The estrogen receptor (ER) is a ligand-activated transcription factor that acts as a homodimer. Truncated estrogen receptor product-1 (TERP-1) is a pituitary-specific, estrogen-induced, isoform of rat ERα that is transcribed from a unique start site and contains only the C-terminal region of the full-length receptor. TERP-1 does not affect transcription directly but suppresses ligand-activated ERα and ERβ activity. Because TERP-1 contains a dimerization domain and part of the coactivator binding pocket, we hypothesized that it modulates ER function by direct interactions with full-length ER or the steroid receptor coactivator, SRC-1. Localization studies demonstrate that TERP-1 is present in the cytoplasm and nucleus of transfected cells and colocalizes with nuclear ER. Protein binding studies show that TERP-1 forms heterodimers with both ERα and ERβ and inhibits ERα binding to its cognate DNA response element. TERP-1 also binds SRC-1, and increasing levels of SRC-1 decrease the TERP-1-ERα interactions, in agreement with the rescue of TERP-1-suppressed ERα transcriptional activity by SRC-1. Mutational analysis of TERP-1 and ERα in the activation helix and the AF-2 dimerization helix indicates that TERP-1 acts predominantly through dimerization with ERα. Therefore, TERP-1 suppression of ER transcription occurs primarily by formation of inactive heterodimers and secondarily by competition for coactivators.

Estrogen receptors (ERs) are ligand-activated transcription factors that bind to cognate estrogen response elements (EREs) on DNA to influence target gene activity in a variety of responsive tissues including breast, uterus, liver, and pituitary (1). ER isoforms, ERα and ERβ, belong to the nuclear receptor superfamily that includes both steroid and nonsteroid nuclear receptors such as thyroid hormone receptors (TR), retinoic acid receptors (RAR), and retinoid X receptors (RXR), as well as an increasing number of orphan receptors with no known ligand (2, 3). Nuclear receptor family members share distinct structural and functional domains. The N terminus contains an activation function (AF-1), which is ligand-independent. A DNA-binding domain (DBD) consisting of two zinc fingers is located in the central region of the receptor, and a second activating function (AF-2) is located within the ligand-binding domain (LBD) at the C terminus of the protein. The AF-2 function requires ligand binding for transcriptional activity, and the contribution of AF-1 and AF-2 to receptor activity is both cell type- and promoter-dependent. Nuclear localization signals are located in both the DBD and LBD, and dimerization domains exist in the DBD and in helices 10/11 of the LBD (1–4).

Nuclear receptor activity can be modulated by several mechanisms including ligand binding, formation of heterodimers with other receptors, and binding of and competition for coregulatory proteins. ER and other steroid receptors are thought to act primarily as homodimers, although ERα-ERβ dimers can be observed on DNA and in solution (5–7). Because the isoforms are expressed in a tissue- and cell-specific manner (8, 9) and have differential affinities for synthetic and environmental ligands (10), dimer formation can have important biological consequences. For example, ER isoforms α and β have markedly different responses to estrogen antagonists, and the ratio of the two isoforms could determine cellular responses to these drugs (10–12).

Cellular specificity of receptor responses may occur in part by selective expression of coregulatory proteins that interact with the receptors in the C-terminal regions and thus influence interaction of the ligand-receptor complex with the transcriptional machinery (13, 14). Several coactivator and corepressor proteins that bind to distinct receptor regions have been isolated. Corepressor proteins, such as nuclear receptor corepressor (NCoR; Ref. 15) and silencing mediator of retinoic acid and thyroid hormone receptors (SMRT), bind to the NCoR (15, 16) box in the receptor hinge region between the DBD and LBD. Corepressors are recruited to unliganded receptors, such as the TR and RAR, and act as potent transcriptional repressors in the absence of ligand (15, 16). NCoR and SMRT do not appear to have a significant role in ligand-activated ER activity but can contribute to the regulation of transcription in antagonist-bound ER complexes (17). Coactivator proteins, including steroid receptor coactivator-1 (SRC-1; Ref. 18), the related glucocorticoid receptor activating protein-1 (GRIP-1; Ref. 19), and

