We have previously reported that antiestrogens stimulate quinone reductase (NAD(P)H:quinone-acceptor oxidoreductase (QR or NQO1); EC 1.6.99.2) enzymatic activity, an action that may provide protective effects against the toxicity and mutagenicity caused by quinones. We have now investigated the transcriptional regulation of the QR gene by antiestrogens. In transfection experiments employing the 5’-flanking (863-base pair) region of the human QR gene promoter with its electrophile/antioxidant response element (EpRE/ARE) or deleted or mutated constructs, we observe that antiestrogens induced an increase in QR gene promoter reporter activity in estrogen receptor (ER) negative breast cancer and endometrial cancer cells transfected with ER, and this induction by antiestrogens was repressed by estradiol. The stimulation of QR transcriptional activity required the 31-base pair electrophile-responsive region from the human QR gene promoter and a functional ER. Intriguingly, antiestrogens were stronger activators of the QR EpRE via the ER subtype ERβ than ERα. Oligonucleotide gel mobility and antibody shift assays reveal that the ER binds to the EpRE but is only a minor component of the proteins bound to the EpRE in ER-containing MCF-7 breast cancer cells. While binding of ERβ to the estrogen response element was weaker when compared with ERα, ERβ and ERα showed similar binding to the EpRE. Together these findings provide evidence that QR gene regulation by the antiestrogen-occupied human QR gene EpRE and hepatoma cell extracts indicate a complex of proteins that binds to the EpRE. While binding of ERβ to the estrogen response element was weaker when compared with ERα, ERβ and ERα showed similar binding to the EpRE. Together these findings provide evidence that QR gene regulation by the antiestrogen-occupied ER is mediated by the EpRE-containing region of the human QR gene and indicate that the ER is one of the complex of proteins that binds to the EpRE. In addition, that ERβ is a more potent activator at EpRE elements than is ERα suggests that the different levels of these two receptors in various estrogen target cells could impact importantly on the antioxidant potency of antiestrogens in different target cells. These findings have broad implications regarding the potential beneficial effects of antiestrogens since EpREs mediate the transcriptional induction of numerous genes, including QR, which encode chemoprotective detoxification enzymes.

Phase 2 detoxification enzymes such as NAD(P)H:quinone-acceptor oxidoreductase (quinate reductase (QR)), glutathione-S-transferases (GSTs), epoxide hydrolase, and UDP-glucuronosyltransferases are induced in cells by electrophilic compounds and phenolic antioxidants (reviewed in Refs. 1 and 2). These widely distributed enzymes detoxify electrophiles, thereby protecting cells against the toxic and neoplastic effects of carcinogens. We have previously shown that increases in QR enzyme activity can be induced by low concentrations of antiestrogens in breast cancer cells (3). Induction of QR enzymatic activity showed unusual reversed pharmacology, being markedly up-regulated by antiestrogen and suppressed by estrogen in breast cancer cells.

The electrophile response element (EpRE) motif has been identified in the regulatory region of the genes encoding QR and the GST-Ya subunit (GST-Ya) (4, 5). This element has been shown to mediate basal expression and its activation by phenolic antioxidants (6–10), and it appeared to be essential for antiestrogen stimulation (3). The human QR gene EpRE motif, unlike the EpRE in the rat QR gene and the GST-Ya gene, contains one copy of the perfect consensus sequence for AP1 binding (10). Although band shift assays with the rat GST-Ya gene EpRE indicate that the major EpRE-interacting and activating protein is not AP1 (12, 13), band shift assays with the human QR gene EpRE and hepatoma cell extracts indicate a complex that was specifically competed by the 12-O-tetradecanoylphorbol-13-acetate (TPA) response element (TRE) (9).

The antiestrogen regulation of quinone reductase enzymatic activity represents a potentially important pharmacological mechanism for this group of anticancer drugs that had not been previously recognized. Thus, studies, now reported here, were conducted to further dissect the molecular mechanism(s) involved in antiestrogen induction of QR activity. Portions of the human QR gene promoter and 5’-flanking region including the EpRE-containing region were transfected into ER-negative breast cancer and endometrial cancer cells, and we show that the ER and the EpRE-containing region are responsible for mediating its antiestrogen responsiveness. We assess the response to various ligands in the presence of the ER subtypes, ERα or ERβ, and compare antiestrogen activation via ERα or ERβ at an EpRE site. Gel shift assays suggest that antiestrogen-mediated induction of QR gene transcriptional activity in

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MCF7 cells involves a direct transcriptional effect where both ERα and ERβ are components of the protein complex that binds the EpRE. These studies have broad implications regarding potential antiestrogen regulation of a variety of genes whose transcription is under the control of electrophile/antioxidant response elements.

