Human Estrogen Receptor Transactivational Capacity Is Determined by both Cellular and Promoter Context and Mediated by Two Functionally Distinct Intramolecular Regions

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We have used a series of human estrogen receptor (ER) mutants to evaluate the cell- and promoter-specific transcriptional activities of the TAF1 and TAF2 transactivation regions within the human ER. We show that the manifestation of TAF1 or TAF2 function depends strongly upon promoter context; on certain promoters, both the TAF1 and TAF2 activators are required for wild-type transcriptional activity, whereas on other promoters, the TAF1 and TAF2 activators function independently. Using these constructs, we show that the antagonist activity of the triphenylethylene-derived antiestrogens, *e.g.* tamoxifen, arises from their intrinsic inability to activate ER TAF2 function. However, on certain promoters, these antiestrogens efficiently activate gene transcription through ER. Consistent with this observation, the TAF2 function of the ER is not required on all promoters. In these TAF2-independent promoter contexts, TAF2 function may be provided by a separate transcription factor bound to the promoter. These data suggest that 1) TAF1 may be the major transcriptional activator of the ER; and 2) TAF2 functions as a transcriptional facilitator. On promoters where TAF2 function is provided independently of the ER, the TAF1 function of the ER can function independently of TAF2 activity, allowing triphenylethylene-derived antiestrogens to demonstrate partial agonist activity.

These observations provide a possible molecular explanation for the tissue-specific partial agonist properties of tamoxifen and related triphenylethylene antiestrogens observed *in vivo*. (Molecular Endocrinology 8: 21–30, 1994)

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INTRODUCTION

The estrogen receptor (ER) is a member of the intracellular receptor superfamily of transcription factors whose functions are regulated by their cognate steroid, vitamin, or thyroid ligands (1). This family of regulatory proteins shares a common mechanistic feature, in that they are transcriptionally inactive within the cell before hormonal exposure. Occupancy by hormone results in activation of the receptor, thus allowing it to productively associate with specific DNA sequences in the regulatory regions of target genes. The resultant positive or negative effects of the DNA-bound receptor on specific gene transcription are cell type and promoter context dependent (2).

The cDNA for ER has been cloned and used in the reconstitution of estrogen-responsive transcription units in heterologous mammalian cells (3, 4). This has enabled a detailed examination of the functional domains within the ER protein (5). Successful extension of these studies to lower eukaryotes such as *Saccharomyces cerevisiae* supports the premise that unique mammalian cell-specific processes are not required for receptor function (6, 7). Despite this, it is clear that hormone-activated receptors do not function identically in all cells. Most revealing in this regard are studies with the antiestrogen tamoxifen. This compound, which

functions as an antagonist of many estrogen-mediated responses, demonstrates partial agonist activities in certain tissues (8). Differences in pharmacokinetics or pharmacodistribution of tamoxifen or the formation of metabolites with estrogen agonist properties could account for a subset of these mixed agonist and antagonist effects *in vivo*. However, these differences in tamoxifen pharmacology may also relate to the fundamental molecular differences in the way different cells interpret the tamoxifen signal, possibly as a consequence of distinct transcription factor pools, receptor levels, posttranslational modifications, chromatin structure, or the promoter context in which the estrogen response elements (EREs) reside.

Functional examination of the domains of the ER in several systems has revealed the likely structural features within the receptor that may interface with critical cellular components to generate a variety of hormone-responsive end points (9). In particular, two distinct transactivation domains have been defined: a region at the amino-terminus of the receptor, termed TAF1, and a region confined to the carboxyl-terminal 60 amino acid residues, termed TAF2 (9–11). Each of these regions may interact directly with unique transcriptional targets, although those cellular targets remain to be defined. However, recent work by our group using a reconstituted estrogen response system in yeast has identified a genetic locus designated SSN6, which allows a 100-fold increase in TAF1 activity when mutated (12). Postulated mammalian homologs of this yeast protein could determine tissue-specific differences in TAF1 transcriptional activity. Experiments in HeLa cells and chick embryo fibroblasts have suggested that the transcriptional activities of the TAF1 and TAF2 sequences within the ER are manifested in a cell- and promoter-specific manner (11). Rigorous examination of TAF1 and TAF2 functions in mammalian cells, however, has not yet been accomplished. In the present study, we examine the dependence of TAF1 and TAF2 activities on cell and promoter context in mammalian cells. In addition, we evaluate the role of ligand in the manifestation of these differences in TAF1 and TAF2 activation.

RESULTS

Transcriptional Activation by Amino- and Carboxyl-Terminal Truncated Receptors

Truncated forms of the human ER were prepared which lacked either the TAF1 (ER179C; see Fig. 1A) or the TAF2 (ERN282G; see Fig. 1A) activation sequence. These constructs as well as the wild-type ER (ER-wt) encode proteins structurally identical to those used previously in mammalian (8) and yeast (13) cells. The transcriptional activities of these ER derivatives were assessed using a reporter plasmid (ERE-tk-LUC) containing one copy of the vitellogenin ERE (19) inserted up-stream of the thymidine kinase promoter. The reporter plasmid and increasing concentrations of ER or mutant ER expression vectors were transiently transfected into the ER-negative cell lines CV-1 (monkey kidney fibroblasts), HepG2 (human hepatocellular carcinoma), and HS578T (human breast cancer cells), and their activities were assessed, as documented in Fig. 1, B–D. All transfections were performed in the absence or presence of 17β-estradiol, the latter concentrations ranging in 1-log steps from 10^{-11}-10^{-5} M. However, due to the number of data points obtained in this way (>2500), we present only the activities obtained using 10^{-7} M 17β-estradiol, a concentration that elicited the maximal transcriptional response in all cell lines examined.