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‡ The abbreviations used are: ER, estrogen receptor; ERα, estrogen response element; TR, thyroid hormone receptor; RAR, retinoic acid receptor; RXR, retinoid X receptor; AF, activation function; DBD, DNA-binding domain; LBD, ligand-binding domain; NCoR, nuclear receptor corepressor; SMRT, silencing mediator of retinoic acid and thyroid hormone receptor; SRC-1, steroid receptor coactivator-1; GRIP-1, glucocorticoid receptor activating protein-1; SHP, short heterodimer partner; PPAR, peroxisome proliferator-activated receptor; TERP-1, truncated estrogen receptor product-1; CMV, cytomegalovirus; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; E, estradiol; FITC, fluorescein isothiocyanate; GST, glutathione S-transferase; OHT, 4-hydroxyamoxifen; ICI, ICI 182,780; REA, repressor of estrogen receptor activity.
The GST-ER
CATGACCCTT-3
activity through both direct and indirect mechanisms and that
start site and contains exons 5–8 of ER
vector using TERP-1-specific 5
(Invitrogen) and subsequently subcloned into the pcDNA expression
(N-ASP-TYR-LYS-ASP-ASP-ASP-LYS-C; Eastman Kodak Co.).

specific 5
subcloned into a cytomegalovirus (CMV) promoter-containing pcDNA
a
pituitaries as described previously (31). ER
length ER
investigations we have addressed the suppressive actions of
and promoter-specific manner at ratios that are observed phys-
struct was generated by PCR amplification from the CMV vector using
(30).
PPAR activation by competing with SRC-1 for binding to PPAR
inhibits activation of the nuclear receptor peroxisome prolifera-
may involve heterodimerization and competition for coactiva-
tors without influencing DNA binding (26, 27). The nuclear
protein, receptor interacting protein-140 (28, 29), stimulates
binding to DNA (25). In contrast, SHP inhibition of ER activity
expression constructs (shown in figure legends), and total DNA was
standardized with pcDNA vector. 16 h later, cells were washed with
phosphate-buffered saline (PBS; pH 7.4), incubated for 24 h, and then
collected to assess luciferase activity or fixed for immunofluorescence.
Where indicated, cells were treated with 10 nM during the 24-h period
post transfection. Luciferase activity was assessed on a Turner-20E
luminometer using a Promega luciferase assay kit, and samples were
normalized by assessing lysate protein with Bio-Rad protein dye. Data
are presented as arbitrary light units and normalized for protein levels.

Immunofluorescence—Cos-1 cells were transfected with CMV vectors
expressing TERP-FLAG (4 μg) and ERα (1 μg) and were incubated in
the absence or presence of 10 nM E. Cells were fixed with 4% parafor-
aldehyde in PBS (10 min), permeabilized with 0.2% Triton X-100 (2
min), and blocked with 5% nonfat dry milk (1 h; Carnation) in PBS.

TERP-FLAG was detected using the monoclonal antibody anti-FLAG M2
(25 μg/ml; VWR) and an ERα antibody, ER C1355 (1:7500). ER C1355
was developed by our laboratory and is directed against the last
14 amino acids of rat ERα (32). ERα was detected using ER C1355 and
ER 715 (1:500; gift of Dr. Jack Gorski), which is directed against the
hinge region (amino acids 189–284) of rat ERα (36). All antibody incu-
bations were performed in PBS containing 2% bovine serum albumin
(Sigma). 1-h incubations at room temperature were performed with
anti-FLAG, and overnight incubations at 4 °C were performed with ER
715. Anti-mouse IgG-conjugated Texas Red (1:500; Calbiochem) was used
to detect TERP-FLAG (anti-FLAG), and anti-rabbit IgG-conju-
gated FITC (1:400; Calbiochem) was used to detect ERα (ER 715).
Alternatively, IgG-conjugated Texas Red or FITC-conjugated Texas
Red or ERα (E355) in individual experiments. For single and
double label immunofluorescence, a Nikon Microphot-SA micro-
scope was used. The Texas Red and FITC filters were from Chroma
Technologies. Cellular sectioning was performed with an IX70 micro-
scope (Olympus America). To acquire the image, Isee software from
with Applied Preci-
Inc. was used for processing the data.

