The truncated estrogen receptor product-1 (TERP-1, or TERP) is a pituitary-specific isoform of estrogen receptor α (ERα), and its expression is regulated by estrogen. TERP modulates the transcriptional activity of ERα but has no independent effect on transcription of estrogen-response element-containing promoters. At low concentrations, TERP stimulates ERα transcriptional activity in transient transfection assays. At TERP concentrations equal to or greater than full-length ERα, TERP forms dimers with ERα and reduces both ligand-dependent and -independent transcription. A dimerization mutant of TERP, TERP L509R, stimulated ERα transcription at all concentrations. We hypothesized that TERP stimulates ERα transcriptional activity by titrating suppressors of ERα activity. We found that repressor of estrogen receptor activity (REA), originally isolated from human breast cancer cells, is present in mouse pituitary gonadotrope cell lines. Levels of REA vary slightly throughout the rat reproductive cycle, but mouse pituitary gonadotrope cell lines. Levels of REA vary slightly throughout the rat reproductive cycle, but TERP mRNA and protein vary much more dramatically. In transfection experiments, REA suppressed ERα transcriptional activity, and TERP L509R was able to alleviate transcriptional suppression by REA. In glutathione S-transferase pull-down assays, TERP bound to REA more efficiently than did ERα at equivalent concentrations, suggesting that REA will preferentially bind to TERP. Our findings suggest that the stimulation of putitary ERα activity by low concentrations of TERP can occur by titration of corepressors such as REA.

The ligand-bound estrogen receptor (ER) activates transcription and exerts its biological actions in a number of responsive tissues such as uterus, mammary glands, brain, and pituitary (1–3). Like other nuclear receptors, the ER has five conserved structural domains. The N-terminal A/B region contains the ligand-independent activation function-1 (AF-1) and is the most variable in length and in sequence. It is thought to contribute to the ligand-independent activation of the receptor and is involved in promoter- and cell-specific activity (4–6). The C-terminal ligand-binding domain (LBD) consists of 12 conserved helices. It contains the ligand-dependent activation function-2 (AF-2) and is also important in heat-shock protein binding, nuclear localization, and dimerization of the receptor (7). Both AF-1 and AF-2 domains contribute to the transcription activation of ER. The most conserved domain is the DNA-binding domain (DBD), which has two zinc fingers and recognizes specific DNA sequences, or estrogen-response elements (EREs), on hormone-responsive genes. The hinge region links the DNA-binding and ligand-binding domains and allows rotation of the DBD (7). Activation of the receptor by ligand requires a conformational change in the LBD in which helix 12 moves toward helix 3 and helix 4, forming a hydrophobic pocket and a surface that allows interaction with coactivators and other regulatory proteins (5, 8). There are two subtypes of the estrogen receptor, ERα and ERβ, which are encoded by separate genes (2). The DBD is nearly identical and the LBD is similar, but the N-terminal regions of the receptor subtypes are different both in structure and in function. The AF-1 domain of ERα is very active in stimulation of transcription in different cell types, whereas the AF-1 activity of ERβ is negligible under the same conditions (9). In the pituitary, ERα is the predominant isoform, and the AF-2 domain is the most important region for transcriptional activation (10, 11).

Nuclear receptor activity is regulated by binding of ligands and coregulator proteins and the formation of heterodimers with other receptors. Coregulatory proteins may either enhance or suppress ER-dependent activity. Coactivators such as steroid receptor coactivator-1 (SRC-1) interact with nuclear receptors in an agonist- and AF-2-dependent manner (8, 12) through their conserved LXXLL receptor interaction motif (12). These coactivators have been shown to activate nuclear receptors by chromatin remodeling via histone acetyltransferase activities or through direct interaction with the basal transcription machinery (12–14).