**EXPERIMENTAL PROCEDURES**

**Chemicals and Materials**—Cell culture media were purchased from Life Technologies, Inc. Calf serum was from Hyclone Laboratories (Logan, UT), and fetal calf serum was from Sigma. The antiestrogens ICI 182,780 and tamoxifen (TOT) were kindly provided by Dr. Alan Wakeling and Zeneca Pharmaceuticals (Macclesfield, United Kingdom), and the antiestrogen LY 117018 was kindly provided by Lilly. TPA and CHAPS were obtained from Sigma. tert-Butylhydroquinone (TBHQ) was obtained from Aldrich. Custom oligonucleotides were purchased from Life Technologies, Inc.

**Plasmids**—pNQO1-CAT 0.863 (containing 863 base pairs of the QR gene promoter, which contains one copy of the EpRE between −476 and −437), pNQO1-CAT 0.365 (containing 365 base pairs of the QR gene promoter and missing the EpRE), pNQO1hARE-tk-CAT (containing the region between −476 and −446 of the QR gene promoter introduced upstream of the thymidine kinase gene promoter in the pBLCAT2 vector), and pNQO1hAREmut-tk-CAT (containing a mutated TRE element) were described previously (9, 10). To construct pNQO1hAREmut-tk-CAT, the single-stranded oligomer 5′-CTT GCC TGG AGG AGG ATT-3′ was used in place of ER-BSII-SK.

**Experimental Designs**—The expression vector for the wild type human estrogen receptor α (WT-ERα), the mutant ERα that lacks activation function-2 activity (ERαAF2), and the ERα DNA binding mutant, ERαAF2mut (missing amino acids 185–251) have been described previously (3, 15). The expression vector for ERβ was constructed by inserting the full-length cDNA encoding the human ERβ (530 residues, pNGV1-ERβ) (16), and including 53 additional N-terminal amino acids as in GenBank (accession number AF051427) into the BamHI site of pBluescript II SK+. This plasmid encoding the β-galactosidase gene was used as an internal control for transfection efficiency in all experiments. Flag-ERβ-SS1-SK+ was constructed by ligating the blunt end HindIII/NcoI fragment from Flag-ERβ-CMV5 into the HindIII site of pBluescript II SK+.

**Cell Culture and Transfections**—MCF7 cells and MDA-MB-231 human breast cancer cells were maintained as described previously (3, 17). Human endometrial adenocarcinoma (HEC-1) cells were from the American Type Culture Collection. Cell growth medium was minimal essential medium plus phenol red supplemented with 5% fetal calf serum, 5% heat-inactivated calf serum and 10 mM HEPES, 100 units/ml penicillin, 100 μg/ml streptomycin, and 25 μg/ml gentamicin. MDA-MB-231 and HEC-1 cells were transfected, and chloramphenicol acetyltransferase (CAT) assays were conducted as described previously (17, 18).

**RNA Isolation and Northern Blot Analysis**—Gel-purified reamplified Qα cDNA was random primer-labeled using the Ready-to-Go DNA labelling kit from Amersham Pharmacia Biotech for Northern analysis. Total RNA was isolated from cells using the RNA extraction kit from Amersham Pharmacia Biotech. Twenty μg of total RNA was separated by electrophoresis, transferred to nitrocellulose support, and hybridized with random primer-labeled cDNA (19). Full-length cDNA for the human QR was kindly provided by David Ross (University of Colorado, Denver).

**In Vitro Transcription and Translation**—In vitro transcription and translation of the ERα was performed using the Promega TNT kit (Madison, WI). Briefly, 1 μg of ERα-BSII-SK+ or ERα cDNA in pBluescript II SK+ vector; see Ref. 20) or Flag-ERβ-BSII-SK+ was mixed with 25 μl of TNT rabbit reticulocyte lysate, 2 μl of TNT buffer, 1 μl of amino acid mixture, and 1 μl of T7 RNA polymerase (20 units/μl). For control lysates, pBluescript II SK+ vector lacking the ER cDNA was used in place of ER-BSII-SK+. In the reaction, the final reaction of 50 μl was incubated for 90 min at 30 °C. The translation efficiency of ERα and ERβ was checked by adding 4 μl of [35S]methionine (15 μCi/μl; ICN, Costa Mesa, CA) in parallel reactions and analyzing 1 μl of the lysate by SDS-polyacrylamide gel electrophoresis.