GST Pull-downs—BL21 bacterial cells were transformed with con-
structs expressing GST, GST-TERP, GST-TERP-E547K, GST-TERP-
L509R, or GST-ERα-LBD. Luria Broth (100 ml) containing 50 μg/ml
ampicillin was inoculated with 1 ml of bacteria and incubated in an
orbital shaker at 37 °C. After 6–7 h of growth, 200 ml of the culture
were collected, and the cells were washed with 0.1 M Tris-HCl, pH 7.5,
and 10 mM EDTA, and a 10% (v/v) solution of lysozyme was
added. The mixture was shaken for 30 min at 37 °C. The supernatant
was removed, and the cells were washed with 0.1 M Tris-HCl, pH 7.5,
and 10 mM EDTA, and a 10% (v/v) solution of lysozyme was
added. The mixture was shaken for 30 min at 37 °C. The supernatant
was removed, and the cells were washed with 0.1 M Tris-HCl, pH 7.5,
and 10 mM EDTA, and a 10% (v/v) solution of lysozyme was
added. The mixture was shaken for 30 min at 37 °C. The supernatant
was removed, and the cells were washed with 0.1 M Tris-HCl, pH 7.5,
and 10 mM EDTA, and a 10% (v/v) solution of lysozyme was
added. The mixture was shaken for 30 min at 37 °C. The supernatant
was removed, and the cells were washed with 0.1 M Tris-HCl, pH 7.5,
TERP-L509R, and GST-ERα-LBD) with ER C1355. For pull-down experiments, approximately 1 μg of GST alone or 1 μg of GST fusion protein was used in each sample incubation. Bovine serum albumin (20 μg/ml) was added to each incubation containing [35S]methionine-labeled (0.04 μCi/50-μl reaction) in vitro translated proteins (TNT Rabbit Reticulocyte Transcription/Translation Kit; Promega) or approximately 100 μg of transfected Cos-1 whole cell extracts. Cos-1 cells were transfected with 1.6 μg of ERα in 60-mm dishes with 10% newborn calf serum for whole cell extract collection (37). Where indicated, 10 nm estradiol (E), 1 μt 4-hydroxyamoxymestrol (OH), 10 μM ICI 182,780 (ICI), or ethanol vehicle was added to the incubations. Total volume was adjusted to 150 μl with GST wash buffer (10 mM MgCl2, 150 mM KCl, 20 mM HEPES, 10% glycerol, and 0.12% Nonidet P-40). Beads and proteins were incubated for 1.5 h at 4 °C and then centrifuged and washed four times in GST wash buffer. Beads were resuspended in 10 μl of SDS loading buffer and boiled for 5 min. Proteins were electrophoresed on SDS-containing 12% acrylamide gels at 150 V, along with standard protein molecular weight markers (Benchmark, Life Technologies, Inc.). Gels containing [35S]methionine-labeled proteins were dried and exposed to film overnight at room temperature. Whole cell extract proteins were transferred to nitrocellulose, and Western blotting with E treatment (data not shown).

observed with the C1355 or ER715 antibodies with or without...total volume was adjusted to 150 μl with GST wash buffer (10 mM MgCl2, 150 mM KCl, 20 mM HEPES, 10% glycerol, and 0.12% Nonidet P-40). Beads and proteins were incubated for 1.5 h at 4 °C and then centrifuged and washed four times in GST wash buffer. Beads were resuspended in 10 μl of SDS loading buffer and boiled for 5 min. Proteins were electrophoresed on SDS-containing 12% acrylamide gels at 150 V, along with standard protein molecular weight markers (Benchmark, Life Technologies, Inc.). Gels containing [35S]methionine-labeled proteins were dried and exposed to film overnight at room temperature. Whole cell extract proteins were transferred to nitrocellulose, and Western blotting with E treatment (data not shown).

FIG. 1. ERα protein structural domains and antibody specificities. The protein structural and functional domains of ERα and TERP-1 and the AP-1, AP-2 and TERP-FLAG constructs are shown. TERP-1 contains a 31-base pair unique untranslated sequence, indicated by the thin line at the N terminus. TERP-1 contains two potential translational start sites, at amino acids 393 and 401, and the translated protein includes most of the ER ligand-binding domain. The hatched area on TERP-FLAG represents the 8-amino acid FLAG epitope. Regions detected by the three antibodies are also shown.