Corepressors negatively regulate transcriptional activity via histone deacetylase-dependent or -independent pathways (12, 15). The nuclear receptor corepressor (NCoR) and silencing mediator for retinoid X receptor and thyroid hormone receptor (SMRT) suppress the transcriptional activity of unliganded
nuclear receptors and ER bound to antagonists or selective estrogen receptor modulators through recruitment of histone deacetylase-containing protein complexes (13, 16), contributing to chromatin compaction. A recently identified ERα-associated protein, template-activating factor-1β (TAF-1β), binds to the DBD and has been shown to inhibit acetylation of histones and ERs, resulting in the suppression of transcription (17). Examples of repressor molecules that do not act directly through histone deacetylases include repressor of tamoxifen transcriptional activity (18), the LIM/homeodomain protein islet-1 (19), DEAD box RNA helicase DP97 (20), receptor-interacting protein 140 (RIP140) (21, 22), and repressor of estrogen receptor activity (REA) (23). REA is the only ER-specific repressor protein known to date and was identified in an MCF-7 human breast cancer cell cDNA library by yeast two-hybrid screening. REA interacts with the ERα or ERβ LBD in the presence of both agonists and antagonists (23, 24). It does not have intrinsic transcription-repression activity and is thought to compete with SRC-1 binding to ER via the LXXLL motif at its N terminus (24).

ER transcriptional activity can also be modulated by the formation of heterodimers. For example, ERβ influences overall ER activity in two ways. It may competitively bind to EREs and prevent ERα binding, or it may form ERα/ERβ heterodimers with lower transcriptional activity than ERα homodimers, possibly because of the less active N-terminal domain of ERβ (25). An ERα isoform cloned in our laboratory, the truncated estrogen receptor product-1 (TERP-1, hereafter called TERP), has also been shown to form heterodimers with and modulate ERα and ERβ activity (26, 27). This pituitary-specific isoform of ERα contains a unique untranslated exon 1 fused to exon 5 through exon 8 of ERα (26). The resulting protein lacks the DBD and does not bind ligand efficiently. TERP has no independent transcriptional activity on ER-containing promoters but modulates ER transcriptional activity biphasically (27, 28). In transient transfection assays, TERP stimulates ERα activity at low concentrations, where the ratio of transfected TERP to ERα is less than 1:1, but inhibits ERα activity at high concentrations, where the ratio of TERP to ERα is greater than 1:1 (28).

Levels of TERP mRNA change dramatically in response to estrogen (10, 29, 30), and it seems likely that TERP may have biological importance in the pituitary (31). TERP is transcribed from a promoter located in the intron between exons 4 and 5 of the rat ERα gene (32). The TERP promoter contains EREs, suggesting that ER may directly regulate TERP expression. The ratio of TERP/ERα mRNA varies during the reproductive cycle from undetectable during metestrus to 4-fold higher than ERα during proestrus, when estrogen levels are highest.

Previously, we and others demonstrated that the suppressive effects of TERP on ERα transcriptional activity, observed with TERP/ERα ratios greater than 1:1, is due to the formation of TERP/ERα heterodimers that inefficiently bind to an ERE (27, 32). We hypothesized that the stimulatory effects of TERP observed at TERP/ERα ratios < 1:1 must occur through a separate mechanism. Here we show that low concentrations of TERP bind efficiently to the repressor molecule REA. We suggest that by the titration and sequestration of repressor proteins, TERP can stimulate ER transcriptional activity.

EXPERIMENTAL PROCEDURES

RNA Extraction—Cos-1, aT3, and LpT2 cells were collected with guanidinium (4 M GdnSCN, 25 mM sodium citrate, 0.5% Sarkosyl, and 0.1 M β-mercaptoethanol) and passed through an 18-gauge needle. The homogenate was layered on CsCl cushions (5.7 M CsCl and 0.01 M EDTA, pH 7) and centrifuged at 35,000 rpm at 20 °C for 18 h (31). The pellet was removed and resuspended in 0.5 mM sodium acetate, pH 5.5, and precipitated in ethanol overnight at −70 °C. The precipitate was washed with 70% ethanol, dried, and rehydrolyzed in sterile water.

Plasmid ERα and TERP DNAs were generated by PCR and subcloned into a cytomegalovirus promoter containing pcDNA3.1 expression vector as described previously (27). PCR products of ERα and TERP were subcloned into the pGEX2T vector downstream of the glutathione S-transferase gene as described (27). Single point mutations of TERP at the dimerization domain (L509R) or at helix 12, the AF2-binding site (E547K), were generated by site-directed mutagenesis as described (27). Mouse REA cDNA was generated by RT-PCR amplification from αT3 total RNA using REA-specific N-terminal and C-terminal primers (5′-ATGGCCCAAGAATCTGAAG-3′ and 5′-CCTCATAAGGGTTAAAGAAG-3′, respectively). The 899-bp PCR product was ligated to the pCR 2.1 vector (Invitrogen) and sequenced in both directions with cloned sequences and the sequence for human REA isolated from human breast cancer MCF7 cells (GenBankTM accession number NM007273). REA was subsequently subcloned into the pcDNA3.1 expression vector and the pGEX-2T vector. The reporter vector for transfection experiments contains two copies of the vitellogenin consensusERE fused to a 105-bp thymidine kinase promoter, (ERE/TK-Luc).