**Gel Shift Assays**—Nuclear extracts from MCF7 cells for use in the gel shift assays were prepared as described previously (21). The single-stranded oligomers, either 5′-AAT TAA ATC GCA GTG ACA GTG ACT CAG CAC AAC AGA ACC-3′ or 5′-AGC TAG TCA GCG GAG CAC AGT GAT ATC-3′, which contain the −476 to −437 region of the human QR gene, or 5′-AGC TAG TCA GGT AGT GAT CCC GAA-3′, which contains the consensus ERE, were annealed to their complement, and cloned at the BamHI site of pBLCAT2. The estrogen response element (ERE)-containing reporter, (ERE)-pS2-CAT, has been described previously (14).

**RESULTS**

**Antiestrogens Increase QR Gene mRNA Expression in Breast Cancer Cells**—As shown in Fig. 1, QR mRNA expression is markedly induced in ER-containing MCF7 breast cancer cells briefly exposed to the antiestrogens TOT and ICI 182,780. Although several QR mRNA species have been reported in certain cell lines, we were able to detect only one 2.7-kilobase mRNA species in MCF7 cells (Fig. 1). The 2.7-kilobase mRNA species corresponds to the most abundant and dioxin-inducible mRNA species detected in HepG2 hepatoma cells (23). A 4.2- and 1.9-fold increase in QR mRNA was detected in cells treated with the antiestrogens TOT and ICI 182,780 for 24 h, respectively. No increase in QR mRNA levels was evident in cells treated with the estrogen estradiol (E2; Fig. 1); E2 even appeared to slightly reduce the control level of QR mRNA in the cells. As expected for an estrogen receptor (ER)-mediated proc-
Along with an expression vector for the wild type human estrogen receptor (WT-ER) estrogen receptor.

Aconcentrations of TOT, E2, TPA, or TBHQ as indicated. Cell extracts were prepared and analyzed for CAT activity and aldehyde internal control reporter to correct for transfection efficiency. Cells were then treated for 24 h with control ethanol vehicle (C) or varying concentrations of TOT, E2, TPA, or TBHQ as indicated. Cell extracts were prepared and analyzed for CAT activity and β-galactosidase activity as described under "Experimental Procedures." Values are the means ± S.E. from three separate experiments.

To examine the possible transcriptional regulation of the QR gene by antiestrogen, a reporter construct that contains the 863-base pair 5'-flanking and promoter regions of the human QR gene upstream of the CAT gene (Fig. 2) was introduced into an ER-negative breast cancer cell line, MDA-MB-231 cells. In cells cotransfected with an expression vector for the wild type ERα, TOT induced an increase in pNQO1-CAT 0.863 reporter activity (Fig. 3, left). E2 inhibited the TOT-mediated, but not the TBHQ-mediated, increase in QR transcriptional activity. No increase in CAT activity was evident in cells after treatment with TPA, a known inducer of AP1 activity (24). Cells that were co-transfected with the control expression vector lacking the ER cDNA (pCMV5), with an expression vector for a mutant ERα that has impaired activation function-2 activity (S554fs), or with an expression vector for a mutant ERα that has impaired DNA binding ability (DBDmut) did not show activation from pNQO1-CAT 0.863 in response to antiestrogens (Fig. 3), although the effect of TBHQ was still observed. Thus, antiestrogen-mediated activation of QR gene promoter activity requires a transcriptionally active ERα. Treatment with either TOT or TBHQ did not induce transcription from a deletion mutant of the pNQO1-CAT 0.863 reporter construct, denoted pNQO1-CAT-0.365, that lacks the base pair -366 to -863 portion of the 5'-flanking region (Fig. 3, right). As expected (10), this truncated construct has reduced basal promoter activity. Our results indicate that the region between kilobase pairs -0.863 and -0.365 of the QR gene is essential for induction by both TOT and TBHQ.