The ER-wt was active in all cell lines tested. Using this protocol, we were unable to detect significant TAF1-mediated transcriptional activity in CV-1, HepG2, HS578T (Fig. 1, B–D), or HeLa cells (data not shown) when assayed in the context of the ERN282G deletion. In contrast, however, the TAF2 activation function using ER179C exhibited substantial activity in these cells (Fig. 1, B–D). The magnitude of TAF2 transcriptional activity in ER179C was cell type dependent. However, even at DNA concentrations that produced a maximal transcriptional response, this isolated activator exhibited lower efficacy than ER-wt. In HepG2 cells, ER179C was about 35% as efficacious as ER-wt (Fig. 1C), whereas in CV-1 and HS578T, the ER179C demonstrated 70% and 65% of ER-wt activity, respectively (Fig. 1, B and D). Transfection efficiency and recombinant expression levels were similar for the three receptor forms, as estimated by indirect immunofluorescence microscopy and flow cytometry (data not shown). Our results are consistent with the hypothesis that the TAF1 and TAF2 sequences represent functionally distinct transcriptional activators. Clearly, fully effective wild-type receptor activity on the ERE-tk promoter requires both activator regions, or alternatively, intact receptor context is necessary for each activator to exhibit maximal transcriptional activity.

In addition to the partial efficacy observed with the above mutated ERs, increasing concentrations of the ER-wt in CV-1 and HS578T cells led to a progressive decrease in hormone-dependent transcriptional activity (Fig. 1, B and D). This compromise of receptor function has been observed by others and probably results from sequestration of transcription factors present in limiting amounts by the overexpressed hormone-activated receptor (11). This squelching or transcriptional interference phenomenon supports the idea that ER requires additional cellular transcription factors that exist in limiting concentrations for appropriate function. The failure of the ER-wt to squelch in the HepG2 cell line (Fig. 1C) suggests either an increased abundance of a required cofactor or the involvement of an additional cell-specific component in this process.

Transcription Activation by a Mutant ER Defective in TAF2 Activity

Previously, TAF1 and TAF2 functions were defined as individual domains within the ER that were capable of

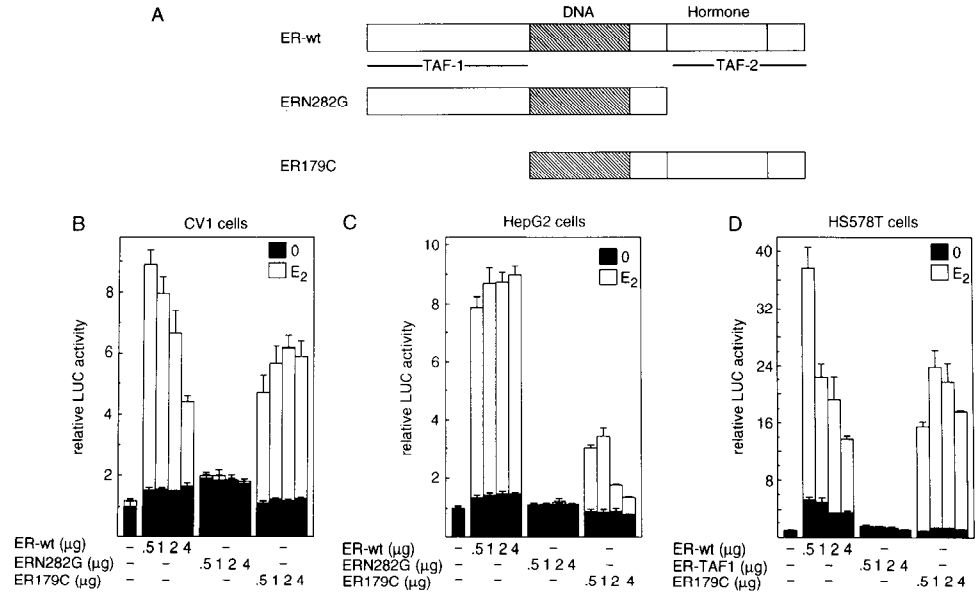

Fig. 1. Transcription Activation by ER-wt and Truncated ER Mutants

A, Schematic organization of ER-wt, ERN282G, and ER179C. CV-1 (B), HepG2 (C), and HS578T (D) cells were transiently cotransfected with increasing concentrations of the different receptor expression vectors, as indicated, together with 9.5 μg/ml ERE-tk-LUC reporter plasmid and 5 μg/ml pRSV-β-gal expression vector as an internal control for transfection efficiency. Carrier DNA (pGEM4) was added to adjust the total amount of DNA to 20 μg/ml (see *Materials and Methods*). Cells were treated with or without 10^{-7} M 17β-estradiol, as indicated, for 36 h and assayed for β-galactosidase and luciferase activity (luciferase activity was normalized for β-galactosidase activity). The relative luciferase activity is calculated by dividing the normalized luciferase value at a given point by that obtained in the absence of transfected receptor or ligand. A single experiment representative of four independent experiments is detailed above. The data shown indicate the mean ± SEM of triplicate estimations.

