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Inhibitory Cross-Talk between Steroid Hormone Receptors: Differential Targeting of Estrogen Receptor in the Repression of Its Transcriptional Activity by Agonist- and Antagonist-Occupied Progestin Receptors

W. LEE KRAUS,[1] KAREN E. WEIS,[1] AND BENITA S. KATZENELLENBOGEN[1,2]*

Department of Physiology and Biophysics[1] and Department of Cell and Structural Biology,[2] University of Illinois, Urbana, Illinois 61801

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Although estrogen receptor (ER) and progestin receptor (PR) are members of different steroid hormone receptor subfamilies, there is considerable biological evidence for cross-talk between the estrogen and progestin hormone-receptor signaling pathways. We have developed a model system to analyze the mechanisms underlying this cross-talk, specifically the repression of ER-mediated transcriptional activity by PR complexed with agonistic or antagonistic ligands. Estrogen- and progestin-responsive reporter vectors containing a variety of promoters were transfected into primary cultures of rat uterine cells and 3T3 mouse fibroblasts with expression vectors for PR (the A and/or B isoforms) as well as ER. Our results demonstrate that both PR isoforms can act as potent ligand-dependent repressors of ER activity. The magnitude of the repression was dependent on the PR isoform (i.e., PR A or PR B), ligand type (i.e., agonist or antagonist), PR levels, and ligand concentration but was unaffected by the ER levels. The promoter context was important in determining both the magnitude and PR isoform specificity of the repression for agonist-occupied PR but not for antagonist-occupied PR. Ligand-occupied PR A was a stronger repressor of ER-mediated transcriptional activity than was ligand-occupied PR B, and antagonist-occupied PR was a more effective repressor than agonist-occupied PR. Mechanistic studies suggest that liganded PR represses ER activity by interfering with its ability to interact productively with the transcriptional machinery, a process known as quenching. The data do not support competitive repression, direct repression, or squelching as the mechanism of PR's inhibitory effect. Experiments with ER mutants demonstrated that the N-terminal portion of ER was required for repression by agonist-occupied PR but not by antagonist-occupied PR. These results, as well as other differences between the two PR-ligand complexes, suggest that they differentially target ER when repressing ER transcriptional activity. These findings underscore the mounting evidence for the importance of interactions between members of the steroid hormone receptor family.

Estrogen receptor (ER) and progestin receptor (PR) belong to a large superfamily of conserved nuclear proteins, including receptors for the steroid, retinoid, and thyroid hormones (5, 8). These proteins, which share common functional domains responsible for properties such as ligand binding, dimerization, DNA binding, and transactivation, act as ligand-modulated transcription factors in the tissues where they are expressed (5, 8, 38). These receptors recognize and bind to specific DNA sequences, termed hormone response elements, which function as hormone-responsive enhancers (9, 38). PR is somewhat unique among members of the steroid receptor family in that it is expressed as two isoforms in most species, a larger B form and a smaller A form (10, 11, 33). The difference between the two isoforms is an N-terminal extension, the size of which (approximately 164 amino acids) varies slightly, depending on the species. This region contributes to the differential activity of the two isoforms that is observed in certain cell and promoter contexts (13, 36, 41).

* Corresponding author. Mailing address: Department of Physiology and Biophysics, University of Illinois, 524 Burrill Hall, 407 S. Goodwin Ave., Urbana, IL 61801. Phone: (217) 333-9769 or (217) 333-7838. Fax: (217) 244-9906. Electronic mail address: benita_katzenellenbogen@qms1.life.uiuc.edu.

Although ER and PR are members of different steroid hormone receptor subfamilies and recognize distinct hormone response elements, there is considerable biological evidence for cross-talk between the estrogen and progestin hormone-receptor signaling pathways. In many cases, progestins suppress the stimulatory effects of estrogens in target cells. For example, estrogen increases the expression of both c-*fos* and PR mRNA in uterine cells, and progestins block these effects (14, 16, 21). Although a number of endpoints for progestin antagonism of estrogen action have been examined, the molecular mechanisms underlying the antagonism have not been elucidated. The effects appear to be mediated via PR, but it is unknown if the ER protein or some other component of the estrogen-ER signaling pathway is the target for repression. In addition, it is unclear how a ligand-activated protein like PR, which under many circumstances functions as a transcriptional activator (9), can behave as a transcriptional repressor. It is also unclear how PR, which has been shown to synergize with ER in some systems (4, 6, 35), can act as a repressor of ER activity in other systems.

We have developed a model system to analyze the cross-talk between the estrogen and progestin signaling systems and to examine the mechanisms underlying the repression of ER activity by PR. Our system utilizes transient transfection of ER and PR, as well as reporter vectors containing estrogen and

progestin response elements, into several cell types. We demonstrate that both PR isoforms can act as potent ligand-dependent repressors of ER activity and that agonist- and antagonist-occupied PRs differentially target ER for repression. Our studies, which address mechanistic aspects of the inhibitory cross-talk between ER and PR, underscore the mounting evidence for the generality of interactions between members of the steroid hormone receptor family.

(Portions of this work were presented at the 76th Annual Meeting of the Endocrine Society, Anaheim, Calif. [19a].)

MATERIALS AND METHODS

Reagents and radioisotopes. All general reagents were of molecular biology grade and were purchased from Sigma Chemical Co. (St. Louis, Mo.), U.S. Biochemicals (Cleveland, Ohio), and Fisher Scientific (Houston, Tex.). Custom oligonucleotides were purchased from National Biosciences Inc. (Plymouth, Minn.). DNA restriction and modifying enzymes were from New England Biolabs (Beverly, Mass.), Gibco/Bethesda Research Laboratories (Gaithersburg, Md.), and U.S. Biochemicals. DNA sequencing reagents were from U.S. Biochemicals. ^{35}S-dATP for DNA sequencing was from Amersham (Arlington Heights, Ill.), and [^{14}C]chloramphenicol for chloramphenicol acetyltransferase (CAT) assays was from DuPont/New England Nuclear Corp. (Boston, Mass.). Sera, antibiotics, and other cell culture reagents were from Sigma and Gibco/BRL. 17β-Estradiol (E_2) was from Sigma. R5020 was from DuPont/NEN. RU486 was from Roussel-UCLAF (Romainville, France).

Plasmid constructions and DNA preparation. All cloning was done by standard techniques (30). When it was necessary to make termini compatible, 3′ and 5′ overhangs generated by restriction digestion were blunted with T4 DNA polymerase and the Klenow fragment of *Escherichia coli* DNA polymerase, respectively. The insertion of double-stranded oligonucleotides was confirmed by dideoxynucleotide triphosphate DNA sequencing. Other manipulations were confirmed by restriction digest analyses.

PR_{Dist}-CAT, PR_{Prox}-CAT, and TK-CAT, which contain the −131 to +65, +461 to +675, and −150 to +56 regions of the rat PR (PR_{Dist} and PR_{Prox}) and herpes simplex virus thymidine kinase (TK) genes, respectively, have been described previously (18). PS2-CAT was constructed by replacing the TK promoter of TK-CAT, which was released by *Bam*HI-*Bgl*II digestion, with a *Bam*HI fragment (−90 to +10) from a genomic clone of the human pS2 gene (12) (kindly provided by Pierre Chambon, Strasbourg, France). ERE_2-PR_{Dist}-CAT, ERE_1-PR_{Prox}-CAT, and ERE_2-PR_{Prox}-CAT were made by annealing the oligonucleotides 5′-AGCTAGTCAGGTCACAGTGACCTGATC-3′ and 5′-AGCTGATCAGGTCACTGTGACCTGACT-3′ and cloning one or two copies of the double-stranded ERE oligomer via its *Hin*dIII-compatible overhangs into the *Hin*dIII site of PR_{Dist}-CAT or PR_{Prox}-CAT. PRE_2-PR_{Dist}-CAT, ERE_2PRE_2-PR_{Dist}-CAT, PRE_2-PR_{Prox}-CAT, ERE_1PRE_2-PR_{Prox}-CAT, and ERE_2PRE_2-PR_{Prox}-CAT were made by annealing the oligonucleotides 5′-AGTCAGAACACAGTGTTCTGATCAGCT-3′ and 5′-GATCAGAACACTGTGTTCTGACTAGCT-3′ and cloning two copies of the double-stranded PRE oligomer via its *Sst*I-compatible overhangs into the *Sst*I sites of PR_{Dist}-CAT, ERE_2-PR_{Dist}-CAT, PR_{Prox}-CAT, ERE_1-PR_{Prox}-CAT, and ERE_2-PR_{Prox}-CAT, respectively. PRE_2 ERE_2-PR_{Dist}-CAT was made by annealing the oligonucleotides 5′-GATCCAAAGTCAGGTCACAGTGACCTGATCAAAGA-3′ and 5′-GATCTCTTTGATCAGGTCACTGTGACCTGACTTTG-3′ and cloning two copies of the double-stranded ERE oligomer via its *Bam*HI-compatible overhangs into the *Bam*HI site of PRE_2-PR_{Dist}-CAT. GAL_2PRE_2-PR_{Dist}-CAT was made by annealing the oligonucleotides 5′-GATCAAAGTCAGAACACAGTGTTCTGATCAA-3′ and 5′-GATCTTGATCAGAACACTGTGTTCTGACTTT-3′ and cloning two copies of the double-stranded PRE oligomer via its *Bam*HI-compatible overhangs into the *Bam*HI site of GAL_2-PR_{Dist}-CAT (18). ERE_2PRE_2-PS2-CAT and ERE_2PRE_2-TK-CAT were made by replacing the *Xmn*I-*Bam*HI fragments of PS2-CAT and TK-CAT with the *Xmn*I-*Bam*HI fragment of ERE_2 PRE_2-PR_{Dist}-CAT, which contains the ERE_2PRE_2 sequence. PRE_2(Dist)ERE_2-PS2-CAT was made by cloning the PRE_2 cassette from PRE_2-PR_{Prox}-CAT, which was released by *Eag*I-*Sal*I digestion, into the *Afl*III site of ERE_2-PS2-CAT.