RESULTS

TERP-1 Can Colocalize with ERα in the Nucleus—The mechanism by which TERP-1 suppresses ER transactivation could be direct or indirect and will be governed by its intracellular location. TERP-1 contains one of the two nuclear localization signals present in ERα and could reside in either the cytoplasm or nucleus. Therefore, we used double label immunofluorescence to localize TERP-1 in transfected Cos-1 cells in the presence or absence of ERα and ligand. Because TERP-1 contains the identical amino acid sequence for most of the LBD of ERα (amino acids 393–600), it was necessary to differentiate the two proteins by an epitope tag (Fig. 1). We constructed the TERP-FLAG expression vector in which the 8-amino acid FLAG sequence is fused to the C terminus of TERP-1. TERP-FLAG and TERP-1 have similar effects on ERα transactivation as measured by transient transfection assays, and immunofluorescence of TERP-1 or TERP-FLAG using the C-terminal-specific C1355 antibody demonstrated similar localization patterns (not shown). In a series of parallel experiments, the same pattern for TERP-FLAG was observed with either the C1355 or FLAG antibodies, and exclusively nuclear staining for ERα was observed with the C1355 or ER715 antibodies with or without E treatment (data not shown).

Fig. 2 depicts cells cotransfected with ERα and TERP-FLAG and treated with E. Transfection conditions were identical to those in which TERP-1 suppresses ERα activity (7). In these cells, TERP-FLAG (red color) appeared in both the cytoplasm and nucleus (Fig. 2A, B, C, and E), and ERα (green color) was found only in the nucleus (Fig. 2D). Panels A and B show the same transfected cell, with colocalization of TERP-FLAG and ERα in the nucleus as indicated by the yellow color in Fig. 2B. The subcellular location of TERP-FLAG was not altered by E treatment or the presence of the full-length ERα, and TERP-FLAG did not alter ERα localization in the nucleus. A second cotransfected cell (Fig. 2C–E) shows individual fluorescence of red TERP-FLAG in both cytoplasm and nucleus (Fig. 2C) and green nuclear ERα (Fig. 2D). Identical subcellular relationships were observed for the two proteins in five independent experiments. Nuclear localization of TERP-FLAG in this cell was further verified by cell sectioning and computer imaging. The mosaic (Fig. 2E) shows every third cellular section (0.4-μm sections) and demonstrates consistent staining for TERP-FLAG throughout the cell and distinct dark nucleoli. Thus, TERP-1 does not inhibit ER activation by sequestering ER protein in the cytoplasm, and the colocalization of both TERP-FLAG and ERα in the nucleus suggests that direct interactions between the two proteins could occur.

GST-TERP Binds ERα and ERβ—Because TERP-1 protein can colocalize with ERα and can modify the transcriptional activity of both ERα and ERβ (7), we tested the ability of TERP-1 to form dimers with both full-length receptors. A GST-TERP fusion protein was constructed and used in pull-down experiments with transfected Cos-1 whole cell extracts and in vitro translated [35S]methionine-labeled ER proteins. GST-TERP specifically bound ER regardless of the protein source. For example, both ERα from transfected Cos-1 cells and cell-free translated ERα strongly bound GST-TERP but did not effectively interact with GST alone (Fig. 3). TERP-1 cannot bind E, but its biological effects require E and an activated ER and are inhibited by the antiestrogens OHT and ICI (7). Therefore, we examined the interactions of TERP-1 and both ER isoforms in the presence and absence of E, OHT, and ICI (Fig. 3). An equal amount of purified GST alone or GST fusion protein was incubated with equivalent amounts of in vitro translated [35S]methionine-labeled ER protein. Formation of TERP-1-ERα heterodimers was not ligand-dependent but was ligand-enhanced. This was seen most dramatically in the pres...
ence of ICI, where binding was increased approximately 8.5-fold as measured by densitometry, and this trend was seen in each of three experiments. In contrast, TERP-1 binding to ERβ was completely ligand-independent. These data show that TERP-1 interaction with either ER isoform does not require ligand, in agreement with the ability of ER isoforms to form dimers in the absence of ligand, and in contrast to the ligand-dependent interactions of receptors and coregulatory proteins.

