DNA ligand independently, the receptor is transcriptionally active.

At Lower Temperatures ER Has a Transcriptionally Active Conformation

Next we examined the conformation of these transcriptionally active unliganded receptors. We also performed protease digestion studies under low-Mg^{++} and low-temperature conditions. Figure 6 shows the results of such an experiment. As shown previously hormone-free ER is completely degraded by added protease when incubated at 37 C with high Mg^{++}, whereas a 32-kDa resistant band is visible when the receptor is bound by hormone (lanes 8, 9, 11, and 12). At low Mg^{++} and 25 C both liganded and unliganded receptors show the 32-kDa resistant band when incubated with protease

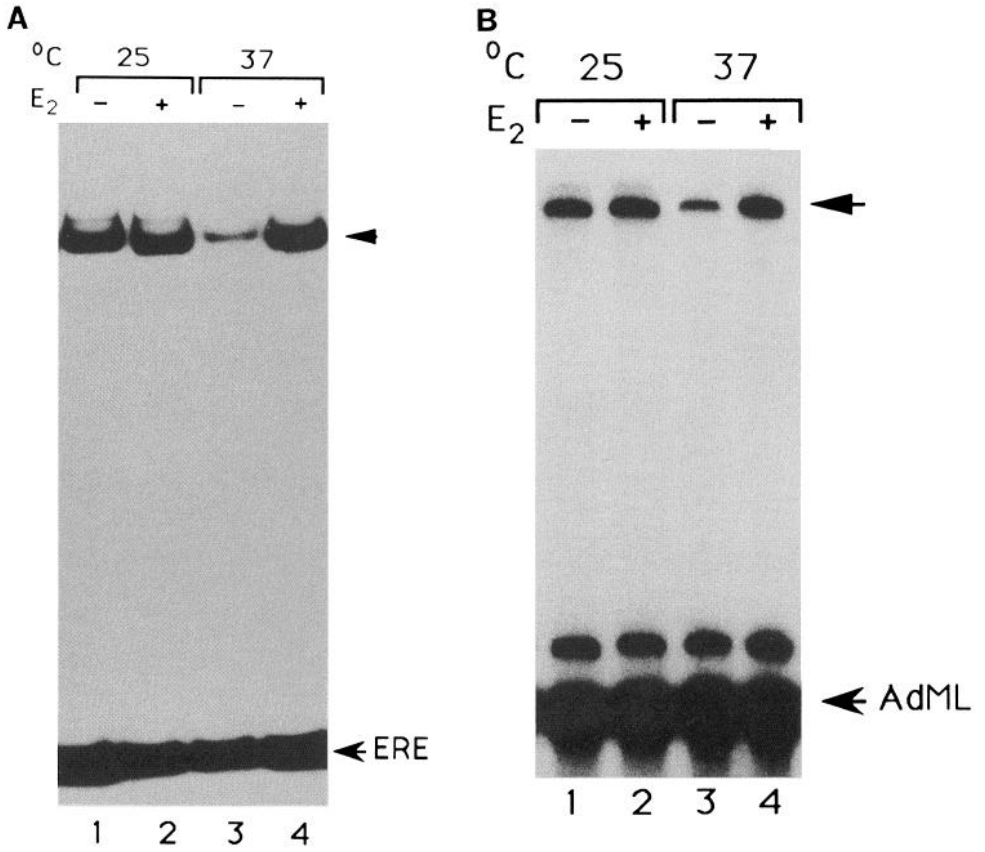

Fig. 5. Hormone-Induced ERE Binding and Transcriptional Activation by the ER Is Temperature Dependent

A, Whole cell extracts containing ER were incubated without (–, lanes 1 and 3) or with (+, lanes 2 and 4) 100 nM 17-β-estradiol at 25 C (lanes 1 and 2) or 37 C (lanes 3 and 4) with a concentration of 10 mM Mg^{++}. Specific DNA binding was analyzed by EMSA. The *arrowhead* denotes the ERE-ER complex. B, Whole cell extracts containing ER were incubated without (–, lanes 1 and 3) or with (+, lanes 2 and 4) 100 nM 17-β-estradiol at 25 C (lanes 1 and 2) or 37 C (lanes 3 and 4) with a concentration of 10 mM Mg^{++}, before transcription at 30 C. The *arrows* denote correctly initiated transcripts from the $pERE_2LovTATA$ or pAdML200 (AdML) templates.