The Rous sarcoma virus promoter-driven rat ER expression vector, pRSV-rER, has been described previously (18). The cytomegalovirus promoter-driven rat ER expression vector, pCMV-rER, was made by cloning the 2.1-kb *Eco*RI fragment from pUCER6 (15) (kindly provided by Masami Muramatsu, Saitama Medical School, Saitama, Japan), which contains the entire rat ER open reading frame, into the *Eco*RI site of pCMV5 (2) (kindly provided by David Russell, University of Texas Southwestern Medical Center, Dallas). The cytomegalovirus promoter-driven expression vectors for wild-type human ER (pCMV-hER) (26) and human ER(1-554) (pCMV-hERΔF) (25) have been described previously. The cytomegalovirus promoter-driven expression vector for human ER(109-595), pCMV-hER(109-595), was made by digesting pCMV-hER with *Sst*II, followed by religation. The sequences flanking the new start codon were then restored to those of the wild-type ER by PCR-based mutagenesis. The Rous sarcoma virus promoter-driven human PR (hPR) B and hPR A expression vectors (pRSV-hPRB and pRSV-hPRA) were constructed by releasing the hPR B cDNA or the hPR A cDNA from pHPR-65, a pGEM3z-based vector containing the full-length hPR cDNA (kindly provided by Geoff Greene, University of Chicago, Chicago, Ill.), by *Sph*I-*Eco*RI and *Tth*111I-*Eco*RI digestion, respectively. The DNA fragments were blunted, *Kpn*I linkers were added, and the fragments were cloned into the *Kpn*I site of pTZ19 (24) (kindly provided by Byron Kemper, University of Illinois, Urbana). The cDNAs were then released from pTZ19 by *Kpn*I digestion and cloned into the polycloning site of pRSV (27), which was modified to contain a *Kpn*I site. The GAL-VP16 expression vector has been described previously (28). The cytomegalovirus immediate early promoter and enhancer-driven β-galactosidase expression vector, pCMVβ, was from Clonetech (Palo Alto, Calif.).

DNA preparation. Supercoiled plasmid DNA was prepared for transfection on CsCl density gradients as described previously (30) or with plasmid DNA preparation kits (Qiagen, Chatsworth, Calif.).

Cell culture and transfection. Primary cultures of rat uterine cells were prepared as described previously (3). For transfections, the cells were plated in 100-mm-diameter dishes in phenol red-free improved minimal essential medium containing 5% charcoal-dextran-treated calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and gentamicin (100 μg/ml) at a density of 3×10^6 cells per dish. Forty-eight hours later, the medium was changed, and the cells were transfected 12 h later by the calcium phosphate coprecipitation technique (7). Each plate received precipitate containing expression vectors, which included one or more of the following: pRSV-hPRA (up to 1 μg), pRSV-hPRB (250 or 500 ng), and pRSV-rER (up to 1 μg) as well as 10 μg of CAT reporter plasmid, 3 μg of pCMVβ (used as an internal control for the determination of transfection efficiency), and carrier DNA (pTZ19) to 20 μg total. The cells were incubated with the precipitates for 5 h and then subjected to a 2.5-min glycerol shock (20% in serum-containing medium). Hormonal treatments were added in fresh medium following the glycerol shock.

3T3 cells were maintained in minimal essential medium containing 5% charcoal-dextran-treated calf serum, 5% charcoal-dextran-treated fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and gentamicin (100 μg/ml) and were plated for transfection in 100-mm-diameter dishes at a density of 6.0×10^5 cells per dish. Forty-eight hours later, the medium was changed, and the cells were transfected 5 h later by the calcium phosphate coprecipitation technique (7). Each plate typically received precipitate containing 10 μg of CAT reporter plasmid, 100 ng of pRSV-rER or 300 ng pCMV-rER, up to 1 μg of the pRSV-hPR expression vectors, 1 μg of pCMVβ, and pTZ19 to 15 μg total, unless otherwise noted. For examination of the ER mutants, the cells were transfected with 30 to 300 ng of the pCMV-ER expression vectors instead of pRSV-rER. The cells were incubated with the DNA precipitates for 14 h and then subjected to a 3-min glycerol shock (20% in Hanks balanced salt solution). Hormonal treatments were added in fresh medium following the glycerol shock.

CAT assays. Twenty-four hours after the glycerol shock and the addition of the hormonal treatments, the cells were harvested and extracts were prepared as previously described (27), with a final volume of 200 μl. CAT assays, normalized for the β-galactosidase activity of each extract, were performed as previously described (27).

RESULTS

Repression of ER-mediated transcriptional activity by ligand-occupied PR A and PR B. To examine the ability of PR to repress ER-mediated transcriptional activity, we performed transient transfection experiments with estrogen response element (ERE)- and progestin response element (PRE)-containing promoter-CAT reporter vectors. The reporters were transfected into primary cultures of rat uterine cells with expression vectors for PR A and/or PR B (pRSV-hPRA and pRSV-hPRB) as well as for ER (pRSV-rER). The transfected cells were treated with vehicle, E_2 (10^{-9} M), the progestin R5020 (10^{-8} M), the antiprogestin RU486 (10^{-8} M), or various combinations of the agents for 24 h. In the first set of experiments, the CAT reporter vectors contained either the distal promoter of the rat PR gene (PR_{Dist}) (18) or the promoter of the human pS2 gene (PS2) (12), both of which are normally regulated by sex steroid hormones in vivo. In addition, the reporters contained two consensus EREs and two consensus PREs located upstream of the promoters (ERE_2PRE_2-PR_{Dist}-CAT and ERE_2PRE_2-PS2-CAT) (Fig. 1 and 2A). Treatment of the uterine cells with E_2 resulted in a substantial increase in CAT activity (ca. 25- to 30-fold) from both reporters, regardless of which PR isoform was present (Fig. 2B and C). Treatment with R5020 alone resulted in low (ca. 2.5-fold for PR A alone and for PR A plus PR B) or modest (ca. 5-fold for PR B) induc-

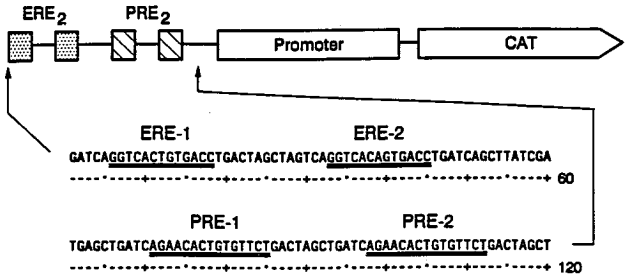

FIG. 1. Schematic diagram of the ERE_2PRE_2-Promoter-CAT reporters. The ERE_2PRE_2-Promoter-CAT reporters contain two consensus EREs and two consensus PREs linked upstream of a variety of promoters (i.e., the rat PR gene distal promoter, the human pS2 gene promoter, the rat PR gene proximal promoter, and the viral TK promoter) and the CAT gene. The nucleotide sequence of the ERE_2PRE_2 region is shown.

tions of CAT activity, whereas treatment with RU486 alone reduced the basal activity by ca. 50% in most cases (Fig. 2B and C).

Interestingly, estrogen-stimulated CAT activity in cells transfected with the PR A expression vector was repressed as much as 70% when the cells were cotreated with R5020 and as much as 85% when the cells were cotreated with RU486, depending on the promoter in the reporter (Fig. 2B and C). R5020-mediated repression was not observed for cells transfected with the PR B expression vector when the reporter contained the PR gene distal promoter (Fig. 2B) but was observed when the reporter contained the pS2 gene promoter (Fig. 2C). The greater inhibition observed with PR A was not due to greater expression of PR A; in fact, PR B was expressed from the expression vectors slightly better than PR A, as determined by immunoblotting (data not shown). RU486-mediated repression was observed with the cotransfected PR B expression vector in both promoter contexts (Fig. 2C). When both the PR A and PR B expression vectors were included in the transfections, the results mirrored those obtained when only the PR A expression vector was included (Fig. 2B and C), suggesting a dominant effect of PR A over PR B. When the cells were treated with both R5020 and RU486, the magnitude of repression of E_2-stimulated CAT activity was the same as when the cells were treated with RU486 alone (data not shown). The magnitude of repression of ER activity by liganded PR was the same when the positioning of the EREs and PREs in the reporter construct was switched (e.g., in PRE_2ERE_2-PR_{Dist}-CAT), indicating that the repression was not dependent on the orientation of the EREs and PREs (data not shown). Together, these results suggest that in uterine cells, a cell type in which coordinate interactions between the estrogen and progestin signaling pathways are normally observed, ligand-occupied PR A and PR B can act as potent repressors of ER-mediated transcriptional activity.