supporting transcription of an ER-responsive promoter (8, 11). In the mammalian cells tested here, we were unable to show a distinct activity of the TAF1 activation sequence when analyzed in the context of the ERN282G deletion. We considered, therefore, that analysis of this transactivator outside the context of the full-length receptor might not reflect its true biological activity. Previously, Danielian *et al.* (9) demonstrated that it was possible to obliterate TAF2 activity by changing three amino acids between residues 535 and 550 in the carboxyl-terminus of the mouse ER. The resultant mutant receptor was, however, capable of binding DNA and hormone with wild-type affinity, indicating that these changes did not lead to gross structural abnormalities in the protein. Therefore, we used site-directed mutagenesis to recreate the same amino acid changes in the carboxyl-terminus of human ER at residues 538, 542, and 545 (9) and designated this mutant ER-TAF1. This triple mutation was also introduced into ER179C, creating an estrogen receptor lacking functional TAF1 and TAF2 sequences. This latter construct, designated ER-Null, allowed specific determination of the effects of these mutations on TAF2 function in the context of ER179C.

The transcriptional activities of the mutant ERs were assessed by transient transfection into CV-1 cells together with the ERE-tk-LUC reporter. ER-null was completely inactive in CV-1 cells (Fig. 2B). Thus, we anticipated that introduction of these mutations into ER-wt would allow an examination of TAF1 activity in the context of the full-length receptor without interference from TAF2 activity.

The full-length receptor containing the triple mutation (ER-TAF1) was subsequently used for analysis of TAF1 function in the context of the intact receptor. Constructs encoding ER-wt, ER-TAF1, ER179C, or ER-null together with the ERE-tk-LUC reporter were transfected into CV-1, HepG2, or HS578T cells, and their activities assessed. The results are shown in Fig. 2. In all cell lines, ER179C was transcriptionally active, as observed previously (Fig. 2, B–D), whereas ER-null was inactive. Interestingly, ER-TAF1 exhibited significant transcriptional activity (Fig. 2, B–D). Thus, the activity of the TAF1 activator in the context of the full-length receptor, as observed here, was different from that after analysis as a deletion mutant (ERN282G; Fig. 1). This suggests that TAF1 activity does not function independently, but, rather, requires additional carboxyl-terminal sequences for appropriate function. Surprisingly, increasing the concentration of transfected ER-TAF1 DNA did not result in a receptor-dependent squelching of transcription. This observation implies that both TAF1 and TAF2 activators, and possibly the context of the full-length receptor, are required for this squelching phenomenon.

To make comparisons among results with these ER variants meaningful, it is important that the level of each variant protein expressed is comparable. Consequently, a comparison was made of the expression level of each

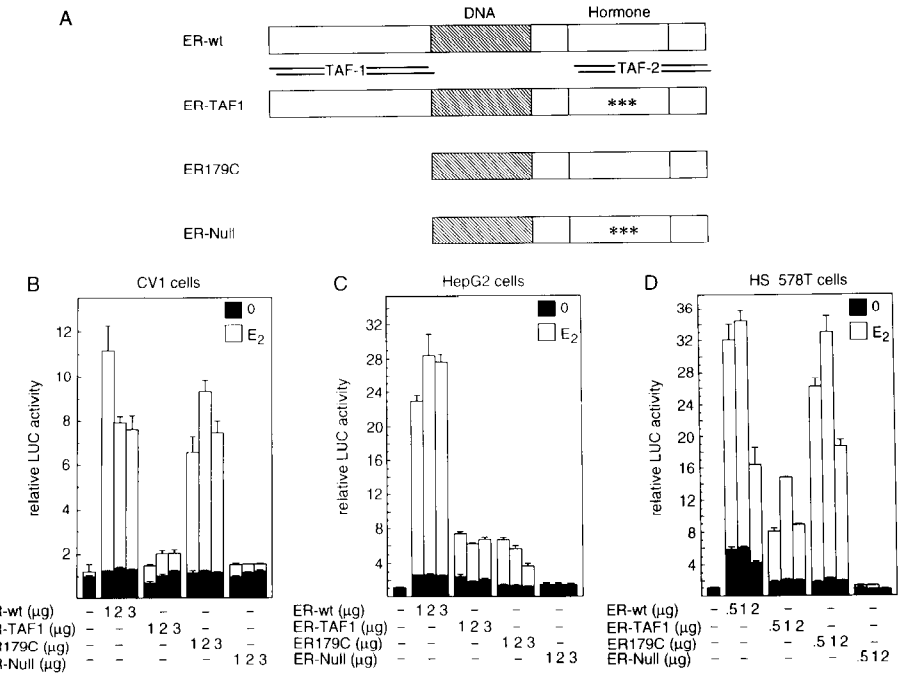

Fig. 2. Transcription Activation by Mutant ER Defective in TAF2 Activity

A, Schematic organization of ER-wt and mutant ERs used in this experiment. CV-1 (B), HepG2 (C), and HS578T (D) cells were transiently cotransfected with increasing concentrations of receptor expression vectors, as indicated, together with ERE-tk-LUC reporter plasmid, pRSV-β-gal expression vector, and carrier DNA, as described in Fig. 1. Cultures were treated with or without 10^{-7} M 17β-estradiol and assayed for luciferase and β-galactosidase activities 36 h later. All values were normalized for transfection efficiency by simultaneous estimation of pRSV-β-gal transcriptional activity. The relative luciferase activity is calculated by dividing the normalized luciferase value at a given point by that obtained in the absence of transfected receptor or ligand. A single experiment representative of four independent experiments is detailed above. The data shown indicate the mean ± SEM of triplicate estimations.

of these receptors by transfecting the expression vectors into CV-1 cells and analyzing the hormone-binding activities of each receptor in the resulting cytosolic extracts (data not shown). The K_d values of ER-wt, ER-TAF1, and ER179C for estradiol were identical. The ER-wt and ER-TAF1 were synthesized at comparable levels, as measured by maximal hormone-binding activity, whereas ER179C and ER-null were expressed at about 25% of ER-wt levels. As all of the transcriptional responses reported for each receptor were the maximal responses achievable (in the linear portion of the response curve), it is unlikely that receptor expression levels were a significant factor in the outcome of our experiments.