RT-PCR—Reverse transcription from rat pituitary RNA was performed as described (31). PCR conditions and primers for ERα, TERP, and actin were described (10), and the cycle number for their PCR reactions are 29, 39, and 26, respectively. PCR conditions for REA consisted of a single cycle of 3 min at 94 °C followed by 34 cycles of 1 min at 94 °C, 1 min at 56 °C, and 2 min at 72 °C with 10 min extension step at 72 °C. The optimal cycle number for quantification of REA was determined based on PCR with 200 ng of female rat pituitary RNA over a range of 25–40 cycles. For each RNA sample, the linear range of the amplification curve was verified independently. For quantification, a minimum of two separate experiments and amplifications were performed for each RNA sample, and mRNA values from densitometric quantification were normalized for β-actin mRNA from the same samples and cDNA amplifications.

Cell Culture and Transfections—Mouse αT3 gonadotrope cells, originally obtained from Dr. Pamela Mellon (University of California, San Diego, CA) (33), were maintained in Dulbecco's minimal essential medium (DMEM) containing 20% fetal bovine serum, 0.5% gentamicin, and 100 μg/ml streptomycin. For transfection experiments, αT3 cells were plated in 30-mm wells at a density of 500,000 cells/well in phenol red-free DMEM with 5% charcoal-stripped newborn calf serum. The ER-negative monkey kidney Cos-1 cells were maintained in DMEM with 10% newborn calf serum. Cos-1 cells were plated in 30-mm wells at a density of 200,000 cells/well in phenol red-free DMEM with 5% stripped newborn calf serum in transfection experiments. 24 h after plating, cells were transfected with 1 μg/well luciferase reporter plasmid, various expression constructs, and pcDNA3.1 vector (for standardization of total DNA) using calcium phosphate methods. Approximately 16–18 h later, cells were washed with phosphate-buffered saline and incubated with or without 17β-estradiol (E2) for 24 h before collection with 1× cell lysis buffer (Promega Corp., Madison, WI). Luciferase activity was measured using a Turner 20e luminometer (Sunnyvale, CA) and normalized for total lysate protein assessed with Bio-Rad protein dye. Data were pooled from at least three independent experiments of duplicate or triplicate samples and are presented as arbitrary light units and normalized for protein levels.

GST Pull-down Assays—pGEX2T (GST alone), GST-EraLBD, GST-TERP, and GST-REA constructs were transfected into BL21 bacteria cells and grown in Luria broth containing 50 μg/ml ampicillin in an orbital shaker at 37 °C for 5 h. After induction with 0.1 mM isopropyl β-thiogalactopyranoside, the bacteria were grown overnight at 30 °C. Cells were collected at 5,000 rpm for 30 min at 4 °C, the pellets were resuspended and incubated on ice for 15 min in buffer containing 50 mM Tris, pH 7.5, 0.5 mM EDTA, 300 mM NaCl, 10 mg/ml lysozyme, and 1 μg/ml dithiothreitol. After 20 μl/ml of 10% Nonidet P-40 was added, the bacterial lysate was incubated on ice for 10 min and frozen at 70 °C in an ethanol bath for 1 h. After thawing at room temperature, the lysate was resuspended on ice in buffer containing 1.5 M NaCl, 12 mM MgCl2, DNase I (5 μg), 10 μg/ml leupeptin, 1 μg/ml pepstatin A, and 0.1 mM phenylmethylsulfonyl fluoride. Lysates were homogenized by passing through a 20-gauge needle and centrifuged at 10,000 × g, 4 °C for 30 min. Soluble lysate was conjugated with glutathione-agarose beads at 4 °C overnight and assessed for protein concentration by the method of Bradford (15). For GST pull-down experiments, recombinant Era, Frontex blotting (GST-EraLBD and GST-TERP) with Era C1355 (31). For pull-down experiments, ~0.5 μg of GST alone or GST fusion proteins were incubated in a 150-μl mixture containing 10 μg Era, 20 μg/ml bovine serum albumin, (15S)methionine-labeled (1,175 Ci/μmol).
mmol: 0.04 mCi/50-μl reaction) in vitro translated proteins (TNT rabbit reticulocyte transcription/translation kit; Promega) and a GST wash buffer containing 10 mM MgCl₂, 150 mM KCl, 20 mM Hepes, 10% glycerol, and 0.12% Nonidet P-40. After 1.5 h of incubation at 4 °C, the beads were washed in GST wash buffer four times and resuspended in SDS loading buffer. The samples were boiled for 2 min prior to electrophoresis on SDS-containing 12% polyacrylamide gels at 145 V. Gels were dried and exposed to film overnight at −70 °C and visualized by autoradiography. The protein band intensity was quantified by densitometry. Trichloroacetic acid precipitation was performed to estimate [35S]methionine incorporation in the in vitro translated proteins. Briefly, 2 μl of in vitro translated protein was incubated for 10 min with 98 μl of 1 M NaOH at 37 °C followed by a 30-min incubation with 25% trichloroacetic acid/2% tryptone on ice. The precipitate was washed on a GF/C filter with cold 5% trichloroacetic acid and 70% ethanol and assayed in a scintillation counter. For competition experiments, the molar ratio of incubated protein was calculated based on the [35S]methionine incorporation and the number of methionines encoded in each protein. GST-REA was then incubated with saturating amounts of ERα L509R and increasing amounts of TERP L509R. Data shown in the figures are the representative results from at least 3–5 independent experiments.