Reconstitution of PR-mediated repression of ER activity in a heterologous cell type. Since the primary cultures of rat uterine cells express ER and PR basally (at ca. 30 fmol/mg of protein and 40 fmol/mg of protein, respectively, as determined by radioligand binding assays), we wanted to examine the ability of PR to repress ER activity in a cell type that does not express these receptors, such as 3T3 mouse fibroblasts. The use of the 3T3 cells also allowed us to test the generality of the repression in a heterologous cell type (i.e., one in which interactions between the estrogen and progestin pathways are not normally observed). The cells were transfected with the CAT reporters containing the PR gene distal and pS2 gene promoters, as well as the ER and PR expression vectors, and were subsequently treated with the hormones and antihormones as described above for the uterine cells. The results from the 3T3 cell experiments mirrored those obtained from the rat uterine cell experiments (Fig. 3B and C; compare with Fig. 2B and C), except that in the 3T3 cells, little repression of E_2-stimulated CAT activity by R5020-occupied PR A was observed in the context of the PR gene distal promoter (Fig. 3B) and no repression by R5020-occupied PR B was observed in the con-

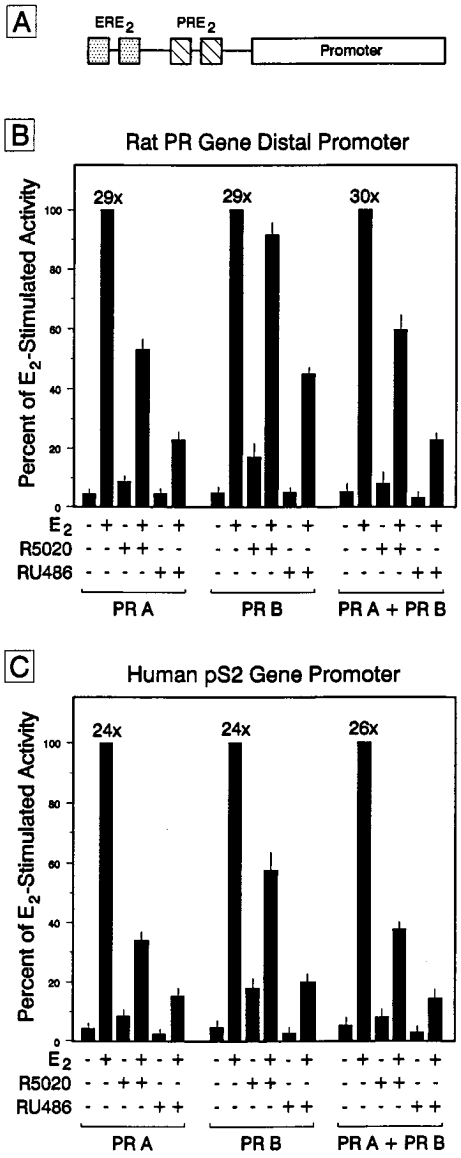

FIG. 2. Repression of ER-mediated transcriptional activity in uterine cells by ligand-occupied PRs. (A) A schematic diagram of the ERE_2PRE_2-Promoter-CAT reporter. (B and C) Each 100-mm-diameter dish of rat uterine cells was transfected with 500 ng of pRSV-hPRA (labeled PR A), 500 ng of pRSV-hPRB (PR B), or 250 ng each of pRSV-hPRA and pRSV-hPRB (PR A + PR B) in addition to 10 μg of ERE_2PRE_2-PR_{Dist}-CAT (B) or ERE_2PRE_2-PS2-CAT (C), 100 ng of pRSV-rER, and 3 μg of pCMVβ, as described in Materials and Methods. The cells were treated with one or more of the following as indicated for 24 h: control vehicle, E_2 (10^{-9} M), R5020 (10^{-8} M), and RU486 (10^{-8} M). The CAT activity in each sample was determined as described in Materials and Methods. Each bar represents the mean + the standard error of the mean for three or more separate determinations. The fold induction in response to E_2 treatment is indicated above the bars.

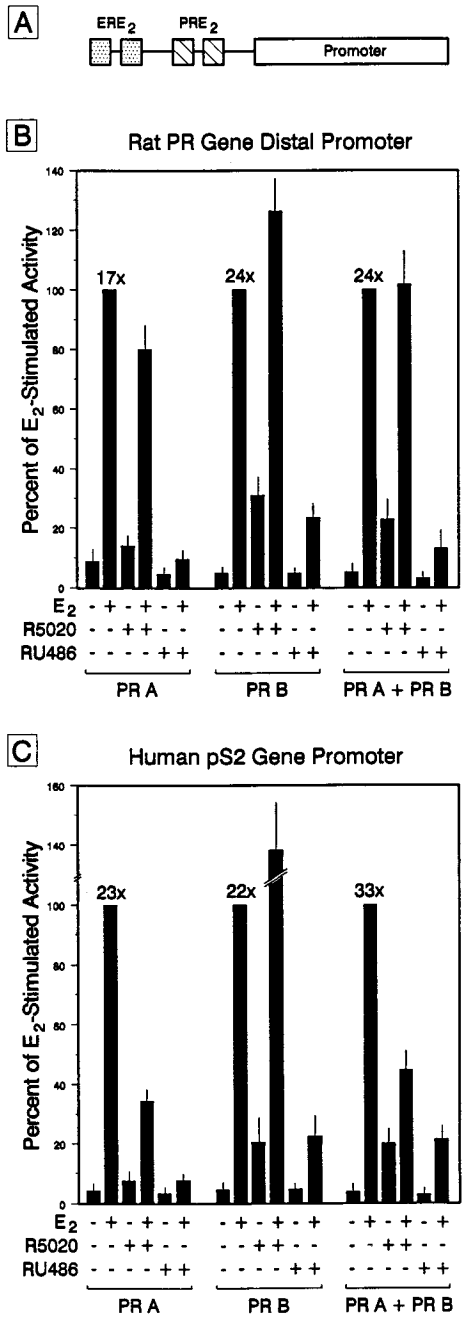

FIG. 3. Repression of ER-mediated transcriptional activity in 3T3 cells by ligand-occupied PRs. (A) A schematic diagram of the ERE_2PRE_2-Promoter-CAT reporter. (B and C) Each 100-mm-diameter dish of 3T3 cells was transfected with the same amounts of pRSV-hPRA (labeled PR A), pRSV-hPRB (PR B), pRSV-hPRA plus pRSV-hPRB (PR A + PR B), ERE_2PRE_2-PR_{Dist}-CAT or ERE_2PRE_2-PS2-CAT, and pRSV-rER as described for uterine cells in the legend to Fig. 2 as well as with 1 μg of pCMVβ. The cells were then treated with one or more of the following as indicated for 24 h: control vehicle, E_2 (10^{-9} M), R5020 (10^{-8} M), and RU486 (10^{-8} M). The CAT activity in each sample was determined as described in Materials and Methods. Each bar represents the mean + the standard error of the mean for three or more separate determinations. The fold induction in response to E_2 treatment is indicated above the bars.

text of the pS2 gene promoter (Fig. 3C). As in the uterine cells, PR A was a more effective repressor than PR B and RU486-occupied PR was a more effective repressor than R5020-occupied PR. Thus, the repression of ER-mediated transcriptional activity by ligand-occupied PR was also observed in a heterologous system.

Examination of dose-dependent effects of receptor, ligand, and target reporter levels on the repression of ER activity by PR. To assess the effects of altering the PR levels on the repression of ER-mediated transcriptional activity, uterine cells and 3T3 cells were transfected with the ERE_2PRE_2-PR_{Dist}-CAT and ERE_2PRE_2-PS2-CAT reporters, respectively, a fixed amount of ER expression vector, and increasing amounts of PR expression vector. In the absence of the PR expression vector, little or no repression of E_2-stimulated CAT activity by cotreatment with R5020 or RU486 was observed (Fig. 4A). With increasing amounts of PR expression vector, greater repression was observed, with maximal repression occurring in the presence of 250 ng or more of the PR expression vector (Fig. 4A). Approximately 0.40 and 0.15 fmol of receptor per mg of protein per 100 ng of expression vector were produced from the Rous sarcoma virus promoter-driven ER and PR expression vectors, respectively, as determined by radioligand binding assays of extracts from transfected 3T3 cells. The relative expression of ER and PR was confirmed by immunoblotting (data not shown). Thus, at the minimal PR expression vector amount required for maximal repression of ER activity (i.e., 250 ng), PR and ER were expressed at roughly equal levels.

To assess the ability of PR to repress ER-mediated transcriptional activity over a wide range of ER levels, uterine cells were transfected with the ERE_2PRE_2-PR_{Dist}-CAT reporter, a fixed amount of the PR A expression vector, and increasing amounts of the ER expression vector. The cells were then treated with E_2, a combination of E_2 and R5020, and a combination of E_2 and RU486. A 20-fold induction in CAT activity by E_2 was observed with endogenous ER alone, and transfection of increasing amounts of the ER expression vector resulted in increased E_2-stimulated CAT activity (ca. 35-fold induction maximum; Fig. 4B). Repression of E_2-stimulated CAT activity by cotreatment with R5020 or RU486 occurred at all ER levels, although the percent repression was greatest when the E_2 stimulation was the greatest (Fig. 4B).