Sequence analysis of the 5'-flanking region of the human QR gene indicates the presence of a single copy of the EpRE motif at positions −467 to −437 (9, 10). The EpRE motif (shown in Fig. 6) contains a TRE and a TRE-like element (see Fig. 2). The EpRE-containing region was introduced upstream of a heterologous promoter, thymidine kinase, and the CAT gene. Although this EpRE-containing construct showed significant basal CAT activity in 231 cells, TOT and TBHQ were able to induce transcription 2.2- and 3.4-fold, respectively, over basal levels (Fig. 4, left). Mutation of the perfect TRE element (middle portion of Fig. 4) reduced basal CAT activity and eliminated induction by TBHQ and TOT. Mutation of the TRE-like element (right portion of Fig. 4) also reduced basal activity and eliminated the TBHQ- and TOT-induced increase in QR gene transcriptional activity. TPA, which increases AP1 activity, did not induce an increase in EpRE-mediated CAT gene expression of the wild type (unmutated) gene construct in these cells (left portion of Fig. 4). This observation does not appear to be due to the absence of AP1 activity, because we have previously shown that TPA induced TRE-mediated CAT reporter gene activity in 231 cells (3).

Regulation of QR Gene Transcriptional Activity by ERα and ERβ—Another subtype of the ER, ERβ, has been recently cloned (16, 25, 26). ERβ exhibits lower transcriptional response to estradiol and TOT when compared with ERα in the context of several ERE-containing gene reporter constructs (16, 25–27). However, ERβ has been reported to show increased TOT agonism from reporter constructs containing an AP1 site (27). We thus were interested in examining ligand activation of ERβ and

![Fig. 2. Diagram of the QR reporter gene constructs.](image-url)

![Fig. 3. Antiestrogen induction of pNQO1-CAT reporter gene activity in estrogen receptor negative cells is mediated by the estrogen receptor. A, MDA-MB-231 breast cancer cells were transfected with the pNQO1CAT 0.863 or pNQO1CAT 0.365 reporter construct along with an expression vector for the wild type human estrogen receptor (WT-ERα). Cells were also transfected with an expression vector for a DNA binding mutant of ERα (ERαDBDmut, missing amino acids 185–251), an expression vector for a mutant ERα that lacks activation function-2 activity (ERαAF2), or the empty expression vector missing the estrogen receptor cDNA (pCMV). The cells were also transfected with a β-galactosidase internal control reporter to correct for transfection efficiency. Cells were then treated for 24 h with control ethanol vehicle (C) or varying concentrations of TOT, E2, TPA, or TBHQ as indicated. Cell extracts were prepared and analyzed for CAT activity and β-galactosidase activity as described under "Experimental Procedures." Values are the means ± S.E. from three separate experiments.](image-url)
ERs from an EpRE, as compared with activation from an ERE.

With a reporter construct containing EREs upstream of the promoter for the estrogen-regulated pS2 gene (Fig. 5A), ERα and ERβ both showed stimulation by the estrogen, estradiol, and as expected, ERβ showed a lower transcriptional response. Interestingly, ERα was weakly stimulated by the antiestrogen TOT and by another antiestrogen, LY 117018 (LY) at this ERE-containing gene construct, whereas the antiestrogens failed to elicit ERβ transcriptional activity at this ERE-containing gene (Fig. 5A). The transcriptional response of ERβ and ERs to these ligands was very different in the context of the QR gene promoter construct pNQO1-CAT 0.863. Of note, although antiestrogens stimulated QR activity via both ERα and ERβ, the magnitude of increase in QR gene transcriptional activity in response to TOT or LY 117018 was higher with ERβ than with ERs (Fig. 5B), and no stimulation by estradiol was observed via ERβ or ERα at an EpRE.

Analysis of the Interaction of Nuclear Factors from Breast Cancer Cells with the EpRE—The antiestrogen-mediated increase in QR mRNA levels might occur through an ER-mediated transcriptional effect that could involve the binding of ER to the EpRE. Thus, gel shift assays (Fig. 6) were conducted to determine if the ER is part of the transcriptional complex that binds to the EpRE.