ER-TAF1 and ER179C Activities Are Promoter Specific

The above results using the ERE-tk-LUC reporter indicated that the TAF1 activator of the ER functions, albeit with less than full efficacy, in the absence of an intact TAF2 function. In addition, TAF1 activity appeared to be cell type dependent. Therefore, we extended our studies to examine the efficacy of the individual activator functions on other estrogen-responsive promoters. To this end, we chose the estrogen-responsive complement factor 3 (C3) promoter, in which a putative ERE has recently been identified (21). The activities of ER-wt, ER-TAF1, and ER179C were evaluated on C3 promoter-directed transcription, as depicted in Fig. 3. In HS578T cells, the C3 promoter could be activated equally well by ER-wt, ER-TAF1, or ER179C (Fig. 3C). In HepG2 cells, in contrast, although ER-TAF1 was as efficacious in transcription as ER-wt, ER179C was silent (Fig. 3B). These data suggest that on the C3 promoter, there is a strong cell type bias for the ER transactivator functions. In CV-1 cells, it appears that the combination of the two activation sequences is required for maximal activity (Fig. 3A). Cumulatively, these data suggest that the TAF1 and TAF2 activators within ER demonstrate a dependence upon both cell type and promoter. In addition, it is clear that TAF1 is the dominant activator of ER-mediated regulation of C3 expression.

Analysis of the relative contribution of the individual ER transcription activators to ER function was extended to include two additional promoters, namely the adenovirus major late promoter, which contains a single ERE, and the vitellogenin promoter, which contains a natural ERE (Fig. 4). Both of these promoters were responsive to estrogen in the presence of ER-wt. However, unlike activity on the C3 promoter, the individual

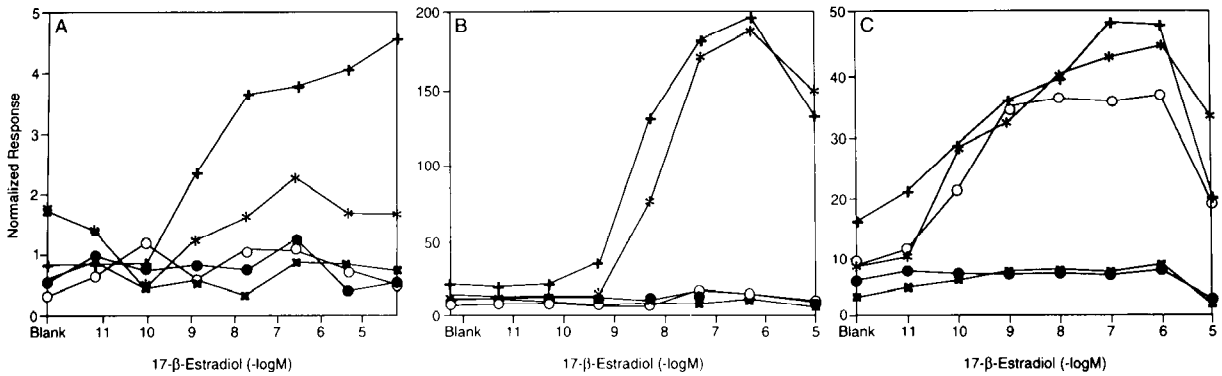

Fig. 3. Activities of TAF1 and TAF2 on the Human C3 Gene Promoter

CV-1 (A), HepG2 (B), and HS578T (C) cells were transiently cotransfected with 0.5 μg ER-wt (+), ER-TAF1 (*), ER179C (○), or ER-null (X); 9.5 μg C3-LUC reporter plasmid; 5 μg pRSV-β-gal; and carrier DNA to a total amount of 20 μg DNA. In addition, a minus receptor control was included (●). Cultures were treated with or without 17β-estradiol for 36 h and assayed for luciferase activity. The data shown are representative curves of experiments that were repeated six times with similar results. The curves represent averages of quadruplicate data points averaged and normalized for transfection efficiency by simultaneous estimation of pRSV-β-gal transcriptional activity.

transactivation domains of ER were minimally active in both cell lines examined. These results highlight further the promoter specificity of the ER transactivation domains.