(lanes 2, 3, 5, and 6). Thus, ER that is now capable of ligand-independent transcriptional activation also has an LBD conformation that is indistinguishable from ligand-activated ER.

DISCUSSION

Recently it was shown by Allan *et al*. (14) that PR and retinoic acid receptor undergo a conformational change in their LBDs upon binding their respective ligands. They concluded that the direct effect of steroid binding is a structural change in the receptor, which is necessary for subsequent HSP dissociation, receptor dimerization, and DNA binding. In this paper we have used two sources of ER and different experimental conditions to demonstrate a correlation between conformational changes in the receptor and transcriptional competence. We found that ER exhibits hormone-induced conformational changes. The proteolytic digestion patterns observed here are similar to those that were previously observed with the PR and retinoic acid receptor (14). Estrogen treatment of ER enhances a protease-resistant band of 32 kDa. A deletion of the C-terminal 30 amino acids of the receptor results in a 3-kDa reduction in the size of the resistant fragment. From this we concluded that the protease-resistant 32-kDa band corresponds to the entire LBD.

Apparently, upon binding hormone the LBD of the receptor folds into a more compact structure that is resistant to protease digestion. This conformation likely results in dissociation of the HSPs and unmasking of the dimerization and DNA binding domains of the receptor and may play an important role in interactions between the receptor and coactivators or the general transcription machinery. This interaction presumably stabilizes the transcriptional machinery at the promoter (25).

In addition, our results show that under conditions where ER can bind to an ERE and can activate transcription without the need of a ligand, proteolytic digestion analysis results in a pattern similar to that of a liganded and activation-competent receptor. This argues that besides HSP dissociation, receptor dimeri-

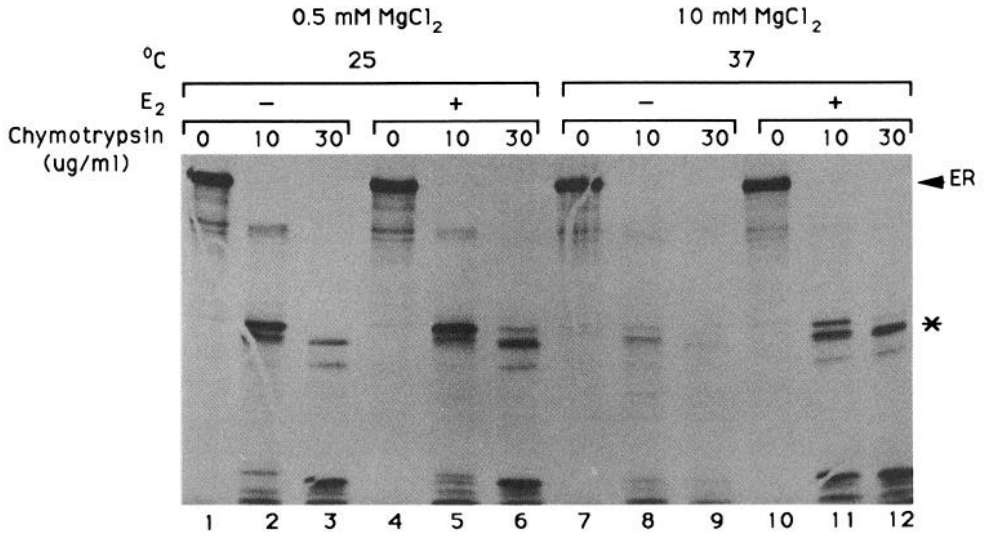

Fig. 6. Hormone-Dependent Conformational Changes in the ER Are Dependent on Mg^{++} Concentration and Temperature

Translated ER was incubated with 100 nM 17-β-estradiol (+, lanes 4–6 and 10–11) or 0.01% (vol/vol) ethanol (–, lanes 1–3 and 7–9) at 25 C with 0.5 mM Mg^{++} (lanes 1–6) or 37 C with 10 mM Mg^{++} (lanes 7–12), before digestion with the indicated levels of chymotrypsin. An equal volume of water was added to the undigested controls (lanes 1, 4, 7, and 10). Analysis was as in Fig. 2.

zation, and DNA binding, the hormone plays a direct allosteric role in transcriptional activation. This is supported by a prior finding that, in the absence of ligand, monoclonal antibodies directed against different regions of PR can induce high affinity binding to its response element *in vitro* (13). Importantly, this antibody-stimulated complex could not activate transcription. Thus, the results suggested that the ultimate role of the hormone lies in events after DNA binding, presumably in transcriptional activation.