We also examined the dose dependence for repression by R5020 and RU486. In this set of experiments, the uterine cells were transfected with the ERE_2PRE_2-PR_{Dist}-CAT reporter and a fixed amount of the ER and PR A expression vectors. The cells were treated with E_2 and increasing amounts of R5020 and RU486. As can be seen from Fig. 4C, dose-dependent effects were observed for both ligands. Similar dose responses were observed in the 3T3 cells with the ERE_2PRE_2-PS2-CAT reporter (data not shown). Finally, we examined the effect of altering the amount of target reporter plasmid used in the transfections. As can be seen from Fig. 4D, the amount of target DNA available to the receptors had no effect on the magnitude of the observed repression. Taken together, these results indicate that progestin- and antiprogestin-mediated repression of ER transcriptional activity requires the presence of PR and that it occurs over a wide range of PR, PR ligand, ER, and target DNA levels.

Evaluation of promoter and cell-type specificity for repression of ER activity by ligand-occupied PR. Our initial studies with the uterine cells and 3T3 cells suggested that the repressive effects of PR on ER transcriptional activity were promoter context specific (e.g., compare Fig. 2B with Fig. 2C and Fig. 3B with Fig. 3C) and, to some extent, cell type specific (e.g., compare R5020-occupied PR B in Fig. 2C and Fig. 3C). To examine this further, we used two additional reporter constructs containing either the rat PR gene proximal promoter (PR_{Prox}) (18) or the heterologous TK promoter. These report-

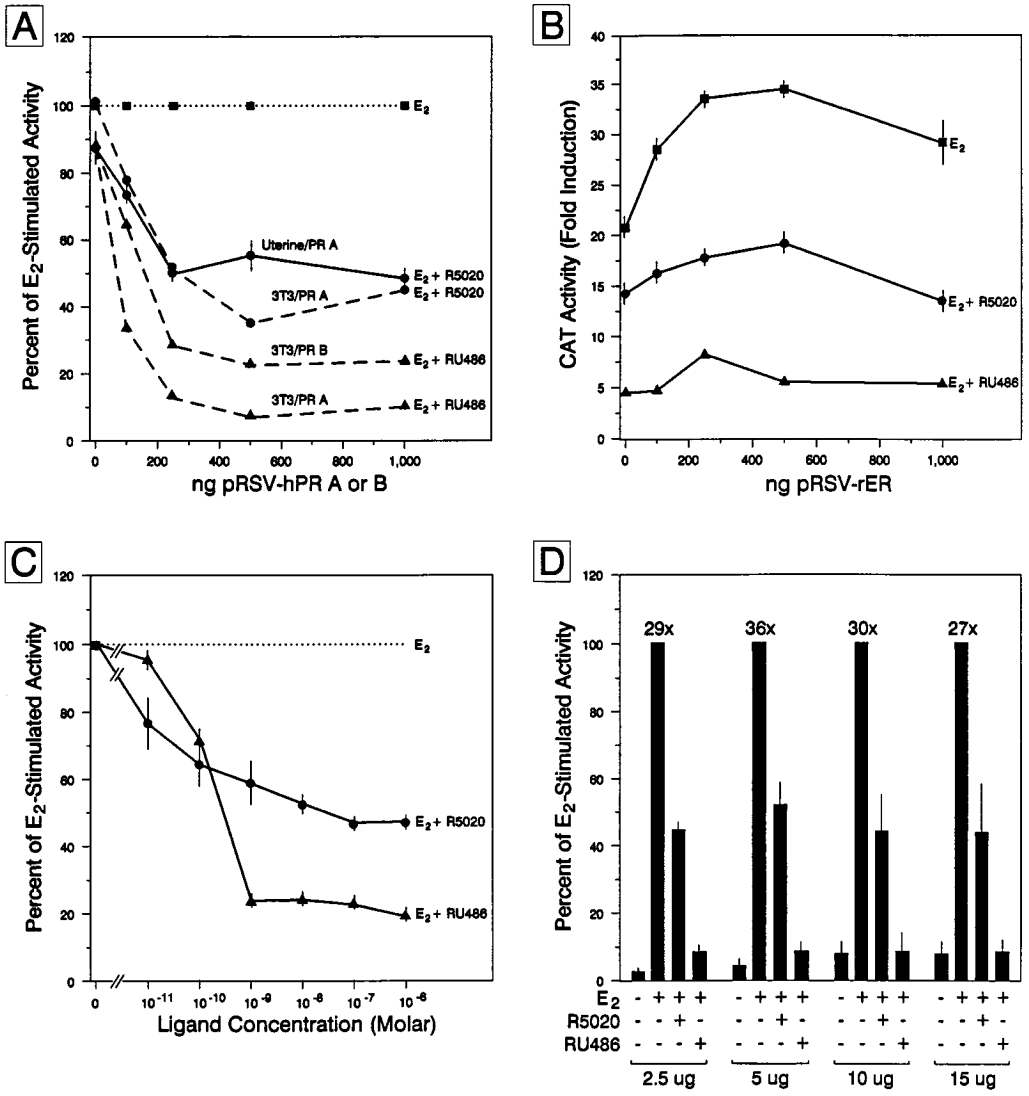

FIG. 4. Dose-dependent effects of receptor, ligand, and target reporter levels on the repression of ER-mediated transcriptional activity by ligand-occupied PR. (A) Rat uterine cells were transfected with the ERE_2PRE_2-PR_{Dist}-CAT reporter and pRSV-rER as described in the legend to Fig. 2 as well as with increasing amounts of pRSV-hPRA. 3T3 cells were transfected with the ERE_2PRE_2-PR_{Dist}-CAT reporter and pRSV-rER as described in the legend to Fig. 3 as well as with increasing amounts of pRSV-hPRA or pRSV-hPRB. The cells were subsequently treated with control vehicle, E_2 (10^{-9} M), R5020 (10^{-8} M), RU486 (10^{-8} M), and with combinations of the agents as indicated. Each line is marked with the cell type and the PR isoform used. (B) Uterine cells were transfected with the ERE_2PRE_2-PR_{Dist}-CAT reporter and pRSV-hPRA as described in the legend to Fig. 2 as well as with increasing amounts of pRSV-rER. They were then treated with control vehicle, E_2 (10^{-9} M), R5020 (10^{-8} M), RU486 (10^{-8} M), and with combinations of the agents as indicated. (C) Uterine cells were transfected with the ERE_2PRE_2-PR_{Dist}-CAT reporter, pRSV-rER, and pRSV-hPRA as described in the legend to Fig. 2. They were then treated with E_2 (10^{-9} M) plus vehicle or increasing amounts of R5020 and RU486 as indicated. (D) 3T3 cells were transfected with different amounts of the ERE_2PRE_2-PS2-CAT reporter, pRSV-rER, and pRSV-hPRA as described in the legend to Fig. 3, except that the total amount of DNA added per dish was 17 μg. The cells were subsequently treated with control vehicle, E_2 (10^{-9} M), R5020 (10^{-8} M), RU486 (10^{-8} M), and with combinations of the agents as indicated. The fold induction in response to E_2 treatment is indicated above the bars. For all panels, the treatment duration was 24 h. The CAT activity in each sample was determined as described in Materials and Methods. Each point represents the mean ± the standard error of the mean for three or more separate determinations or the mean from two separate determinations (no error bars).

ers were transfected into the uterine cells and 3T3 cells, and the ability of both R5020 and RU486 to repress E_2-stimulated CAT activity was assessed. The results are presented in Table 1. RU486 was a good repressor of E_2-stimulated CAT activity in the context of the PR gene proximal promoter and the TK promoter in both uterine cells and 3T3 cells. R5020, however, showed only moderate to no repressive ability with the same reporter constructs. The results presented in Fig. 2 and 3 and in Table 1 suggest that the extent of repression of ER-mediated transcriptional activity by agonist-occupied PR was strongly dependent on the promoter context and only somewhat dependent on the cell context. In contrast, repression by antagonist-occupied PR remained strong and was not affected by these parameters.

Assessment of the requirement for PR DNA binding and the effect of PR on activator binding to adjacent sites. To determine if the binding of PR to its response element was a requirement for the repression of ER-mediated transcriptional activity, we used a PR_{Dist}-CAT reporter construct containing two EREs upstream of the promoter but lacking PREs (ERE_2-PR_{Dist}-CAT) (Fig. 5A). This construct was cotransfected into the uterine cells with the PR A expression vector. The cells

TABLE 1. Analysis of cell type and promoter specificity for the repression of E_2-stimulated CAT activity by ligand-occupied PR A

Promoter[a]	Repression (%)[b]			
	Rat uterine cells		3T3 cells	
	R5020	RU486	R5020	RU486
PR_{Dist}	50 ± 3.8	81 ± 2.0	23 ± 9.7	99 ± 7.2
pS2	70 ± 1.0	86 ± 1.5	69 ± 4.1	96 ± 2.2
PR_{Prox}[c]	36 ± 6.3	89 ± 0.7	15 ± 8.3	87 ± 3.6
TK	25 ± 5.6	67 ± 5.6	None[d]	81 ± 11

[a] The promoters in the ERE_2PRE_2-Promoter-CAT reporters were PR_{Dist}, rat PR gene distal promoter; pS2, human pS2 gene promoter; PR_{Prox}, rat PR gene proximal promoter; and TK, herpes simplex virus TK promoter.