Regulation of ER-TAF1 and ER179C Activities by ER Agonists

Triphenylethylene-derived ER antagonists, such as tamoxifen and nafoxidine, exhibit partial agonist activities (20). Therefore, we tested whether these compounds might preferentially activate through either TAF1 or TAF2 transactivator regions. A series of these compounds was evaluated in HepG2 cells using the ER-TAF-specific receptor mutants and the C3 promoter. On this promoter, nafoxidine and 4-hydroxytamoxifen were both efficient activators of ER-wt-mediated C3 gene transcription (Fig. 5, B and C). However, none of these compounds was as efficacious as estrogen in this setting (Fig. 5A). In addition, estrogen was an efficient activator of ER-TAF1, whereas the partial estrogen agonists were weakly effective. The C3 promoter was not activated in HepG2 cells by ER179C in the presence of either estradiol or the partial estrogen agonists. These data imply that although TAF1 activity is necessary, it is not sufficient for activation of the C3 promoter by these partial estrogen agonists. Thus, the detailed mechanisms of action of these antagonists may be different from that of estrogen (Fig. 5). The differences in hormone responsiveness of these receptor derivatives, however, do not relate to alterations in the affinity of the proteins for ligands. This was accomplished by performing competitive binding analysis using yeast-produced recombinant ER-wt and ER-TAF1. We were unable to obtain sufficient amounts of these proteins from transfected mammalian cells to perform a complete analysis. As shown in Fig. 6, A and B, the affinity and specificity of ligands for the ER-wt and ER-TAF1 were indistinguishable.

DISCUSSION

Reconstitution of ER function in heterologous cells coupled with analysis of mutant receptors has allowed definition of the principal functional domains within the protein (3–5). Domains responsible for DNA binding, hormone binding, nuclear localization, and dimerization have been defined using this approach (3–5, 22). Less progress has been made in precisely defining the sequences that are required for transcriptional activation (8–11). Analysis of ER deletion constructs has identified unique amino- and carboxyl-terminal sequences, termed TAF1 and TAF2, respectively, which can independently stimulate transcriptional activation. The amino acid sequence motifs in these regions do not resemble known transactivation sequences and, thus, are not enlightening as to their mechanism of action. In addition, it is unclear whether these sequences are responsible for transactivation within the context of the full-length receptor or are artifacts of the deletion itself. As previous studies have shown that up to 1% of a random peptide library can function in the appropriate context as transcriptional activators, it is possible that receptor deletions may expose cryptic or irrelevant activation sequences (23). Recently, Danielian *et al.* (9) disrupted TAF2 function in the mouse ER through substitution of three amino acids in the carboxyl-terminus. As supported here, introduction of these same mutations into the human ER similarly affected TAF2 function without altering the mutant receptor's binding affinity for estrogen, tamoxifen, or 4-hydroxytamoxifen. The surprising result, however, was that the TAF1 function in the mutant ER retained considerable tran-

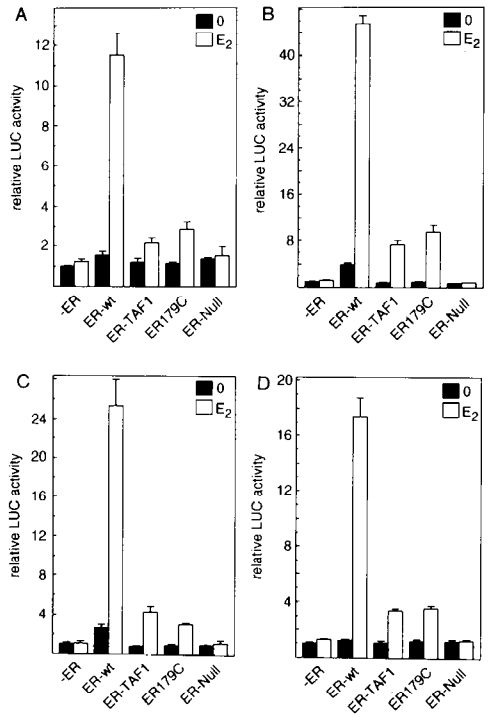

Fig. 4. Activities of ER-TAF1 and ER179C on Different Promoter Constructs

CV-1 (A) and HepG2 cells (B) were transiently cotransfected with 0.5 μg of the indicated receptor expression vector, 9.5 μg pA_2-LUC reporter plasmid, 5 μg pRSV-β-gal, and carrier DNA to a total amount of 20 μg. CV-1 (C) and HepG2 (D) cells were cotransfected, as described above, using the pEREMLT-LUC reporter. Cultures were treated with or without 10^{-7} M 17β-estradiol for 36 h and assayed for luciferase activity (luciferase activity was normalized for β-galactosidase activity). The relative luciferase activity is calculated by dividing the normalized luciferase value at a given point by that obtained in the absence of transfected receptor or ligand. A single experiment representative of two independent experiments is detailed above. The data shown indicate the mean ± SEM of triplicate estimations.

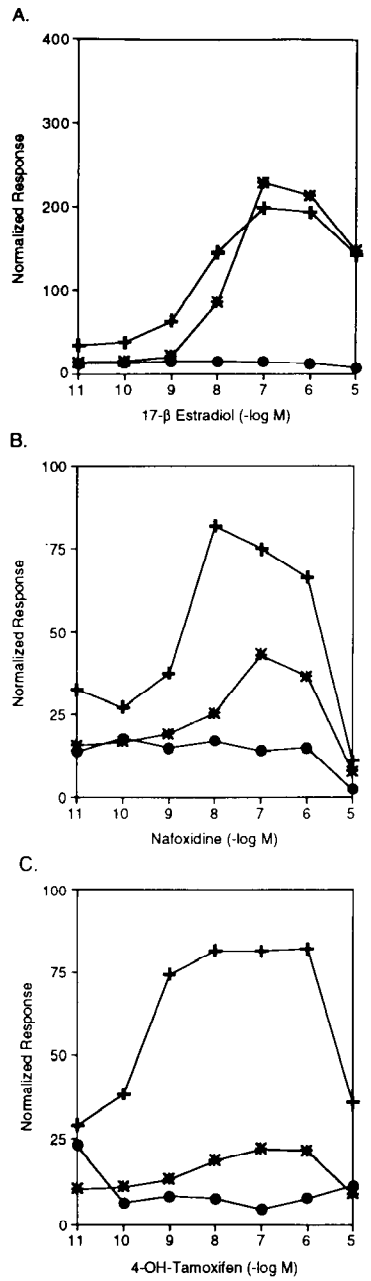

Fig. 5. Activation of ER-TAF1 and ER-179C by Triphenylethylene-Derived Estrogen Partial Agonists