Fig. 6A shows the nucleotide sequence of the −476 to −437 EpRE motif from the QR gene and the ERE oligonucleotide containing one consensus ERE. The DNA-protein complexes detected in TOT-treated cells by gel shift assays with this ERE oligonucleotide were not different from complexes detected using nuclear extracts from untreated MCF7 cells (data not shown). Previous reports also indicate no detectable differences in protein-EpRE interactions using TBHQ-treated versus untreated nuclear extracts from HepG2 cells (13). Gel shift assays (Fig. 6B) with the radiolabeled EpRE and nuclear extracts from MCF7 cells treated with antiestrogens revealed a shifted complex that was specifically competed by unlabeled EpRE (Fig. 6B, lane 3) and by high concentrations (200× but not 50×) of ERE (lane 4 versus lane 6) but not by excess unlabeled TRE (lane 5), mutated ERE (lane 7), or mutated EpRE (mut 1 or mut 2 of Fig. 2; data not shown). Unlabeled EpRE was a more effective competitor than was unlabeled ERE (compare lanes 3 and 6 with a 50-fold excess of EpRE or ERE). These observations may reflect the fact that a portion of the EpRE (−465 to −453) resembles an ERE. All nucleotides from −465 to −453 of the EpRE are identical to the ERE (underlined nucleotides), except for the outermost 5’ and 3’ nucleotides (positions −465 and −453), where a different purine or a different pyrimidine is found, respectively. Therefore, at a sufficiently high concentration, the ERE oligonucleotide may be able to displace the EpRE from proteins that normally bind to it. A consistently observed but light supershifted band (SSB) was evident in the presence of a monoclonal antibody to the ER (lane 8). However, there are other proteins that interact with the EpRE and are not shifted by ER antibody.

To further verify the binding of the ER to the EpRE, gel shift assays were also performed using unlabeled in vitro translated ER (Fig. 6C). A DNA-protein complex was observed that was competed out by unlabeled EpRE (50×) or ERE (200×) (Fig. 6C, lanes 4–6). Moreover, this complex was supershifted with ER antibody (Fig. 6C, lane 7). This specific band was not seen with control lysate (ivt-CTRL, lanes 8–10) from in vitro transcription-translation reaction with the vector lacking the ER cDNA, confirming that the band observed in lane 4 can be attributed to ER binding to the radiolabeled EpRE. The major protein complex detected (lane 4) migrated similarly to the major DNA-protein complex detected in gel shift assays where 32P-ERE was used as the DNA template for binding of in vitro translated ER (lane 11). This 32P-ERE-protein complex was fully competed with excess radiolabeled ERE (lane 12) and was supershifted with ER antibody (lane 13). Thus, together with the results presented in Fig. 6B, these gel shift assays indicate that the ER binds to the EpRE and is one of several proteins that interacts with the EpRE. The binding of ER to the EpRE appears to be much weaker than its binding to the ER, since equal cpm and ng amounts of radiolabeled 32P-ERE and 32P-ERE were used in the binding reactions, and the DNA-ERE complex is much less intense with the 32P-ERE oligomer (Fig. 6C).

Because of higher transcriptional activation of the QR gene by ERβ when compared with ERα (as seen in Fig. 5B), we examined the DNA binding ability of ERα and ERβ to the EpRE. For these experiments, we used Flag epitope-tagged ERβ. Our gel shift assays, using 32P-labeled EpRE or 32P-labeled ERE and nonradiolabeled ERα or ERβ prepared by in vitro transcription and translation (Fig. 7), indicate that although ERβ showed much weaker binding with the ERE when
compared with ERα. ERβ binding to the EpRE was comparable with that observed with ERα (Fig. 7). The presence of ERα and ERβ in the DNA-protein complex is indicated by the supershifted bands observed in the presence of ER antibody H222 or Flag antibody M2, respectively. Thus, the higher transcriptional activation from the EpRE by ERβ as compared with ERα cannot be attributed to differences in the relative EpRE binding ability of ERβ and ERα but perhaps to receptor-type specific interactions with other protein factors in the EpRE transcriptional complex.

**DISCUSSION**

Antiestrogens induce an increase in QR mRNA in breast cancer cells, and our studies indicate that the regulation of QR activity by antiestrogens occurs at the transcriptional level and is mediated by the ER and an EpRE element in the QR gene.

Gel shift analyses reveal that the ER can bind to the EpRE. Therefore, the antiestrogen-mediated increase in QR activity may occur through a direct transcriptional effect by the ER when bound to antiestrogens and/or may involve antiestrogen-ER enhancing the activity of other factors that interact with the EpRE.