TERP-1 Inhibition of ERα Activity through the AF-1 and AF-2 Domains—TERP-1 modulation of AF-1 and AF-2 transactivation was tested in cotransfection studies and compared with effects on the full-length ERα (Fig. 4). All three constructs (proteins shown in Fig. 1) stimulated promoter activity of the luciferase reporter gene. Both ERα- and ERα-AF2-stimulated activities of the ERα-containing construct were increased by E, and both activities were inhibited by cotransfected TERP-1 by an average of 70–80%. Interestingly, high concentrations of TERP-1 also inhibited AF-1 activity in the same experiments by approximately 40%, suggesting that some less effective transcriptional complex was formed. Both AF-1 and AF-2 have less activity than the full-length ERα in the same experiments, indicating that interaction between the two activating functions of the receptor is required for full biological activity. The data indicate that TERP suppresses both AF-1 and AF-2 activity. The inhibition of ERα activity was similar to suppression of ERα-AF2, in agreement with previous data showing that the suppressive effects of TERP-1 require a ligand-bound ER and that the antiestrogen OHT, which suppresses AF-2 but maintains AF-1 activity (14), completely abolishes the effects of TERP-1 (7).

TERP-1 Binds to the Coactivator SRC-1 and Competes for Binding with ERα—Cotransfection of increasing amounts of SRC-1 in Cos-1 cells (Fig. 5A). Previous work demonstrated that this rescue is dose-dependent (7). Because TERP-1 includes regions of ERα that are important for coactivator binding, particularly helix 12 (amino acids 544–552 in rat ERα), we examined the

![Image](image_url)
ability of TERP-1 to interact directly with SRC-1 in pull-down assays with in vitro translated [35S]methionine-labeled proteins (Fig. 5B). In addition to binding ERα, GST-TERP also bound SRC-1. The interaction between TERP-1 and SRC-1 was specific, because the corepressor proteins SMRT and NCoR did not bind to GST-TERP (not shown). Suppression of ERα activity could occur by competition of TERP-1 and ERα for available coreactivators. To test this possibility, we performed competition experiments with GST-TERP in the presence of a constant amount of ERα and increasing SRC-1 (Fig. 5B). Increasing amounts of SRC-1 displaced ERα binding to TERP-1 up to 64%. To assess whether this competition could be biologically relevant, we compared the ability of the ERα-LBD and TERP-1 to individually bind SRC-1 in the same experiment. As shown in 5C, both molecules can bind SRC-1. ERα-LBD binding to SRC-1 was greatly enhanced by the presence of E as shown previously (18), whereas SRC-1 binding to TERP-1 was not. TERP-1 bound SRC-1 slightly better than unliganded ERα-LBD but less well than liganded receptor. Thus, TERP-1 could compete with ER for binding to coreactivators but only at times when TERP-1 protein levels are high, which can occur physiologically (32). This would limit the ability of coreactivators to bind activated ERα, thus limiting the ability of the ligand-activated receptor to stimulate transcription.

**Point Mutations in TERP-1 Demonstrate That Suppression of ERα and ERβ Occurs Predominantly through Dimerization with the ER—**Because TERP-1 influences the AF-2 function in the C-terminal region of ERα, we examined two potential mechanisms of the actions of the TERP-1. First, because TERP-1 contains the dimerization domain in helices 10/11 (amino acids 479–522), it could interact with the ER directly. The second potential mechanism involves the interactions of coactivators, like SRC-1 with ER or TERP-1. TERP-1 contains the coactivator binding interface and could sequester coactivator proteins away from activated ERs. Therefore, two single point mutations were made in TERP-1 that would individually disrupt the dimerization domain (TERP-1-L509R; Ref. 34) and the helix 12 coactivator binding pocket (TERP-1-E547K; Ref. 22). The same individual mutations were made in the full-length ERα to ensure that the receptors were functionally compromised in luciferase reporter gene assays. Wild type ERα activity was effectively stimulated by E; however, both ERα-E547K and ERα-L509R had compromised E-induced activity (Fig. 6A). GST pull-down experiments showed that ERα interactions with GST-TERP-L509R (Fig. 6B) are greatly reduced relative to wild type GST-TERP, whereas those with GST-TERP-E547K are not. TERP-1 mutated in helix 12 inhibited ERα activity as well as wild type TERP-1 and partially inhibited ERβ activity (Fig. 6C). However, the dimerization mutation in TERP-1 severely compromised its ability to inhibit both ERα and ERβ activity (Fig. 6C) and even slightly increased ERβ activity. The failure of the L509R mutant to inhibit ER was not due to protein instability, because immunoblots of transfected cells demonstrated levels of TERP-1-L509R equal to that of wild type TERP-1 (not shown). Transfection of a TERP-1 construct containing both mutations (TERP-1-RK) had no suppressive effect on ER actions, indicating that these two pathways likely explain all suppressive effects of TERP-1. Overall these data suggest that TERP-1 and ERα interact directly through dimerization domains, and this is the primary pathway of TERP-1 inhibition. In comparison, interaction of TERP-1 with coactivator proteins is a secondary mechanism for TERP-1 actions.