Quantification of Protein Expression—To quantify ERα, TERP, and REA mRNA through the estrous cycle, intact female CD-1 rats (200–225 g of body weight, Charles River Laboratories) were maintained on a 14:10-h light:dark cycle, and estrous cycle stage was determined by vaginal lavage. All animals were required to have two consecutive normal cycles prior to use in the study. Animals were euthanized at 9 a.m. and 5 p.m. of estrous and proestrus and at 9 a.m. on metestrus and diestrus (12–16 animals/group). Total pituitary RNA was prepared from pooled tissue (2–3/sample), and total protein was obtained by direct homogenization of individual pituitaries (three individual samples for each cycle day and time) in 50 mM Tris (pH 7.6) solution containing 2% SDS. Detection and quantitation of ERα and TERP protein were as described previously (11, 31). Protein samples were denatured by boiling with 2% β-mercaptoethanol and separated by electrophoresis on 12% polyacrylamide gels containing 1% SDS and then transferred to nitrocellulose membranes. Proteins were detected by the ERα C-terminal specific antibody C1355 (1:7,500) (31), peroxidase-labeled donkey anti-rabbit IgG, and the SuperSignal West Pico chemiluminescent detection system (Pierce). Full-length ERα and TERP protein were distinguished on the basis of size, quantitated by densitometric analysis, and normalized for β-actin protein in the same samples on the same blot as described previously (10). Data were analyzed from two separate experiments, with three samples/group. Animals were obtained and used in accordance with the guidelines established by the Animal Care and Use Committee of the University of Virginia.

RESULTS

Dimerization Mutant of TERP Stimulates ERα-mediated Transcription—Our laboratory has observed previously that TERP modulated ERα transcriptional activity biphasically (28). High concentrations of TERP were suppressive for ER activity through inactive dimer formation with ERα that disrupted ERα binding to an ERE. We speculated that if TERP could no longer form dimers, its stimulatory effect would be more apparent. A point mutation at leucine 509 of TERP disrup-
ted ERα binding to an ERE. Although the protein of this mutant, TERP L509R, is made at the same level as the wild type, its ability to inhibit ERα is severely compromised (27). Cotransfection of ERα with increasing amounts of TERP L509R in ER-negative Cos-1 cells consistently stimulates ERα transcriptional activity on an ERE-containing promoter in a dose-dependent manner (Fig. 1A), whereas the same amount of wild type TERP is invariably suppressive (Fig. 1B). Similar results were observed in ER-positive mouse pituitary gonado-
trope αT3 cells (Fig. 1C), a cell line in which TERP is normally expressed (10). We postulated that TERP L509R might be interacting with suppressive molecules and thus sequestering them from ERα.