[b] Percent of E_2-stimulated CAT activity above the basal activity that was repressed by cotreatment with R5020 or RU486. E_2, 10^{-9} M; R5020 and RU486, 10^{-8} M. Each value represents the mean ± the standard error of the mean for three or more determinations.

[c] The PR_{Prox}-containing CAT reporter transfected into the rat uterine cells (ERE_1PRE_2-PR_{Prox}-CAT) contained only one consensus ERE upstream of the promoter since PR_{Prox} contains an endogenous ERE that is functional in these cells but is not functional in 3T3 cells.

[d] No repression was observed.

were then treated with vehicle, E_2, R5020, RU486, and combinations of the agents. Cotreatment with R5020 did not repress the E_2-stimulated CAT activity from the reporter without PREs; in fact, the CAT activity with E_2 and R5020 treatment was elevated (ca. 200% of the activity elicited by E_2 treatment alone) (Fig. 5A). This anomalous activity was dependent on the presence of the EREs, suggesting that the effect was not mediated via a cryptic PRE in the reporter (data not shown). RU486-occupied PR A and PR B, which had repressed E_2-stimulated CAT activity from ERE_2PRE_2-PR_{Dist}-CAT in the rat uterine cells, also failed to repress E_2-stimulated CAT activity from the reporter without PREs (Fig. 5A and data not shown). Similar results with reporters without PREs were obtained with the 3T3 cells. These results indicate that, in our model system, binding to PREs is required for repression of ER-mediated transcriptional activity by ligand-occupied PR.

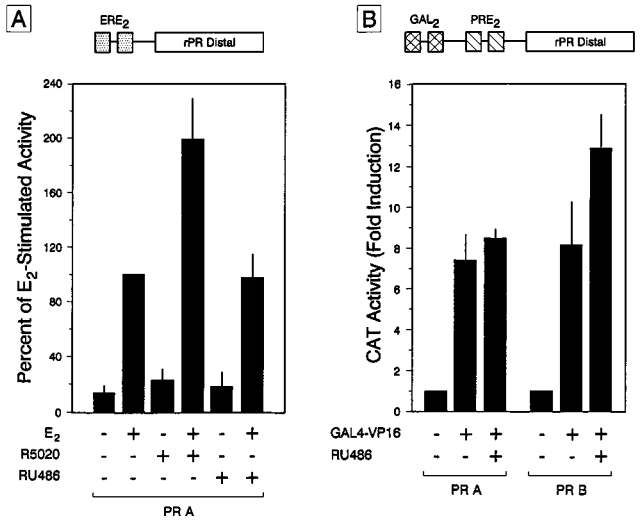

FIG. 5. Assessment of the requirement for PR DNA binding and the effect of PR on activator binding to adjacent sites. (A) Rat uterine cells were transfected with the ERE_2-PR_{Dist}-CAT reporter, which is illustrated schematically, as well as with pRSV-rER and pRSV-hPRA, as described in the legend to Fig. 2. The cells were then treated for 24 h with various combinations of control vehicle, E_2 (10^{-9} M), R5020 (10^{-8} M), and RU486 (10^{-8} M) as indicated. (B) 3T3 cells were transfected as described in the legend to Fig. 3 with the GAL_2PRE_2-PR_{Dist}-CAT reporter, which is illustrated schematically, as well as with 500 ng of pRSV-hPRA or pRSV-hPRB and 500 ng of GAL-VP16 as indicated. The cells were subsequently treated for 24 h with control vehicle or RU486 (10^{-8} M) as indicated. For both panels, the CAT activity in each sample was determined as described in Materials and Methods. Each bar represents the mean + the standard error of the mean for three or more separate determinations.

To determine if the binding of PR to the PREs in the reporter constructs would interfere with the binding of other activators to adjacent response elements, we assessed the effect of PR on the ability of a heterologous activator to bind to adjacent sites in a PRE-containing reporter. The reporter construct, which contained two binding sites for the *Saccharomyces cerevisiae* activator GAL4 adjacent to two PREs (GAL_2PRE_2-PR_{Dist}-CAT) (Fig. 5B) positioned similarly to the elements in the ERE_2PRE_2-Promoter-CAT reporters, was transfected into 3T3 cells with expression vectors for PR A or PR B in the presence or absence of the constitutive hybrid activator GAL-VP16 (28). As can be seen from Fig. 5B, the activity of GAL-VP16 was not inhibited by RU486-occupied PR A or PR B, both of which acted as effective repressors of ER activity under the same promoter context in previous experiments. R5020-occupied PR A and PR B synergized strongly with GAL-VP16 (data not shown). These results suggest that liganded PR does not prevent the access of other activators, like GAL-VP16 and ER, to adjacent response elements in the reporter constructs by steric hindrance.

Examination of the effects of PRE positioning relative to the EREs and promoter on the repression of ER activity by liganded PR. To determine the effects of spacing the PREs in the reporter construct at a distance from the EREs and the promoter, we constructed the PRE_2(Dist)ERE_2-PS2-CAT reporter in which the PREs were placed distally, greater than 2 kb away (Fig. 6). This allowed us to further examine the effect of the relative positioning of the EREs and PREs, as well as interactions between the ER and PR binding sites, on the repression of ER activity by PR. This reporter construct was cotransfected into the 3T3 cells with the PR A expression vector as described above for the other reporters. The cells were then treated with vehicle, E_2, R5020, RU486, and combinations of the agents. ERE_2PRE_2-PS2-CAT was also examined under identical conditions for comparison. As can be seen from Fig. 6, repression by both R5020- and RU486-occupied PR A was observed when the PREs were located distally, although the magnitude of the response was somewhat reduced relative to that with ERE_2PRE_2-PS2-CAT. These results indicate that the repression of ER activity by liganded PR is, for the most part, independent of the location of the PREs.

Examination of the ability of liganded PR to repress the activity of ER mutants. To localize the region(s) of ER that renders it susceptible to repression by liganded PR, we examined the ability of PR to repress the activity of a number of ER mutants. The results obtained with two mutants, an N-terminal deletion, ER(109-595), and a C-terminal deletion, ER(1-554), are presented in Fig. 7. pCMV-based expression vectors for wild-type and mutant human ERs were transfected with the PR A expression vector and the ERE_2PRE_2-PS2-CAT reporter into 3T3 cells. We then assayed the ability of R5020 and RU486 to repress E_2-stimulated CAT activity. E_2-stimulated activity was greatest with the wild-type ER, yielding an 80-fold induction at the largest amount of ER expression vector used; the activities of ER(109-595) and ER(1-554) were ca. 30 and 75% of wild-type activity under the same conditions (data not shown).

Both R5020- and RU486-occupied PR A were good repressors of wild-type ER activity over a wide range of ER levels

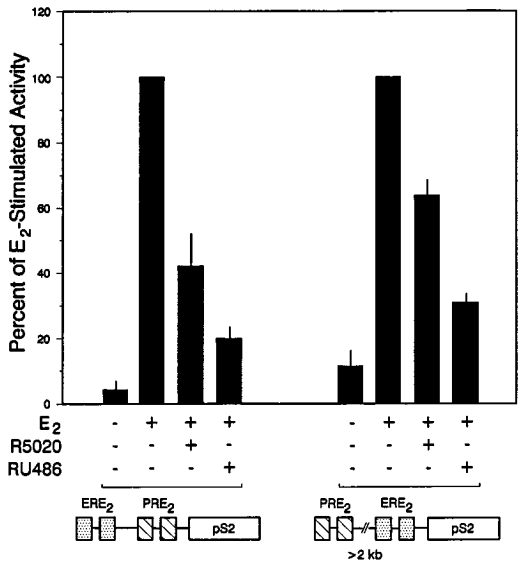

FIG. 6. The effects of PRE positioning relative to the EREs and promoter on the repression of ER-mediated transcriptional activity by ligand-occupied PR. 3T3 cells were transfected as described in the legend to Fig. 3 with the ERE_2PRE_2-PS2-CAT and PRE_2(Dist)ERE_2-PS2-CAT reporters, which are illustrated schematically, as well as with 300 ng of pCMV-rER and 500 ng of pRSV-hPRA. The cells were then treated for 24 h with various combinations of control vehicle, E_2 (10^{-9} M), R5020 (10^{-8} M), and RU486 (10^{-8} M) as indicated. The CAT activity in each sample was determined as described in Materials and Methods. Each bar represents the mean + the standard error of the mean for three separate determinations or the mean + range for two separate determinations.

(Fig. 7B), as noted earlier (Fig. 4B). The C-terminal deletion mutant, ER(1-554), was also effectively repressed by R5020- and RU486-occupied PR A (Fig. 7C). Interestingly, although the activity of the N-terminal deletion mutant, ER(109-595), was repressed by RU486-occupied PR A, it was not repressed by R5020-occupied PR A (Fig. 7C). These results indicate that the N-terminal region of ER is essential for repression by R5020-occupied PR but not for repression by RU486-occupied PR. In addition, these results suggest that agonist- and antagonist-occupied PRs may differentially target ER in the repression of its transcriptional activity.

DISCUSSION

Both PR A and PR B can act as ligand-dependent repressors of ER-mediated transcriptional activity. Our studies demonstrate that both PR A and PR B can act as effective ligand-dependent repressors of ER-mediated transcriptional activity. As our results show, the repression of E_2-stimulated CAT activity from ERE- and PRE-containing reporter vectors by liganded PR was as much as 99%, depending on a number of parameters. The magnitude of the repression varied with the PR isoform (i.e., PR A or PR B), ligand type (i.e., agonist or antagonist), PR levels, and ligand concentration but was largely unaffected by the ER levels. The promoter context was important in determining both the magnitude and PR isoform specificity of the repression for agonist-occupied PR but not for antagonist-occupied PR. The magnitude of the repression by agonist-occupied PR was also affected, to some extent, by the cell context.