**TERP-1 Reduces the Ability of ERα to Bind to an ERE—**One possible mechanism by which direct dimerization of TERP-1 to ERα could suppress ERα activity is by inhibition of ERα binding to its defined DNA element (ERE). To test this hypothesis, electrophoretic mobility shift assays were performed with a labeled ERE and in vitro translated proteins. The amount of ERα in each lane was standardized by immunoblotting with ER C1355, and unprogrammed reticulocyte lysate was added where necessary to maintain a constant total lysate volume. As
expected, TERP-1 does not bind to the ERE, because it does not contain a DBD. In contrast, ERα forms specific high affinity complexes as shown by the shaded arrowhead in Fig. 7, and this complex is shifted upon addition of C1355 antibody as indicated by the open arrowhead in Fig. 7. No smaller DNA-protein complexes potentially representing ERα/TERP-1 dimers on DNA were observed. ERα cotranslated with TERP-1 bound DNA less efficiently than ERα alone. Thus, TERP-1 interacts with ERα through dimerization domains to form ER dimers with reduced DNA binding ability.

**DISCUSSION**

The stimulation of transcription by activated estrogen receptor has both general and cell-specific aspects that contribute to biological responses (8–12). This has particular importance therapeutically with the use of selective estrogen receptor modulators that have both tissue- and cell-specific effects to treat osteoporosis, memory loss, and heart disease and as treatments or possible preventives for steroid-dependent cancers of the breast and uterus (39). Among the major factors contributing to tissue-selective responses are the complement of receptor isoforms present in a given cell type and the number and types of proteins capable of binding to the receptors and modulating their function. Several groups have isolated activating coactivators and inhibitory proteins called corepressors that bind directly to specific sites on nuclear receptors and alter their transcriptional capability (13, 14). Others have defined molecules termed orphan receptors that have some structural similarity to the nuclear receptors and modify their activity by direct interactions with the nuclear receptor ligand-binding domain (25–27, 40, 41). We have identified a novel form of ERα, TERP-1, which acts as a tissue-specific, highly regulated suppressor of ER action (7, 31). Here we show that it exerts its activity primarily by binding to both full-length receptor isoforms, ERα and ERβ, and secondarily by acting as an intracellular buffer to prevent binding of cofactors to the full-length ER forms.

**TERP-1 Suppresses ER Activity Primarily by Direct Protein-Protein Interactions**—Transient transfection studies with constructs representing either the N-terminal AF-1 or ligand-binding region AF-2 domains of ERα indicate that TERP-1 suppression of ERα transactivation is equal to suppression of the C-terminal AF-2 function, although suppression of AF-1 also is observed. Direct physical and functional interactions between the N- and C-terminal regions of the estrogen, progesterone, and androgen receptors and interactions between the N-terminal regions of the steroid receptors and SRC-1 have been reported (42–46), perhaps explaining some inhibition of AF-1 activity by TERP-1 in transfection assays. TERP-1 does not inhibit ERα action by sequestering the full-length receptor in the cytoplasm (Fig. 2) or by inhibiting E binding by ERα, as we previously demonstrated (7). Instead, TERP-1 acts primarily by binding to ERα through the dimerization helix and suppressing the transcriptional activity of ligand-activated receptor.

Although a direct interaction between TERP-1 and ERα was not observed in stringent immunoprecipitation studies, this
TERP-1 Suppresses Estrogen Receptors by Dimerization