HepG2 cells were cotransfected with 0.5 μg of the indicated receptor expression vectors, 9.5 μg C3-LUC reporter, 5 μg pRSV-βgal, and carrier DNA to a total amount of 20 μg. Cultures were treated with 17β-estradiol (A), nafoxidine (B) or 4-hydroxytamoxifen (C) for 36 h and assayed for luciferase activity. The relative luciferase activity was calculated as described in Fig. 1. A single experiment representative of six independent experiments is detailed above. The data shown indicate the mean ± SEM of triplicate estimations.

scriptional activity. This finding is in contrast to our initial observations that deletion of the TAF2 sequence through truncation (ERN282G) obliterated the residual transcriptional activity of the TAF1 activator in all cell lines examined. This suggests that full manifestation of TAF1 activity requires either the TAF2 activator or the structural context of the full-length receptor. In contrast to our data, Berry *et al.* (8) observed that a construct encoding a protein equivalent to ERN282G was constitutively active in avian chick embryo fibroblast cells. This suggests a possible difference in ER function in mammalian and avian cells, highlighting further the importance of cell type to receptor function.

Using the modified receptors, we have been able to identify cell- and promoter-specific differences in the activity of ER-TAF1 and ER179C. In studies controlled for both expression level and transfection efficiency, we observed that both mutant ERs displayed promoter- and cell type-specific differences in activity. The most striking example shown here is the inability of ER179C to function well on any promoter when examined in a HepG2 liver cell background. ER-TAF1 in the same cells, on the other hand, functioned well on the complex

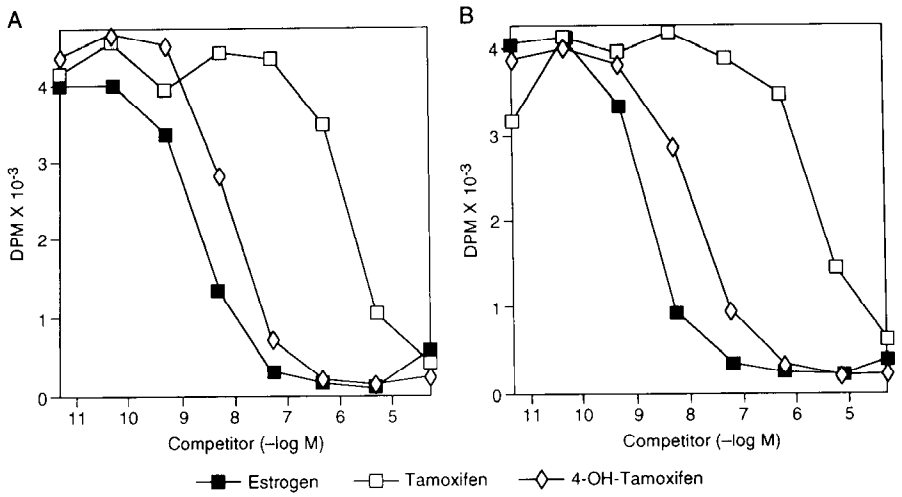

Fig. 6. Displacement of Estradiol Binding to Yeast ER-wt and ER-TAF1 Proteins by Estrogen Agonists

Yeast cytosols prepared from cells expressing ER-wt (A) or ER-TAF1 (B) were incubated overnight at 4 C with 5 nM 17β-[^{3}H] estradiol alone or in the presence of the indicated concentrations of the different ER ligands, as described in *Materials and Methods*. These data represent the average of triplicate determinations at each hormone concentration.

C3 promoter, but was an inefficient activator of an ERE-containing adenovirus major late promoter or the natural vitellogenin promoter within the same cells. The cell type and promoter activity profiles of ER-TAF1 and ER179C are clearly distinct, suggesting dissimilar mechanisms of action, as originally proposed by Tasset *et al.* (11). On the C3 promoter, no apparent synergy was evident between the TAF1 and TAF2 activators, whereas synergy was clearly present when assayed on other promoters. These observations suggest that manifestation of TAF1 and TAF2 transcription activators clearly depends on promoter context. The data obtained in HepG2 cells using the C3 promoter suggest that a transcription factor associated with this promoter can functionally substitute for TAF2. Thus, ER-TAF1 is as efficient a transcriptional activator as its ER wild-type counterpart in this context. However, because the TAF2 activator alone is unable to activate C3 gene transcription, it is clear that no functional replacement for the TAF1 activator exists on this promoter in the HepG2 cell line.