Under the conditions examined, ligand-occupied PR A was a stronger repressor of ER-mediated transcriptional activity than was ligand-occupied PR B. This difference must be, at least in part, a consequence of the N-terminal extension of the PR B isoform, which has been shown previously to contribute to the different activities observed for the two PR isoforms (13, 36, 41) and to contain a distinct transactivation function (31). Since all of PR A is contained in PR B, the N-terminal extension must act to attenuate the repressive activities of the B isoform of the receptor. PR A was generally dominant to PR B in that coexpression of the two isoforms in cases in which their individual repressive activities were not the same resulted in activity similar to that observed with PR A alone. Antagonist-occupied PR was always a more effective repressor of E_2-stimulated CAT activity than agonist-occupied PR. This was exemplified by the fact that repression by antagonist-occupied PR B was observed under all conditions, whereas repression by agonist-occupied PR B was generally not observed. Previous studies have shown that the conformation of the ligand binding domain of agonist-occupied PR differs from that of antagonist-occupied PR (1, 40). These conformational differences could underlie the differences in the magnitudes of repression achieved with agonist- and antagonist-occupied PRs. The characteristics of the repressive actions of R5020- and RU486-occupied PRs are summarized in Table 2.

Mechanism of repression of ER-mediated transcriptional activity by ligand-occupied PR. In addition to the various parameters affecting the magnitude of the repression that were outlined above, our studies have elucidated a number of mechanistic aspects of the repression of ER-mediated transcriptional activity by ligand-occupied PR (Table 2). First, there is an absolute requirement for PR to be liganded, either with an agonist or antagonist. Second, our results indicate that the binding of PR to its response element is required. This binding does not block access to adjacent activator binding sites. Third, the repressive response is maintained when the PREs are positioned distal to the EREs and the promoter. Lastly, repression by agonist-occupied PR, but not by antagonist-occupied PR, requires an intact N-terminal region of ER.

At least four mechanisms for the repression of transcription have been characterized or proposed: competitive repression, direct repression, squelching, and quenching (reviewed in reference 20). Competitive repression occurs when the access of a transcription factor to its binding site is blocked by a repressor protein, which binds to an overlapping site. Direct repression occurs when a repressor protein binds to a defined site in the promoter region and interferes with the formation or activity of the general transcription complex. Squelching results from the overexpression of a transcription factor which sequesters others factors necessary for the activity of an activator. It does not require specific DNA binding or an intact DNA binding domain. Quenching occurs when a repressor protein binds to a site distinct from an activator binding site and prevents the activator, which remains bound to its site, from making proper contact with the transcriptional complex.

Our data support a model in which the repression of ER transcriptional activity by liganded PR occurs by quenching, not by competitive repression, direct repression, or squelching. For example, liganded PR does not appear to interfere with the binding of ER to its response element by competition for the same site or by steric hindrance from an adjacent site, given (i) the requirement for the PREs in the reporter, (ii) the fact that activation by the GAL-VP16 activator via GAL4 binding sites located in the same position as the EREs was not repressed, (iii) the fact that the repression was maintained when the PREs were placed distal to the EREs and the promoter, and (iv) evidence suggesting that ER and PR facilitate each other's binding to their respective elements when the elements are adjacent (35). These observations argue against competi-

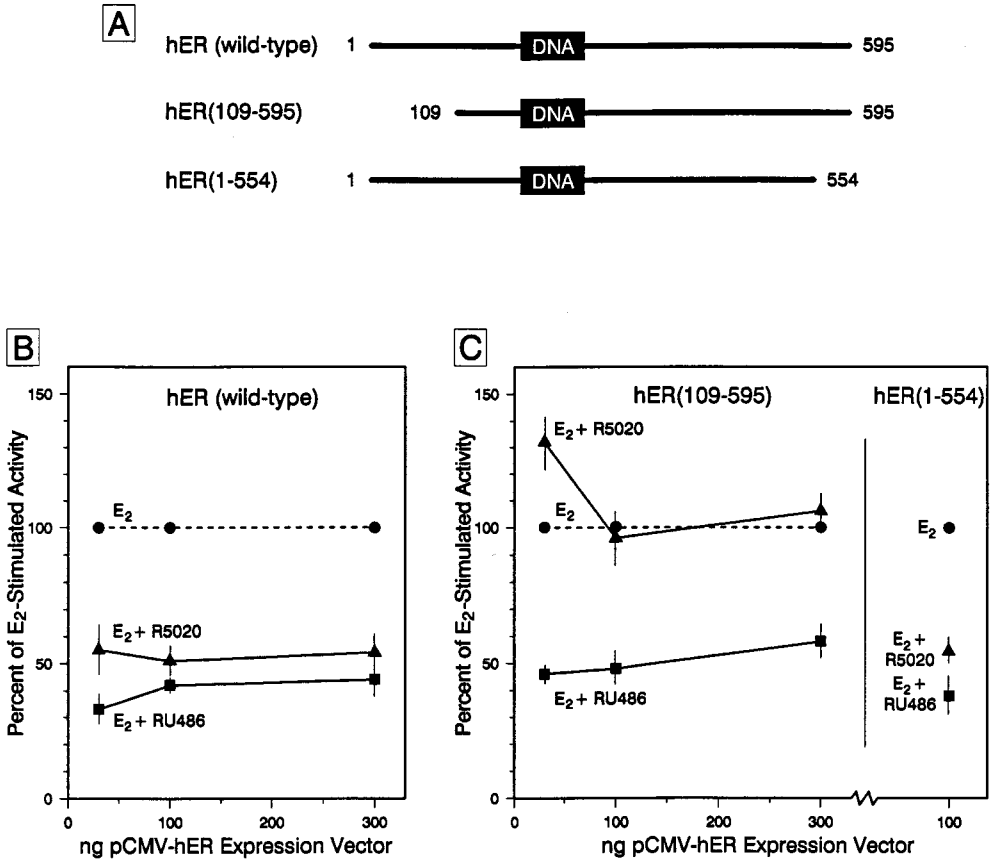

FIG. 7. Repression of the transcriptional activity of ER mutants by ligand-occupied PR. (A) Schematic diagrams of wild-type ER and the N- and C-terminal deletion mutants. The DNA binding domain is shown as a solid box labeled "DNA." (B and C) Each 100-mm-diameter dish of 3T3 cells was transfected with 10 μg of ERE_2PRE_2-PS2-CAT, 500 ng of pRSV-hPRA, and 30 to 300 ng of the pCMV-hER expression vectors as well as 1.6 μg of pCMVβ. The cells were then treated with one or more of the following as indicated for 24 h: control vehicle, E_2 (10^{-9} M), R5020 (10^{-8} M), and RU486 (10^{-8} M). The CAT activity in each sample was determined as described in Materials and Methods. Each point represents the mean ± the standard error of the mean for three or more separate determinations.

tive repression. Our results do not support direct repression as the mechanism either. The repressive effect was not general; it showed specificity for ER, while the activity of GAL-VP16 was unaffected, suggesting that the general transcription complex itself was not the target. Additionally, the requirement for PR to be liganded and bound to its response element and the fact that the repression was observed at relatively low receptor expression levels eliminate squelching by PR overexpression as the mechanism of the repression. Finally, the observed repression was not attributable to a reduction in the levels of ER, since the ER levels did not decrease in response to the progestin and antiprogestin treatments in our experiments (data not shown).

TABLE 2. Response characteristics and mechanistic aspects of repression of ER activity by agonist- and antagonist-occupied PRs

Parameter	Result	
	PR + R5020	PR + RU486
Response characteristics		
Level of repression	Moderate	Strong
PR isoform specific	Yes	No
Promoter specific	Yes	No
Cell type specific	Yes	No
PR level dependent	Yes	Yes
ER level dependent	No	No
PR ligand dose dependent	Yes	Yes
Target DNA level dependent	No	No
Mechanistic aspects		
PREs required	Yes	Yes
Access to adjacent sites blocked	No	No
Repression with distal PREs	Yes	Yes
N terminus of ER required	Yes	No

According to the quenching model for repression, which is consistent with our findings, PR bound at the PREs in the estrogen-responsive reporters targets the ER either directly or indirectly for repression by interfering with its ability to productively interact with the preinitiation complex (Fig. 8). That ER or an ER-associated component of the estrogen signaling pathway and not the basal transcriptional machinery is the target for repression is supported by the fact that repression by PR shows at least some level of specificity for a particular subset of transactivators. It is also supported by the fact that mutations in the ER can render it insensitive to repression by PR.