In contrast to the observations made with antiestrogens, TBHQ-mediated induction of QR gene transcriptional activity did not require the ER and occurred equally well in the presence of functionally inactive ER or in the absence of ER altogether. However, the DNA elements required for antiestrogen-mediated induction of QR gene transcriptional activity that we identified through deletion and mutational studies mapped identically with the elements required for TBHQ-mediated induction. The TRE and the TRE-like element in the EpRE were required for antiestrogen and TBHQ-mediated induction. However, no increase in QR gene transcriptional activity resulted from TPA treatment, which induces the synthesis and/or activity of AP1 transcription factors (24). Although antiestrogens have been reported to affect gene transcription through an AP1 site in some cell types (28), we were not able to induce an increase in CAT activity from TRE-containing reporter constructs in MCF7 cells after antiestrogen treatment (3). We were also not able to compete out interactions between the EpRE DNA elements and MCF7 nuclear factors with excess amounts of consensus TRE oligonucleotide. Therefore, although the EpRE contains a perfect TRE and two TRE-like elements, the major EpRE binding and activating protein factors in MCF7 breast cancer cells are probably not the AP1 transcription factors. However, our studies do not preclude the possibility that AP1 transcription factors are involved in EpRE-mediated transcriptional regulation in other systems, as has been reported in Hepa-1 liver tumor cells (9).

Since the antiestrogen-estrogen receptor-mediated increase in QR gene expression might involve the direct binding of ER to the EpRE, we looked by gel shift analyses for proteins in MCF-7 cells that interact with the EpRE and, in particular, sought to determine whether ER is one of these proteins. We observed a strong gel shift and a conspicuous complex formed between MCF-7 cell proteins and radiolabeled EpRE. A shift of a minor portion of the radiolabeled EpRE-protein complex was observed with antibody to the ER, indicating that the ER was capable of interacting with the EpRE. This was also verified in studies utilizing in vitro transcribed and translated ER. A transcriptional effect is supported also by the requirement for ER DNA binding ability in the activation of the QR gene by antiestrogens (Fig. 3). This interaction may reflect, in part, the similarity in nucleotide sequence between the ERE and the EpRE. AP1 transcription factors are involved in EpRE-mediated transcriptional regulation in other systems, as has been reported in Hepa-1 liver tumor cells (9).

Our observations are consistent with several reports showing that EpRE-mediated chemoprotective gene expression involves the interaction of multiple proteins with the EpRE regulatory site (12, 29, 30). The direct interaction of the ER with the EpRE also appeared to be weaker than the interaction of the ER with the ERE. Thus, a direct transcriptional effect of the ER mediated through the EpRE is possible, because we see some ER interaction with the EpRE, but this interaction appears to be weak when compared with the interaction of other protein(s) with the EpRE, and the ER is only one of several proteins bound there.

Several aspects of antiestrogen regulation of QR transcriptional activity cannot be attributed solely to ER binding to the EpRE and remain to be investigated. This is especially true in light of 1) our previous observation that the time course of
induction of QR enzyme activity is relatively slow (with increases in QR mRNA first detectable at 12–16 h after antioestrogen treatment of MCF7 cells; Ref. 3), 2) our previous observation that antiestrogen activation of GST-Ya gene transcriptional activity is mediated through an EpRE, which is not homologous to the ERE (3), and 3) the observation in the

**Fig. 6. Interaction of nuclear factors from breast cancer cells and interaction of ER with the EpRE.** A, the sequence of the −476 to −437 region of the human QR gene and consensus ERE. B, gel mobility shift assays were performed using a double-stranded oligomer containing the −476 to −437 region of the human QR gene and extracts from MCF7 cells treated with tamoxifen (for 48 h) as described under “Experimental Procedures.” Lane 1, no extract; lane 2, with extract; lane 3, with extract plus a 50-fold excess of unlabeled EpRE; lane 4, with extract plus a 200-fold excess of unlabeled ERE; lane 5, with extract plus a 200-fold excess of unlabeled TRE; lane 6, with extract plus a 50-fold excess of unlabeled ERE; lane 7, with extract plus a 200-fold excess of unlabeled TRE; lane 8, with extract plus mononuclear antibody for the estrogen receptor, H222. The arrows indicate the shifted (SB) and supershifted (SSB) bands. The autoradiographs have been overexposed in order to highlight the estrogen receptor-EpRE supershifted (SSB) band. C, gel mobility shift assays were performed using a double-stranded oligomer containing the −476 to −437 region of the human QR gene (lanes 1–10) or the consensus ERE (lanes 11–13) and MCF7 nuclear extracts (lanes 1–3), in vitro translated ER (lanes 4–7 and lanes 11–13), or control lysate (ivt-CTRL, pBluescript II SK + vector lacking the ER cDNA was used in place of ER-BSII-SK + in the in vitro transcription/translation reaction; lanes 8–10) as described under “Experimental Procedures.” Competitor unlabeled EpRE (50-fold excess) or unlabeled ERE (200-fold excess) or ER antibody H222 included in the reaction are indicated above each lane. The positions of the major shifted complex (SB) and the supershifted complex (SSB) are indicated. Equal cpm and ng amounts of 32P-EpRE and 32P-ERE were used in the binding reactions. The autoradiographs are representative of three separate experiments.