It is unclear at present why ER is structurally and functionally active *in vitro* in the absence of ligand. Numerous previous investigators have noticed this lack of ligand dependence for DNA binding (15–18) and trancriptional activation (8). There may be some inherent instability in the LBD of ER which, paradoxically, renders it active after isolation from cells or *in vitro* translation. This ligand independence has also been noted for purified PR (25) and baculovirus-expressed glucocorticoid receptor (26). Previous data have indicated that the removal of HSPs from receptors would not be sufficient by itself to activate them (12). It is also unlikely that the spontaneous formation of ER dimers is responsible for ligand independence, as antibody-induced DNA-bound receptor dimers are transcriptionally inactive and do not exhibit the conformational change which correlates with activation (13; Allan, G. F., unpublished data). How the conditions described by Brown and Sharp (22) affect ER in the absence of hormone is also unclear. They do not result in receptor degradation (24; see also Fig. 6, lane 7). We cannot rule out the possibility that ER is denatured under these conditions and that the ligand stabilizes the receptor. However, others have shown that wild type ER does not lose the ability to bind hormone after incubation at high temperature (24). Therefore, this suggests either that the active conformation is destabilized or that the binding affinity for the receptor of a ubiquitous estrogenic contaminant is reduced by these conditions.

Antagonists induce a distinctive change in the conformation of the LBD. The difference lies within the carboxy-terminal tail of the receptor, as was shown by the protease digestion studies with the C-terminal deletion mutant. This difference in the conformation of the LBD could be a clue to the inability of these compounds to fully activate the receptor, as it may block interactions of the LBD with the general transcription machinery. One class of antagonists (type I), represented in this paper by NAF (27), displays mixed agonist/antagonist properties. NAF induces binding of the ER to DNA to the same extent as estradiol. From studies by Berry *et al.* (28) with another type I antiestrogen, tamoxifen (29) it was concluded that these antiestrogens can inhibit the action of the hormone-dependent activation function (TAF2) localized in the LBD of ER, but have no influence on the hormone-independent TAF1 in the amino-terminal A/B domain. The other type of antagonists, type II, of which ICI 164,384 is an example (30), displays minimal agonist activity and is therefore considered a pure antagonist. It causes rapid loss of ER in uterine tissue (31), impairs dimerization of the ER (32), and as we show in this paper changes the conformation of the LBD in a manner different than the agonist. These three observations may account for the pure antagonistic properties of ICI 164,384.

Taken together, the data presented here and in previous publications from our laboratory indicate a correlation between the conformation of the LBDs of steroid hormone receptors and their ability to activate transcription. The conformational changes induced by ligands

are likely to expose regions of the receptor that are necessary for interactions with the DNA response element. Estrogens provoke further changes which may be necessary for the receptor to interact with the transcriptional machinery or coactivators, thereby stabilizing the transcriptional apparatus and promoting initiation of transcription by polymerase II. Antiestrogens do not cause this additional conformational change. Consequently, they are unable to unmask the second activation function of the ER and therefore cannot fully activate the receptor, resulting in only partial agonistic activity. Our study indicates that conformational changes in the receptor play an important part in transcriptional activation and a crucial role in antagonist action.

MATERIALS AND METHODS

Materials

Ultrapure nucleotides, 3′-*O*-methyl-GTP, cap analog [m^7G(5′)ppp(5′)G], poly(dI-dC)·(dI-dC), and poly(dG-dC)·(dG-dC) were purchased from Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). [α-^{32}P]UTP and EN^{3}HANCE were from Du Pont-New England Nuclear (Boston, MA). Ribonuclease T1 was from Calbiochem (San Diego, CA). Grace's and serum-free media were obtained from GIBCO (Grand Island, NY). Transcription and nuclease-treated rabbit reticulocyte lysate translation kits were purchased from Promega (Madison, WI). Unlabeled L-methionine and L-[^{35}S]methionine (Tran^{35}S-label, >1000 Ci/mmol) were obtained from ICN Biomedicals (Costa Mesa, CA). Proteases were obtained from Sigma (St. Louis, MO). ^{14}C-Labeled low-range protein molecular weight markers were from Bethesda Research Laboratories (Gaithersburg, MD), and rabbit antirat immunoglobulin G from Zymed Laboratories (San Francisco, CA).