The quenching may occur by (i) direct or indirect inhibitory interaction of PR with ER, (ii) recruitment of an inhibitory factor, or (iii) inactivation of a component of the ER signaling pathway, such as a transcriptional intermediary factor. Any of these modes of action could allow liganded PR to interfere

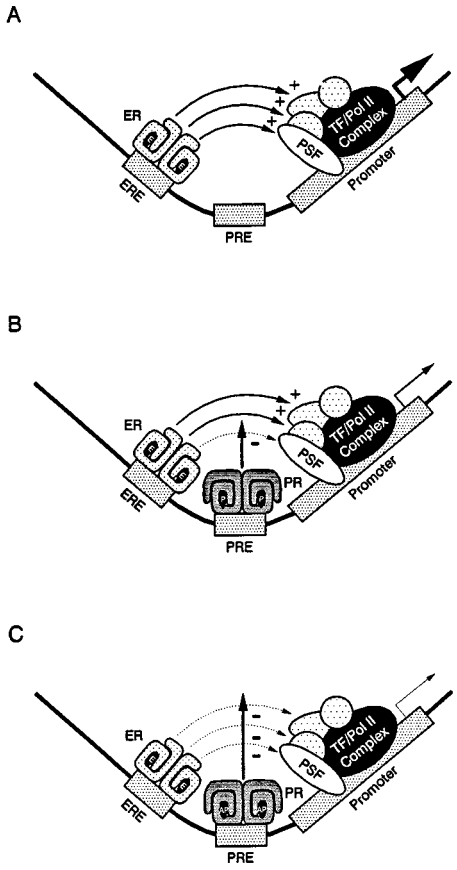

FIG. 8. A model for the repression of ER-mediated transcriptional activity by agonist- and antagonist-occupied PRs. Our findings support a model in which the repression of ER transcriptional activity by liganded PR occurs by quenching. According to this model, liganded PR binds to a site (PRE) distinct from the binding site for ER (ERE) and interferes with the ability of ER to make productive contact with the transcriptional complex. Differences in the magnitude of repression observed for agonist- and antagonist-occupied PRs suggest that agonist-occupied PR only quenches ER-transcription factor interactions that involve the AF-1 of ER or a promoter-specific component of the ER signaling pathway (PSF) while antagonist-occupied PR quenches a wider range of the ER-transcription factor interactions that occur at the promoter. (A) Stimulation of transcription by liganded ER. (B) Repression by agonist-occupied PR. (C) Repression by antagonist-occupied PR. AP, antiprogestin; E, estrogen; ER, estrogen receptor; ERE, estrogen response element; P, progestin; PR, progestin receptor; PRE, progestin response element; PSF, promoter-specific factor; TF/Pol II Complex, general transcriptional machinery.

with the ability of ER to productively interact with the transcription complex. Furthermore, the mode(s) of action that agonist-occupied PR uses to inhibit ER activity may be distinct from those used by antagonist-occupied PR (see below). The fact that the repression is dependent on the absolute levels of PR but is independent of the absolute levels of ER and the ER/PR ratio suggests that it does not involve competition between ER and PR for the same contact sites on the transcriptional machinery. If this were the case, increasing the absolute levels of ER should relieve the repression, an effect that was not observed in our experiments. It is important to note that in our model system, PR was only weakly active as a transcriptional activator. The failure of liganded PR to act as a strong transcriptional activator in certain promoter contexts is most likely an essential part of its ability to act as a repressor of ER activity.

Generality of transcriptional repression by ligand-occupied PR. The generality of transcriptional repression by ligand-occupied PR is an important consideration in light of the therapeutic use of both progestins and antiprogestins. As mentioned above, our results indicate that repression by agonist-occupied PR is strongly dependent on the promoter context and, to a lesser extent, the cell type. Most of the studies presented herein were performed with promoters from genes that are normally regulated by estrogens and progestins in vivo. In addition, we used untransformed (i.e., primary) uterine cells, a cell type in which the reciprocal interactions between the estrogen and progestin receptor systems are normally observed. The fact that we were able to reconstitute these effects in a heterologous cell type (i.e., 3T3 cells) and, to some extent, with a heterologous promoter (i.e., the TK promoter) suggests that PR-mediated repression may be an important modulator of ER activity in other cell types and with other estrogen-regulated promoters.

We have also tested the generality of PR-mediated transcriptional repression with a heterologous transcriptional activator. Our results indicate that liganded PR not only fails to repress but in some cases actually synergistically activates transcription by the GAL-VP16 activator in the same promoter and cell contexts and under the same experimental conditions in which repression of ER-mediated transcriptional activity is observed. Studies by others have shown that PR can interact synergistically with many different types of transcription factors (34). Moreover, synergistic transcriptional stimulation by interaction between ER and PR has been demonstrated under some cell and promoter contexts with reporter constructs containing adjacent EREs and PREs (4, 6, 35). Recent studies have shown repressive effects of PR with other, but not all, members of the steroid hormone receptor family (22, 23, 39, 41). Thus, it seems likely that factors such as the PR isoform, transactivator type, promoter context, and cell type determine whether the actions of PR are negative or positive.

Our findings are consistent with those of other reports demonstrating that liganded PR can have potent inhibitory effects on other transcriptional activators (22, 23, 39, 41). Our results do differ from some of the results of these studies in that the repression in our system was not limited to PR A but was observed with both PR isoforms. In addition, we observed that the repression required the binding of PR to its response element. While the reasons for these differences are not clear, these findings consistently point to an important role for PR as a transcriptional repressor.

Agonist- and antagonist-occupied PRs differentially target ER when repressing its transcriptional activity. Four lines of evidence suggest that agonist- and antagonist-occupied PRs act to repress ER activity through distinct mechanisms, most likely by differentially targeting the ER or a component of the ER signaling pathway. First, the magnitude of the repression was typically greater when the PR was complexed with an antagonist. Previous studies have shown that the conformation of the ligand binding domain of agonist-occupied PR is different from that of antagonist-occupied PR (1, 40). These structural differences may underlie the differences observed for repression by agonist- and antagonist-occupied PR. Second, repression by antagonist-occupied PR was observed under conditions in which agonist-occupied PR was unable to act as a repressor. This was exemplified by the fact that repression by antagonist-occupied PR was observed with both PR A and PR B, whereas repression by agonist-occupied PR was generally specific for PR A. Third, repression by agonist-occupied PR exhibited strong promoter specificity and, to some extent, cell type specificity, whereas repression by antagonist-occupied PR was, for the most part, independent of these variables. Lastly, the ER(109-595) mutant, which lacks a substantial portion of the

N-terminal region, was insensitive to repression by agonist-occupied PR, yet it remained sensitive to repression by antagonist-occupied PR. This suggests that for repression of ER activity, the direct or indirect target of agonist-occupied PR was the N-terminal region of ER, which contains the ligand-independent activation function of the receptor (AF-1) (9, 37). The fact that ER(109-595) remained sensitive to repression by antagonist-occupied PR suggests that it was differentially targeted by this PR-ligand complex. Table 2 lists the different characteristics of the repression for PR occupied with both types of ligands.

The differences that were outlined above for the repression of ER activity by agonist- and antagonist-occupied PRs suggest that these two distinct PR-ligand complexes differentially target ER or a component of the ER signaling pathway when repressing ER activity. It is likely that agonist-occupied PR only quenches a subset of the ER-transcription factor interactions that occur at the promoter, namely those involving AF-1 or a promoter-specific component of the ER signaling pathway (Fig. 8). This would explain the dependence on an intact N-terminal region of ER, as well as the promoter specificity, for the repression of ER activity by agonist-occupied PR. Antagonist-occupied PR, on the other hand, must be capable of quenching a wider range of the ER-transcription factor interactions that occur at the promoter (Fig. 8).

Implications for the coordinate regulation of gene expression by estrogens and progestins in vivo. Estrogens and progestins act in a wide variety of tissues and, in many cases, have opposing actions. For example, estrogens stimulate a wide variety of uterotrophic effects, whereas progestins are generally antagonistic to these actions of estrogens. This interplay between the estrogen and progestin signaling systems is also observed at the level of gene expression. For example, the expression of both c-*fos* and PR mRNAs are increased by estrogens and decreased by progestins in the uterus (14, 16, 21). While the general mechanisms for transcriptional stimulation by estrogens and progestins are well understood, the mechanism(s) whereby progestins antagonize estrogen actions has not been as clearly elucidated. No negative PRE, like the previously identified negative glucocorticoid response element of the prolactin gene (29), through which PR could exert an inhibitory effect has been identified. In contrast, positive PRE-like sequences have been identified in genes for which estrogen-stimulated expression of the genes is inhibited by progestins. For example, both the rat and rabbit PR genes have defined EREs, as well as a number of PRE-like sequences, in their regulatory regions (17, 19, 32). By acting at one or more PREs in an estrogen-regulated gene through the mechanisms described herein, liganded PR may be able to antagonize the estrogen-stimulated expression of the gene. The results obtained with our model system, which are consistent with the known biological actions of estrogen and progestins, suggest that repression of ER-mediated transcriptional activity by liganded PR at the gene is an important process in the interplay between the estrogen and progestin signaling systems.