Promoter and cell type differences in ER transactivator function can be explained by a model of ER function in which TAF1 is the dominant transcriptional activator and TAF2 is a transcriptional facilitator, *i.e.* can accentuate TAF1 (or a cellular equivalent) activity but cannot transactivate on its own. As illustrated in Fig. 7, we suggest that the function of the TAF2 activator may be to prepare the general transcription apparatus for TAF1 function. This preparatory function could involve the recruitment of basic transcription factors, alteration of chromatin structure, or displacement or alteration of a transcriptional repressor. Thus, other transcription factors that act on a specific promoter might provide this preparatory function, obviating the need for TAF2 function. Indeed, if the function of TAF2 is to prepare the transcription apparatus for TAF1 actions, one would predict that it might exhibit little inherent transcriptional activity. In support of this hypothesis, we and others observed that the TAF2 activator is only weakly active on certain minimal promoters (12, 13). Indeed, Metzger *et al.* (24) demonstrated that the transcriptional activity ascribed to TAF2 increases in parallel with promoter complexity. Additional evidence in support of a facilitator role of the TAF2 activator is provided by the yeast model, in which the TAF1 activator is similarly inactive on a minimal promoter. Interestingly, deletion of the SSN6 locus (a promoter-restricted cellular repressor of yeast transcription) results in a 100-fold increase in ER-TAF1 efficacy (to a level comparable to that of ER-wt) while exerting no effect on ER179C activity (12). This observation suggests that the SSN6 cellular mutation mimics the molecular function of the TAF2 activator.

Previous studies in avian cells indicate that the partial agonist activity of tamoxifen is related to its ability to activate the TAF1 region in ER (8). To confirm this hypothesis in mammalian cells, we used the C3 promoter as a transcriptional target in which the TAF2 region contributed little to the overall promoter activity. Clearly, our data suggest that the triphenylethylene-derived antiestrogens can function as partial agonists of ER-wt function. Interestingly, whereas mutations in the TAF2 region have no effect on the ability of estrogen to stimulate transcription from this promoter via the TAF1 activator, they do compromise the partial agonist activity of these antiestrogens. This suggests that although these antihormones do not activate TAF2 in ER, they may require the wild-type receptor context for agonist function. In addition, these data imply that conformations of the receptor induced by the antiestrogens and those caused by estrogens are not identical.

We have previously shown, using an *in situ* DNA binding interference assay, that the triphenylethylene

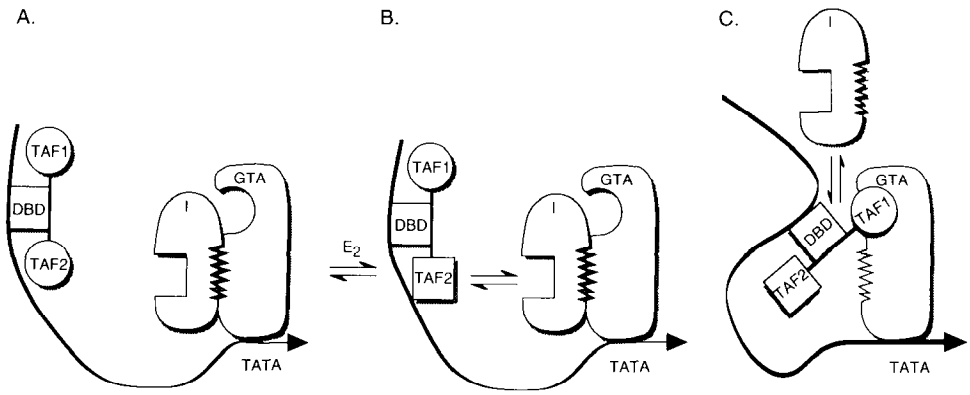

Fig. 7. Model for TAF1 and TAF2 as Functionally Dependent Activators of Transcription

This schematic outlines a hypothesis for the promoter and cell specificity of the individual transactivators of the ER. Interaction of the receptor with ligand initiates a cascade of events that exposes the receptor DNA-binding region (DBD) and promotes association of ER with DNA. Only estrogenic compounds are capable of functionally activating TAF2. Upon activation (B), TAF2 interacts with a transcriptional repressor (I), displacing it or altering its structure (C) to permit the TAF1 activation sequence access to the general transcription apparatus (GTA). In certain cells and on certain promoters, TAF2 function can be supplied by other transcription factors, allowing TAF1 to function independently of TAF2. Therefore, binding of the receptor to DNA is synonymous with transactivation and can be accomplished by both estrogen agonists as well as antagonists that permit delivery of the receptor to DNA. In this model the partial agonist activity of the triphenylethylene-derived estrogen agonists depends on the conformation induced by the ligand and the effect that this conformation has on the presentation of TAF1 to the transcription apparatus.

class of antiestrogens delivers receptor to DNA (Ref. 25 and our unpublished data). If the role proposed for TAF2 is correct, then TAF1 function depends on 1) the cellular context of a given promoter and 2) the capacity of the TAF1 region to interact with other transcription factors on the promoter. It is also possible that on other promoters, antiestrogens may differentially affect TAF1-transcription factor interactions. The differences in the efficacy of the antiestrogens may reflect intermediate conformational changes in receptor structure and, consequently, differences in the degree of TAF1 exposure. In this scenario, then, cell type and promoter context would determine the transcriptional activity of the exposed TAF1 activator.

Our data indicate that tamoxifen is a promoter-specific ER agonist rather than a cell-specific agonist, as has been suggested previously (8). In support of this hypothesis, Schull *et al.* (26) have recently shown that the ability of tamoxifen to act as an estrogen agonist in pituitary cells is restricted to several estrogen-responsive genes. This supports our contention that the triphenylethylene antiestrogens are both cell type- and promoter context-specific ER partial agonists.