REA Is Expressed in Mouse Pituitary Gonadotrope Cell Lines and Suppresses ERα-mediated Transcriptional Activity—We next examined the potential role of the ER-specific repressor protein, REA, in TERP regulation of ERα transcription. REA interacts with ERα at the LBD, most of which is contained in TERP. We first examined the expression of REA in gonado-
trope cells. RT-PCR assays were performed on RNA extracted from mouse pituitary gonadotrope cell lines, αT3 and LβT2, as well as monkey kidney Cos-1 cells, using primers designed from the published sequence of human REA (23). As shown in

![Fig. 1. TERP L509R stimulates ERα-mediated transcription.](image-url)
Fig. 2. Expression and functional analysis of REA. In A, RT-PCR was performed on RNA samples extracted from MCF-7 human breast cancer cells and αT3 and LβT2 mouse pituitary gonadotrope cells, using REA-specific N-terminal and C-terminal primers. The PCR products were electrophoresed on a 1% agarose gel stained with ethidium bromide. Mrk, DNA size marker. In B, αT3 cells were transfected with 1 μg of (ERE)2-vit-TK-Luc, 2 μg of REA, and 2 μg of TERP L509R as indicated. After transfection (16 h), cells were treated with E2 for 24 h, and luciferase activity was assayed. In C, Cos-1 cells were transfected with 1 μg of (ERE)2-vit-TK-Luc and 0.5 μg of ERα expression plasmid in the absence or presence 1–2 μg of REA and 0.5 and 1 μg of TERP L509R as indicated. Data shown are the mean ± S.E. from three independent experiments performed with triplicate wells for each treatment. *, p < 0.05 versus E2-stimulated wild type ER control. +, p < 0.05 comparing TERP L509R stimulated with REA suppressed samples.

Fig. 3. REA interacts with TERP. Approximately 0.5 μg of GST or GST-REA was incubated with in vitro translated [35S]methionine-labeled TERP or progesterone receptor (PR) (A), ERα or ERα L509R (B), and TERP, TERP L509R, or TERP E547K (C), in the presence of 10 nM E2. The migration of proteins bound to GST or GST-REA is indicated by arrows and visualized by autoradiography. Input lanes represent ~10–20% of total protein incubated with the GST or GST-REA. The autoradiogram shown is representative of 3–6 independent experiments.

CTP-1 Stimulates ERα by Titration of Repressors

TERP binds to REA and competes with ERα in binding to REA—in order for TERP to influence ERα activity through REA, TERP must bind REA. Fig. 3A shows that TERP REA binds to in vitro translated [35S]methionine-labeled TERP, but not to progesterone receptor, showing specificity of interaction. In vitro translated TERP is detected as a pair of doublets at 22–24 kDa, which likely represent translation from two methionines, at amino acids 393 and 408 in exon 5 (28, 32). The larger protein is detected preferentially in transfected cells and in normal pituitaries (11, 28, 29, 31, 32). The dimerization mutants of ERα and TERP, ERα L509R and TERP L509R, also interact with GST-REA in a similar fashion (Fig. 3, B and C). These data indicate that monomers of TERP and ERα can also bind REA. A helix 12 point mutant of TERP, TERP E547K, is also capable of interacting with GST-REA (Fig. 3C), suggesting...
that glutamic acid 547 in the region important for coactivator
binding is not required for REA/ERα interaction in the rat
receptor. If TERP sequesters REA physiologically, we expect
that TERP would bind REA more strongly than ERα. In GST
pull-down assays, increasing amounts of TERP L509R decrease
binding of ERα L509R to GST-REA (Fig. 4). The L509R mu-
tants of both TERP and ERα bind to GST-REA similarly to the
wild type TERP and ERα. Thus, the dimerization mutants
were used in competition pull-down experiments to prevent
secondary binding so that TERP does not associate with REA
through ERα or vice versa. Analysis of the GST pull-down data
shows that at TERP L509R:ERα L509R ratios of 0.5:1 or
greater, TERP L509R binds preferentially to REA and can
displace ERα L509R from REA. Similar results were seen in
three independent experiments, and studies performed with
wild-type ERα or TERP also gave similar results (data not
shown). The data suggest that TERP effectively competes with
ERα in binding to REA and that titration of REA by TERP at
low TERP concentrations can result in a stimulatory effect on
ERα-mediated transcription.