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REFERENCES

1. **Allan, G. F., X. Len, S. Y. Tsai, N. L. Weigel, D. P. Edwards, M.-J. Tsai, and B. W. O'Malley.** 1992. Hormone and antihormone induce distinct conformational changes which are central to steroid receptor activation. J. Biol. Chem. **267:**19513–19520.
2. **Andersson, S., D. N. Davis, H. Dahlback, H. Jornvall, and D. W. Russell.** 1988. Cloning, structure, and expression of the mitochondrial P-450 sterol 26-hydroxylase, a bile biosynthetic enzyme. J. Biol. Chem. **264:**8222–8229.
3. **Aronica, S. M., and B. S. Katzenellenbogen.** 1991. Progesterone receptor regulation in uterine cells: stimulation by estrogen, cyclic adenosine 3′,5′-monophosphate, and insulin-like growth factor I and suppression by antiestrogens and protein kinase inhibitors. Endocrinology **128:**2045–2052.
4. **Bradshaw, M. S., S. Y. Tsai, X. Leng, A. D. W. Dobson, O. M. Conneely, B. W. O'Malley, and M.-J. Tsai.** 1991. Studies on the mechanism of functional cooperativity between progesterone and estrogen receptors. J. Biol. Chem. **266:**16684–16690.
5. **Carson-Jurica, M. A., W. T. Schrader, and B. W. O'Malley.** 1990. Steroid receptor family: structure and functions. Endocr. Rev. **11:**210–220.
6. **Cato, A. C. B., E. Heitlinger, H. Ponta, L. Klein-Hitpass, G. U. Ryffel, A. Bailly, C. Rauch, and E. Milgrom.** 1988. Estrogen and progesterone receptor-binding sites on the chicken vitellogenin II gene: synergism of steroid hormone action. Mol. Cell. Biol. **8:**5323–5330.
7. **Chen, C., and H. Okayama.** 1987. High-efficiency transformation of mammalian cells by plasmid DNA. Mol. Cell. Biol. **7:**2745–2752.
8. **Evans, R. M.** 1988. The steroid and thyroid hormone receptor superfamily. Science **240:**889–895.
9. **Gronemeyer, H.** 1991. Transcription activation by estrogen and progesterone receptors. Annu. Rev. Genet. **25:**89–123.
10. **Gronemeyer, H., M.-E. Meyer, M.-T. Bocquel, P. Kastner, B. Turcotte, and P. Chambon.** 1991. Progestin receptors: isoforms and antihormone action. J. Steroid Biochem. Mol. Biol. **40:**271–278.
11. **Ilenchuck, T. T., and M. R. Walters.** 1987. Rat uterine progesterone receptor analyzed by [^{3}H]R5020 photoaffinity labeling: evidence that the A and B subunits are not equimolar. Endocrinology **120:**1449–1456.
12. **Jeltsch, J. M., M. Roberts, C. Schatz, J. M. Garnier, A. M. C. Brown, and P. Chambon.** 1987. Structure of the human oestrogen-responsive gene pS2. Nucleic Acids Res. **15:**1401–1414.
13. **Kastner, P., A. Krust, B. Turcotte, U. Stropp, L. Tora, H. Gronemeyer, and P. Chambon.** 1990. Two distinct estrogen-regulated promoters generate transcripts encoding two functionally different human progesterone receptor forms A and B. EMBO J. **9:**1603–1614.
14. **Kirkland, J. L., L. Murthy, and G. M. Stancel.** 1992. Progesterone inhibits the estrogen-induced expression of c-*fos* messenger ribonucleic acid in the uterus. Endocrinology **130:**3223–3230.
15. **Koike, S., M. Sakai, and M. Muramatsu.** 1987. Molecular cloning and characterization of rat estrogen receptor cDNA. Nucleic Acids Res. **15:**2499–2513.
16. **Kraus, W. L., and B. S. Katzenellenbogen.** 1993. Regulation of progesterone receptor gene expression and growth in the rat uterus: modulation of estrogen action by progesterone and sex steroid hormone antagonists. Endocrinology **132:**2371–2379.
17. **Kraus, W. L., and B. S. Katzenellenbogen.** Unpublished data.
18. **Kraus, W. L., M. M. Montano, and B. S. Katzenellenbogen.** 1993. Cloning of the rat progesterone receptor 5′-region and identification of two functionally distinct promoters. Mol. Endocrinol. **7:**1603–1616.
19. **Kraus, W. L., M. M. Montano, and B. S. Katzenellenbogen.** 1994. Identification of multiple, widely-spaced estrogen-responsive regions in the rat progesterone receptor gene. Mol. Endocrinol. **8:**952–969.

19a. **Kraus, W. L., K. E. Weis, and B. S. Katzenellenbogen.** 1994. Repression of estrogen receptor-mediated transcriptional activity by ligand-occupied progesterone receptors. Endocrinology **134**(Suppl.)**:**323. (Abstract 492.)

20. **Levine, M., and J. L. Manley.** 1989. Transcriptional repression of eukaryotic promoters. Cell **59:**405–408.
21. **Loose-Mitchell, D. S., C. Chiappetta, and G. M. Stancel.** 1988. Estrogen regulation of c-*fos* messenger ribonucleic acid. Mol. Endocrinol. **2:**946–951.
22. **McDonnell, D. P., and M. E. Goldman.** 1994. RU486 exerts antiestrogenic activities through a novel progesterone receptor A form-mediated mechanism. J. Biol. Chem. **269:**11945–11949.
23. **McDonnell, D. P., M. M. Shahbaz, E. Vegeto, and M. E. Goldman.** 1994. The human progesterone receptor A-form functions as a transcriptional modulator of mineralocorticoid receptor transcriptional activity. J. Steroid Biochem. Mol. Biol. **48:**425–432.
24. **Mead, D. A., E. Szczesna-Skorupa, and B. Kemper.** 1986. Single-stranded DNA 'blue' T7 promoter plasmids: a versatile tandem promoter system for cloning and protein engineering. Protein Eng. **1:**67–74.
25. **Pakdel, F., P. Le Goff, and B. S. Katzenellenbogen.** 1993. An assessment of the role of domain F and PEST sequences in estrogen receptor half-life and

bioactivity. J. Steroid Biochem. Mol. Biol. **46:**663–672.
26. **Reese, J. C., and B. S. Katzenellenbogen.** 1991. Differential DNA-binding abilities of estrogen receptor occupied with two classes of antiestrogens: studies using human estrogen receptor overexpressed in mammalian cells. Nucleic Acids Res. **19:**6595–6602.
27. **Reese, J. C., and B. S. Katzenellenbogen.** 1991. Mutagenesis of cysteines in the hormone binding domain of the human estrogen receptor: alterations in binding and transcriptional activation by covalently and reversibly attaching ligands. J. Biol. Chem. **266:**10880–10887.
28. **Sadowski, I., J. Ma, S. Triezenberg, and M. Ptashne.** 1988. GAL4-VP16 is an unusually potent transcriptional activator. Nature (London) **335:**563–564.
29. **Sakai, D. D., S. Helms, J. Carlstedt-Duke, J. A. Gustafsson, F. M. Rottman, and K. R. Yamamoto.** 1988. Hormone-mediated repression of transcription: a negative glucocorticoid response element from the bovine prolactin gene. Genes Dev. **2:**1144–1154.
30. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Sartorius, C. A., M. Y. Melville, A. Rudie Hovland, L. Tung, G. S. Takimoto, and K. B. Horowitz.** 1994. A third transactivation function (AF3) of human progesterone receptors located in the unique N-terminal segment of the B-isoform. Mol. Endocrinol. **8:**1347–1360.
32. **Savouret, J. F., A. Bailly, M. Misrahi, C. Rauch, G. Redeuilh, A. Chauchereau, and E. Milgrom.** 1991. Characterization of the hormone responsive element involved in the regulation of the progesterone receptor gene. EMBO J. **10:**1875–1883.
33. **Schneider, W., C. Ramachandran, P. G. Satyaswaroop, and G. Shyamala.** 1991. Murine progesterone receptor exists predominantly as the 83-kilodalton "A" form. J. Steroid Biochem. Mol. Biol. **38:**285–291.
34. **Schule, R., M. Muller, C. Kaltschmidt, and R. Renkawitz.** 1988. Many transcription factors interact synergistically with steroid receptors. Science **242:**1418–1420.
35. **Slater, E. P., G. Redeuilh, and M. Beato.** 1991. Hormonal regulation of vitellogenin genes: an estrogen-responsive element in the *Xenopus* A2 gene and a multihormonal regulatory region in the chicken II gene. Mol. Endocrinol. **5:**386–396.
36. **Tora, L., H. Gronemeyer, B. Turcotte, M.-P. Gaub, and P. Chambon.** 1988. The N-terminal region of the chicken progesterone receptor specifies target gene activation. Nature (London) **333:**185–188.
37. **Tora, L., J. White, C. Brou, D. Tasset, N. Webster, E. Scheer, and P. Chambon.** 1989. The human estrogen receptor has two independent nonacidic transcriptional activation functions. Cell **59:**477–487.
38. **Truss, M., and M. Beato.** 1993. Steroid hormone receptors: interaction with deoxyribonucleic acid and transcription factors. Endocr. Rev. **14:**459–479.
39. **Tung, L. T., M. K. Mohamed, J. P. Hoeffler, G. S. Takimoto, and K. B. Horwitz.** 1993. Antagonist-occupied human progesterone B-receptors activate transcription without binding to progesterone response elements and are dominantly inhibited by A-receptors. Mol. Endocrinol. **7:**1256–1265.
40. **Vegeto, E., G. F. Allan, W. T. Schrader, M.-J. Tsai, D. P. McDonnell, and B. W. O'Malley.** 1992. The mechanism of RU486 antagonism is dependent on the conformation of the carboxy-terminal tail of the human progesterone receptor. Cell **69:**703–713.
41. **Vegeto, E., M. M. Shahbaz, D. X. Wen, M. E. Goldman, B. W. O'Malley, and D. P. McDonnell.** 1993. Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. Mol. Endocrinol. **7:**1244–1255.