A secondary mechanism for TERP-1 suppression of ER activity is competition for binding to coactivator proteins. Nuclear receptors associate with several different activator proteins, including SRC-1 and GRIP-1, through the C-terminal portion of the receptor, although the precise areas of interaction within ERα vary slightly with each coactivator (29). Co-transfection of SRC-1 partially overcomes the inhibitory effects of TERP-1 on ERα transactivation (Fig. 5A and Ref. 7). TERP-1 contains a portion of the coactivator binding interface, helix 12 of the LBD, but lacks the most N-terminal helices of the LBD that are important for coactivator binding, helices 3 and 5. Mutations in each of these helices inhibit binding of SRC-1 and GRIP-1 to TRβ and GRIP-1 to human ERα (22). Our data indicate that TERP-1 interacts with SRC-1, as well as with ER, and could thus compete with ER for SRC-1 binding. In agreement with this possibility, Jeyakumar et al. (47) demonstrated that a small peptide consisting of amino acids 437–456 of TRβ, equivalent to helix 12, inhibited SRC-1 binding to the entire receptor. Other investigators have also found that RIP140, a coregulatory protein binding at a somewhat different region on PPAR than SRC-1, antagonizes receptor activation by SRC-1 by effectively competing for binding to the receptor (30). The ERα-LBD binds SRC-1 more effectively than does TERP-1 in GST-pull-down experiments. This suggests that competition of TERP-1 and ER for SRC-1 is likely to occur only when SRC-1 levels are low or when TERP-1 levels are high, as has been noted during proestrus (32).

A related mechanism by which the TERP-1 helix 12 region could inhibit ER action is by direct occupation of the coactivator pocket, as has been observed for the homologous helix 12 of the ERα when bound to the selective estrogen receptor modulators tamoxifen or raloxifene (21, 48). The helix 12 mutant, TERP-1-E547K, is as effective at suppressing ERα transactivation as the wild type TERP-1 in these studies but only partially inhibits ERβ transactivation, demonstrating that suppression through helix 12 of TERP-1 is more important for ERβ than ERα. This mechanism could be important in some cellular contexts, when ERβ is high (9, 10), or when coactivator proteins themselves are modulated. For example, SRC-1 levels are altered in clonal pituitary cells after treatment with E or thyroid hormone, and this could alter responsiveness of nuclear receptors in those cells (49). The ability of SRC-1 to rescue TERP-1 actions demonstrates a functional role for this pathway.

TERP-1 Is Functionally Similar to Nuclear Receptor Coregulatory Proteins SHP and DAX-1—Nuclear receptor function can also be modulated by the formation of specific heterodimers with orphan receptors that have structural similarities with the nuclear receptors but do not bind known ligands. Association of RARs, TRs, and vitamin D receptors with the previously characterized RXRs results in heterodimers with increased binding affinity to DNA cognate response elements and enhanced transcriptional responses to retinoic acid, thyroid hormones, and vitamin D (50). RXRs themselves bind to DNA and associate with nuclear receptors via dimerization domains similar to those found in the nuclear receptor LBDs. The ER isoforms do not interact with RXR family members, and ER function is not affected by RXRs.

More recently, a new class of suppressive orphan receptor molecules has been defined. These proteins, including SHP and DAX-1, contain putative LBDs but lack DNA-binding regions. DAX-1 combines preferentially with the orphan receptor steroidogenic factor-1 with which it is coexpressed in the adrenals, gonads, hypothalamus, and pituitary gland (40, 41, 51, 52), whereas SHP is expressed in a variety of tissues and suppresses the activities of RAR, TR, and ER (25–27). SHP and DAX-1 are proposed to exert their effects via several mechanisms. These include dimerization with target nuclear receptors to form nonproductive transcriptional complexes, inhibition of heterodimer binding to DNA, and competition for coactivator proteins.

Like these orphan receptors, TERP-1 does not bind DNA or
ligand and has no transcriptional effects on its own. All three proteins exert their suppressive effects by binding directly to nuclear receptor partners, but the physical nature of the interaction is different. Although DAX-1 and SHP both contain putative dimerization helices, they do not associate with target receptors through those C-terminal regions. DAX-1 association with steroidogenic factor-1 occurs through an N-terminal region that does not contain a dimerization helix (51). Similarly, interaction of SHP with target receptors does not occur through the SHP C-terminal putative dimerization helix 10/11 but instead requires the central interaction domain between amino acids 92 and 148 (25–27, 53). This region does not contain functional NR box motifs (LXXLL) required for other coactivator interactions with nuclear receptors but may require a similar region in activation helix 12 within the AF-2 region of the nuclear receptors (27). Mutation of this region in ERα does not disrupt the interaction with TERP-1 (Fig. 6). SHP interactions with nuclear receptors are generally ligand-dependent, with the exception of its association with ERβ. In comparison, TERP-1 binds to both ERs in the absence of ligand, as has been noted for dimerization of the ER isoform homodimers and heterodimers (38), and occurs through the dimerization helix 10/11 regions of both partners. The protein-protein interactions occur even in the presence of antiestrogens, reinforcing the importance of the dimerization helix in this process and in biological function.