Plasmid Construction

The coding sequence of the hER was subcloned between the *Nco*I and *Sal*I sites of the pT7β*Sal*/*Stu in vitro* translation vector (33) as follows. The entire coding sequence of the wild type receptor (HEGO; 34) was amplified by the polymerase chain reaction (PCR) in order to generate *Nco*I and *Sal*I sites at the 5′- and 3′-ends, respectively. The sequences of the oligonucleotide primer were: 5′-GGACCATGGCCATGACCCTCCACACCAAAGCATC-3′ (5′-primer) and 5′-AGGGTCGACTCAGACTATGGCAGGGAAACCTCT-3′ (3′-primer). The artificial *Nco*I site contains the initiator AUG of the coding sequence and converts the natural threonine codon at position two to an alanine codon. The carboxy-terminal truncation mutant pT7βhERC30 was generated by replacing the final 0.8 kilobases of the wild type coding sequence (corresponding to a *Hin*dIII-*Sal*I fragment in pT7βhER) with a PCR-generated fragment. The sequences of the PCR primers were: 5′-GTGAAGCTTCGATGATGGGCTTACTGACCAACCT-3′ (5′-primer contains *Hin*dIII site) and 5′-GGCGTCGACTCATTGGTCCGTCTCCTCCACGGAT-3′ (3′-primer contains *Sal*I site adjacent to a termination codon replacing codon number 565 of the 595 amino acid receptor).

The entire reading frame of the hER was removed from the resulting vector (pT7βhER) as an *Nco*I-*Eco*RI fragment, blunt-ended with Klenow polymerase, and cloned into the *Sma*I site of the baculovirus transfer vector pVL1393 to generate the vector pVL-hER. Plasmids pLovTATA, pERE$_2$LovTATA, and pAdML200 were described previously (8, 25).

Coupled *in Vitro* Transcription and Translation

ER was transcribed and translated *in vitro* as described (14) after linearization of pT7βhER with *Sal*I.

Baculovirus Expression System

Recombinant baculovirus was isolated from occlusion body-negative plaques after cotransfection of *Spodoptera frugiperda* (Sf) cells with pVL-hER and linear viral DNA according to the InVitroGen (San Diego, CA) transfection protocol. Cells were grown, infected, and extracted as previously described (35).

Western Blot Analysis

Whole cell extracts were subjected to electrophoresis in 0.1% sodium dodecyl sulfate-10% polyacrylamide gels. Proteins were electrotransferred to an Immobilon-P (Millipore, Bedford, MA) membrane in a buffer containing 20 mM Tris-glycine, pH 7.4, and 20% methanol. The membrane was incubated with antibody D75 (23) for 3 h at room temperature, washed, and incubated with rabbit antirat immunoglobulin G for 1 h at room temperature. The membrane was developed using the ECL kit (Amersham, Arlington Heights, IL), and bands were visualized by exposure to Kodak XAR-5 film (Eastman Kodak Co., Rochester, NY) for several seconds at room temperature.

EMSA

EMSAs were carried out as previously described (36). A ^{32}P-labeled synthetic fragment containing the ERE sequence present in the *Xenopus* vitellogenin A$_2$ promoter (37) was used as a probe. Four microliters of unlabeled translation mix were used per 10-μl reaction, containing 0.1 ng DNA probe and 1 μg poly(dI-dC)·(dI-dC) as competitor. Proteins were incubated with ligand in the presence of 0.5 or 10 mM $MgCl_2$ at room temperature or 37 C for 10 min, followed by the addition of DNA and further incubation for 10 min. Two micrograms of baculovirus whole cell extract were used per 10-μl reaction, containing 4 μg poly(dG-dC)·(dG-dC) as competitor and 10 mM $MgCl_2$. The reaction was incubated for 20 min at room temperature or 37 C. DNA-protein complexes were resolved on 4% or 5% (wt/vol) gels.

Limited Proteolytic Digestion of Translated Receptor

Limited digestion of cell-free synthesized [^{35}S]methionine-labeled ER with chymotrypsin was carried out as before (14). Pretreatment of ER with hormone or antihormone was performed at room temperature or 37 C, and the $MgCl_2$ concentration was 0.5 or 10 mM.

In Vitro Transcription

Cell-free transcription of the pLovTATA and pERE$_2$LovTATA template was carried out as before (12, 38). Ten micrograms of baculovirus whole cell extract and 40 μg HeLa cell nuclear extract were incubated with or without hormone at room temperature or 37 C for 10 min. Template DNAs were added, and after another incubation, nucleotides were added (to a final vol of 25 μl and $MgCl_2$ concentration of 10 mM), and transcription proceeded at 30 C for 45 min.

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