Expression of REA Compared with TERP Throughout the
Female Rat Estrous Cycle—Because the relative ratios of
TERP, REA, and ERα will determine the overall ERα tran-
scriptional activity in pituitary cells, we investigated whether
REA expression changes in the female rat pituitary during the
estrous cycle and how this relates to TERP and ERα expression
during this time period. RT-PCR was performed on female rat
pituitary RNA taken from different time points in the estrous
period and normalized for β-actin levels. Protein levels of TERP
and ERα were measured by immunoblotting and were also
normalized for β-actin. As shown in Fig. 5, REA mRNA changes
only slightly throughout the estrous cycle, peaking in the af-
afternoon of proestrus and decreasing in the afternoon of estrus.
The normalized level of REA mRNA is similar to that of ERα
(data not shown), which also changes little during the cycle.
ERα protein levels correlate tightly with mRNA levels and also
change little throughout the cycle. In contrast, TERP mRNA
and protein expression vary dramatically throughout the
estrous cycle (Fig. 5B). TERP mRNA expression correlates with

\[ \frac{\text{TERP L509R:ERα L509R}}{\text{Ratio}} \]

![Figure 4](image4.png)

**Fig. 4.** TERP L509R competes with ERα L509R for binding to REA. Approximately 0.5 μg of GST or GST-REA was incubated with in vitro translated [35S]methionine-labeled proteins in the presence of 10 nM E2. GST-REA was incubated with saturating amounts of ERα L509R and increasing amounts of TERP L509R (0, 0.25, 0.5, 1, 2, and 5 × ERα L509R). Unprogrammed reticulocyte lysate was added to maintain constant total proteins. The migration of proteins bound to GST or GST-REA is indicated, detected by autoradiography, and quantified by densitometry. The autoradiogram shown is representative of three independent experiments.

**Expression of REA, TERP, and ERα in Female Rat Pituitary Throughout the Estrous Cycle**—Because the relative ratios of TERP, REA, and ERα will determine the overall ERα transcriptional activity in pituitary cells, we investigated whether REA expression changes in the female rat pituitary during the estrous cycle and how this relates to TERP and ERα expression during this time period. RT-PCR was performed on female rat pituitary RNA taken from different time points in the estrous cycle and normalized for β-actin levels. Protein levels of TERP and ERα were measured by immunoblotting and were also normalized for β-actin. As shown in Fig. 5, REA mRNA changes only slightly throughout the estrous cycle, peaking in the afternoon of proestrus and decreasing in the afternoon of estrus. The normalized level of REA mRNA is similar to that of ERα (data not shown), which also changes little during the cycle. ERα protein levels correlate tightly with mRNA levels and also change little throughout the cycle. In contrast, TERP mRNA and protein expression vary dramatically throughout the estrous cycle (Fig. 5B). TERP mRNA expression correlates with
protein ratio changes from 0.1 in the morning of metestrus to 4.3 by the morning of estrus. Because REA and ERα expression are relatively constant, the dramatic changes in TERP expression may be the more important determinant in the interaction among ERα, TERP, and REA.

**DISCUSSION**

Our previous studies showed that the TERP:ERα ratio influences ERα transcriptional activity in a biphasic manner in transient transfection experiments (28). For TERP suppressive effects on ERE-containing promoters, TERP dimerization with ERα or ERβ is required (27, 34). We show in this study that the dimerization mutant of TERP (L509R) is stimulatory at all concentrations and mimics the effects of wild-type TERP at low concentrations. Because TERP L509R does not bind to DNA and cannot interact with ERα, these data suggest that TERP is interacting with regulatory molecules involved in ER activation. These molecules can be saturated by TERP titration because the stimulatory effect on transcription plateaus at high concentrations of TERP L509R (Fig. 1A). We suggest that both TERP and TERP L509R recruit repressor molecules and make them less available to ERα.

Cellular levels of coactivators and corepressors help to determine the ERα transcriptional activity and the tissue specificity of selective estrogen receptor modulators (35, 36). Whereas the corepressors NCoR and SMRT bind ER only in the presence of antagonist, the recently identified repressor proteins may provide a more general way to influence ERα signaling because they bind to steroid receptors in the presence of either agonist or antagonist. Receptor-interacting protein 140 (RIP140) interacts with ER AF-2 in the presence of E2 (21, 22) and suppresses nuclear receptor-receptor-mediated transcription by competition with coactivator binding (37, 38). Increasing expression of SRC-1 is sufficient to reverse the suppressive effect of RIP 140 (37, 38). REA is similar to RIP140 in that it does not have intrinsic transcription-repression activity and suppresses ER activity through competition with SRC-1 binding via the LXXLL motif at its N terminus (24, 39).