Formation of heterodimers between nuclear receptors and orphan receptors suppresses transcription through at least three defined mechanisms, including direct transcriptional repression, inhibition of DNA binding, and changes in coactivator or corepressor binding to nuclear receptors. Both DAX-1 and SHP have separate, transferable transcription repressor functions separated structurally from receptor interaction domains, as measured in the yeast two-hybrid system. TERP-1 is unlikely to have a separate, transferable suppressive function, and there is no evidence for suppressive activity with other receptor partners such as TR (7). Neither DAX-1 actions on steroidogenic factor-1 nor SHP inhibition of ER occur by interference with DNA binding (26, 27, 40, 51, 52). However, SHP suppression of TR,RAR, and RXR activity is postulated to occur through the demonstrated direct inhibition of receptor heterodimer binding to DNA (25), as we have demonstrated for TERP-1 and ER.

SHP, DAX-1, and TERP-1 have all been proposed to interfere with the AF-2 function of nuclear receptors, partly by competition for binding of coactivators. In support of this second mechanism, SHP inhibition of ERα and ERβ activity can be rescued by cotransfection with high levels of the coactivator transcriptional intermediary factor-2 (27). TERP-1 also suppresses AF-2 activity of ER by competition for coactivator binding, and these effects can be rescued by addition of SRC-1 (7). DAX-1 may also recruit the binding of corepressors to steroidogenic factor-1 (52), but this activity has not been tested for SHP inhibition of TR and RAR. The previously described corepressors N-CoR and SMRT appear to have a minor role in ER activation by natural ligand, and because TERP-1 does not bind N-CoR or SMRT, this pathway is unlikely to play a major role in TERP-1 suppression. Recently, however, an ER-selective coregulator (repressor of estrogen receptor activity, or REA) has been identified (54). REA interacts with the ligand-binding domain of ER in a ligand-independent fashion and suppresses ER activity. REA or similar molecules could interact with TERP-1, and titration of such repressors by TERP-1 could conceivably explain the stimulatory effects of TERP-1 noted at low TERP-1:ER ratios (7). The relative contribution of the dimerization and coactivator binding pathways to SHP and DAX-1 activities have not been directly evaluated. Our mutation studies indicate that the dimerization pathway is the most important one for TERP-1 suppression of ER activity.

**TERP-1 Is a Novel Tissue-specific Suppressor of ER Action**—Two novel features of TERP-1 distinguish it from SHP and DAX-1. TERP-1 protein represents a portion of a functional receptor, ERα, and has strict tissue-specific expression of the mRNA and protein. Other mRNA variants of both ERα and ERβ have been described, and some of these have altered biological functions when tested in transfection assays (55–57). However, these variants all occur through exon splicing of ER mRNA, comprise only a small percentage of total ER mRNA, and do not appear to be regulated in response to physiological state. Not all of these variants have been verified at the protein level, and they affect the biological activity of full-length ER only at ratios far in excess to those demonstrated in cells. In contrast, TERP-1 is transcribed from a transcriptional start site distinct from that of full-length ERα mRNA (31). TERP-1 mRNA and protein are expressed only in the pituitary, and the physiological levels observed can be equal to or greater than that for full-length ERα (32). Detectable TERP-1 mRNA has been found only in pituitary cells that express ER. Many of these cells express ERα and ERβ, and TERP-1 can suppress the activity of both of these ER forms. It is not known whether expression of full-length ER is a requirement for TERP-1 expression. TERP-1 mRNA levels are dramatically stimulated by E and increase 50-fold on the day of proestrus to become the predominant ER isoform (32). This increase in TERP-1 coincides with the pituitary hormone surge and ovulation and correlates with the physiologic switch from positive to negative feedback in the hypothalamic-pituitary axis at this time (58). SHP, which can also suppress ER function, is expressed in many estrogen-sensitive tissues and is, significantly, not in the pituitary (27). This regulatory function in the pituitary can be provided by TERP-1, which acts a novel, highly regulated, tissue-specific suppressor of ER action.

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