MATERIALS AND METHODS

Receptor Expression Vectors

Complementary DNA sequences encoding the human ER-wt and a TAF1-deleted receptor derivative were excised from the yeast expression plasmids YEpwtER and YEpER179C, respectively, using *Bfr*I and *Sac*I. The DNA encoding the TAF1 receptor derivative was excised from the plasmid YEpERN282G using *Bfr*I and *Kpn*I. Construction of the vectors YEpwtER, YEpER179C, and YEpERN282G was described previously (13). The excised DNA was treated with T4 DNA polymerase and ligated into the *Eco*RV site within the mammalian expression vector pRST7 (a gift from Peter Syka, Ligand Pharmaceuticals, Inc.).

Receptor Mutations

The wild-type ER cDNA was cloned into pGEM-11Zf(+). Specific mutations were introduced into the hormone-binding domain of the receptor by substituting alanine for amino acids located at positions 538, 542, and 545, using site-directed mutagenesis (14), creating the plasmid pGERm. The mutated hormone-binding domains were introduced into ER-wt and ER179C by exchanging the *Bgl*II-*Sac*I C-terminal fragment of these vectors with the analogous mutated fragment from pGERm.

Reporter Plasmids

The reporter plasmid ERE-tk-LUC contains a single copy of the vitellogenin ERE fused up-stream of the *Herpes simplex* thymidine kinase (TK) promoter sequence linked to luciferase (LUC). The C3-LUC reporter, which contains 1.8 kilobases of the human C3 gene promoter (−1807 to 58) (15), was provided by Jon Rosen (Ligand Pharmaceuticals, Inc.). The pA2-LUC contains a 835-basepair fragment (−821 to 14) of the *Xenopus* vitellogenin A2 gene promoter (15). The pEREMLT-LUC contains a single ERE cloned up-stream from the adenovirus major late promoter sequences (−44 to 11) (16).

Cell Culture

CV-1 and HS578T cells were routinely maintained in Dulbecco's Modified Eagle's Medium (Biowhittaker, Walkersville MD) supplemented with 10% fetal calf serum (FCS; Hyclone Laboratories, Logan, UT). HepG2 cells were maintained in Eagle's Minimum Essential Medium (Biowhittaker) containing 10% FCS.

Transient Transfection Assays

Cells were seeded 24 h before transfection in flat-bottom 96-well tissue culture plates (5×10^{-3} cells/well) in phenol red-

free Dulbecco's Modified Eagle's Medium containing 10% FCS. DNA was introduced into cells using calcium phosphate coprecipitation (17). Transfections were performed on a Biomek 1000 Automated Laboratory Workstation (Beckman, Fullerton, CA). Cells were transfected for 6 h and then washed with PBS to remove the precipitate. Cells were incubated for an additional 36 h in phenol red-free medium containing 10% charcoal-treated FCS with or without hormones, as indicated in the text. Cell extracts were prepared as described previously (17) and assayed for luciferase and β-galactosidase activities. All determinations were performed in triplicate in at least two independent experiments and were normalized for transfection efficiency using β-galactosidase as an internal control.

Preparation of Yeast Receptor Proteins

Expression vectors producing ER-TAF1 protein were constructed by replacing the *Bfrl-Mlu*I fragment of YEpE10 (18) with the corresponding fragment from ER-TAF1. This vector and a vector producing wild-type receptor (YEpE10) were transformed into the yeast strain BJ2168, as described previously (6). Individual transformants were picked and grown to 1 OD_{600nm}. Cultures were then induced with 100 μM $CuSO_4$ and 2 mM chloroquine for 16 h at 30 C. Cells were pelleted and washed with cold water. The cell pellets were resuspended in 2–5 vol (wt/vol) 10 mM Tris (pH 7.6), 0.4 M KCl, 2 mM EDTA, 0.5 mM phenylmethylsulfonylfluoride, 1 μg/ml aprotinin, and 2 mM dithiothreitol (DTT) and lysed by vortexing in the presence of 0.45- to 0.5-mm glass beads with intermittent cooling on ice. At least 90% of the cells were disrupted during lysis. Extracts were centrifuged at 13,000 × *g*, and the supernatants were recovered. Protein concentrations were determined by the Bio-Rad protein assay (Bio-Rad, Richmond, CA).

17β-Estradiol Binding Competition Assay

All procedures were performed using a Biomek 1000 automated workstation (Beckman Instruments). Ten-fold serial dilutions of the compounds to be tested were prepared in 10 mM Tris (pH 7.6), 0.3 M KCl, 5 mM DTT, and 100 μl of each diluent, ranging in concentration from 10^{-4}-10^{-11} M, and transferred to polystyrene tubes containing 5 nM 17β-[^{3}H]estradiol (Amersham, Arlington Heights, IL). Extracts of yeast strains (22 μg total protein) producing either ER-wt or ER-TAF1 were added. After an overnight incubation at 4 C, 100 μl of a 6% hydroxylapatite slurry in 10 mM Tris, pH 7.6, and 5 mM DTT were added. The tubes were incubated for an additional 30 min at 4 C, and the pellets were recovered by centrifugation. Hydroxylapatite pellets were washed four times with 1 ml 1% Triton X-100 in 10 mM Tris, pH 7.6, and 5 mM DTT. Finally, the hydroxylapatite pellets were resuspended in 800 μl Ecoscint A scintillation fluid (National Diagnostics, Manville, NJ). The radioactivity was measured on a LS6000IC scintillation counter (Beckman Instruments). Estradiol and nafoxidine were obtained from Sigma (St. Louis, MO), 4-Hydroxytamoxifen was a gift from ICI Pharmaceuticals (Macclesfield, England).

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