In this study, we show that cellular availability of the repressor REA, which is found in multiple cell types and tissues (23, 40, 41) including pituitary cells (Figs. 1 and 5), may be modulated by binding to or sequestration by TERP. Our protein binding data agree with previous observations that REA binding is ER-specific and that the LBD domain of ERα is the most important region for REA binding (23, 39). Neither an intact dimerization domain nor an active helix 12 region in ERα and TERP is required for binding to REA. Most importantly, low levels of TERP and TERP L509R effectively compete with ERα and ERα L509R in binding to REA (Fig. 4). Differential binding efficiencies may be due to three-dimensional structure differences between ERα and TERP. It has been suggested that the C-terminal F domain of ERα prevents recruitment of REA to the unliganded receptor LBD and that deletion of the F domain increases REA binding (24). TERP lacks the N-terminal region of the LBD, and this structural modification may also contribute to enhanced TERP-REA binding relative to ERα. The ability of TERP and TERP L509R to bind REA has functional consequences on ERα transcriptional activity. Increasing TERP L509R expression stimulates ERα transcription in both Cos-1 and αT3 pituitary gonadotrope cells and reverses the suppression by REA (Fig. 2, B and C). Stimulation by TERP L509R can also be reversed by REA (data not shown). TERP may bind other repressors as well. For example, the ERα repressor LIM/homeodomain protein islet-1 (ISL1) also binds to TERP, but the functional consequences are unknown (19).

TERP may be considered an orphan receptor, in that it is structurally similar to a known nuclear receptor (ERα) but is inefficient or unable to bind ligand (26, 28). At least two orphan nuclear receptors, short heterodimer partner (SHP) and DAX-1, have also been shown to modulate ERα activity (42, 43). SHP is expressed in multiple cell types and contains a putative ligand-binding domain but lacks a DNA-binding domain. SHP does not interfere with ERα homodimerization but interacts with ERα through its central LXXLL-related motif and competes for coactivator binding to suppress ERα and ERβ activities (44, 45). DAX-1 is expressed in reproductive tissues and consists of a conserved LBD and a unique DNA/RNA-binding domain containing three leucine-rich repeats, one of which is the LXXLL motif. DAX-1 binds to both ERα and ERβ via the LXXLL motif and is thought to suppress ERα action by preventing coactivator binding and recruiting the corepressor NCoR (43).

TERP is similar to SHP and DAX-1 in that it also forms heterodimers with ERα, but through the dimerization helices 10/11 rather than through LXXLL motifs (27). Both SHP and TERP can interact with the coactivator SRC-1 (27, 44). Whereas titration of SRC-1 is the predominant mechanism of SHP suppression of ER, TERP suppression is primarily via formation of heterodimers. This is because SRC-1 binding to liganded ERα is much greater than SRC-1 binding to TERP (27). In contrast, REA binds to TERP better than to liganded ERα. It is not known whether SHP and DAX-1 bind to REA, although REA binding appears to be specific for ERα and ERβ (23). Only TERP exhibits biphasic modulation of ERα activity, whereas SHP and DAX-1 are always suppressive (42, 43, 46). Physiological regulation of SHP and DAX-1 has not been shown, whereas levels of TERP expression vary with E2 (10, 31) (Fig. 5).

Modulation of REA levels by hormones, as has been reported for SRC-1 and SMRT (47), could also contribute to changes in ER activity. However, we found that REA mRNA expression varies less than 2-fold throughout the estrous cycle and is similar to that of ERα (data not shown). In contrast, TERP expression changes dramatically in response to E2 (26, 31), during pregnancy (29), and throughout the estrous cycle (Fig. 5B). Thus, TERP can provide rapid and efficient regulation of ER activity in the pituitary. We propose a model in which ERα transcriptional activity is regulated by the differential expression of its tissue-specific isoform, TERP (Fig. 6). As TERP is expressed at levels lower than ERα, TERP titrates the repressor protein REA from ERα, allowing SRC-1 to bind and ERα activity to be stimulated. Higher concentrations of TERP will
also bind to and suppress ERα activity by formation of inactive heterodimers that inhibit DNA binding. Because the ratios of TERP:ERα vary with estrogen levels, both of these mechanisms could contribute to the physiological modulation of ERα activity.

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