**Fig. 7. Both ERα and ERβ interact with the EpRE.** Gel mobility shift assays were performed using 32P-labeled double-stranded oligomer containing the −476 to −437 region of the human QR gene (EpRE, lanes 1–10) or 32P-labeled consensus ERE (lanes 11–16) and in vitro translated ERα (lanes 1–4, and lanes 11–13), in vitro translated Flag-ERβ (lanes 5–8 and lanes 14–16), or control lysate (ivt-CTRL, pBluescript II SK + vector lacking the ER cDNA used in place of ER-BSII-SK + in the in vitro transcription/translation reaction; lanes 9 and 10) as described under “Experimental Procedures.” Competitor unlabeled EpRE (50-fold excess) or unlabeled ERE (200-fold excess), ERE antibody H222, or Flag antibody M2 included in the reaction are indicated above each lane. The positions of the major shifted complex (SB) and the supershifted complex (SSB) are indicated. Equal cpm and ng amounts of in vitro translated nonradiolabeled ERα and ERβ were used in these experiments based on separate parallel experiments in which radiolabeled ERα and ERβ were made by in vitro translation. The autoradiographs are representative of three separate experiments.
present studies that the interaction of the ER with the EpRE is weak and that the EpRE interacts with additional proteins. Clearly, the regulation by antiestrogen-ligated ER may be also attributable to changes in the levels and/or the activity of other factors. The factors that interact with the EpRE are only now being characterized (4, 5, 12, 29, 30) and highlight the likely complexity of EpRE-mediated gene regulation. An intriguing possibility is that antiestrogens, and not estrogens, might promote an interaction of the ER with protein activators of QR transcriptional activity.

It is clear that ligand activation of ER transcriptional activity is highly dependent on the nature of the response element. Antiestrogen ligands (TOT, LY 117018), which induce very little activity from ERβ at an ERE, can induce significant activity from ERβ at an EpRE and, as previously reported, from a TRE (27). It has been proposed that ERβ regulates transcription from an AP1 site by a direct interaction with Fos and Jun. However, our studies suggest that transcriptional activation of the QR gene by TOT is not mediated through Fos and Jun. Our studies indicate that the differential transcriptional activation of the QR gene by TOT and ERα does not directly correlate with their EpRE binding ability, since this appeared to be similar. This would be consistent with antiestrogen regulation of QR transcriptional activity not being determined only by ER binding to the EpRE, with ERβ perhaps interacting more effectively with other protein regulators of QR gene activity than does ERα.

We have now observed antiestrogen-mediated activation of EpRE enhancer activity in various promoter contexts, the QR gene promoter (as shown in this study) and the GST-Ya gene promoter (3), as well as in the context of a heterologous promoter, the thymidine kinase gene promoter (this study). The induction of QR gene transcriptional activity by antiestrogens was also evident in more than one cell context. It is of note that tamoxifen has been reported to induce an increase in the mRNA levels of other phase II detoxifying enzymes in rat liver (31). These findings raise the intriguing possibility that antiestrogens might regulate, in several cellular contexts, the activity of numerous proteins that contain EpREs in their regulatory regions and thereby afford substantial chemoprotective benefit to estrogen receptor-containing cells. This activation by antiestrogens may be modulated by differences in the relative levels of ERα and ERβ in different target cells and by additional promoter-specific elements, as observed for several steroid hormone-regulated genes (11, 32–36), thus altering the pharmacological response profile (EC50 and magnitude) at different EpRE-regulated genes.

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