Mapping and Characterization of the Functional Domains Responsible for the Differential Activity of the A and B Isoforms of the Human Progesterone Receptor*

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Paloma H. Giangrande, Giuseppe Pollio, and Donald P. McDonnell‡

From the Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina 27710

In humans, the biological response to progesterone is mediated by two distinct forms of the progesterone receptor (human (h) PR-A, 94 kDa and hPR-B, 114 kDa). These two isoforms are transcribed from distinct estrogen-inducible promoters within a single copy PR gene; the only difference between them is that the first 164 amino acids of hPR-B (B-upstream sequence) are absent in hPR-A. In most cell lines such as MCF-7 (human breast cancer cells), CV-1 (monkey kidney fibroblasts), and HeLa (human cervical carcinoma cells), hPR-A functions as a transcriptional repressor, whereas hPR-B functions as a transcriptional activator of progesterone-responsive genes. Interestingly, in these cell contexts, hPR-A also acts as a trans-dominant repressor of the transcriptional activity of other steroid hormone receptors.

In contrast to hPR-A, which functions predominantly as a ligand-dependent transcriptional repressor, we show in this study that the A isoform of the chicken PR (cPR-A) lacks this trans-dominant repressor function and is a transcriptional activator in all contexts examined. By constructing chimeras between the N-terminal domains of the chicken and human PR, we mapped the trans-dominant repressor function of hPR-A to the first 140 amino acids of the protein. Notably, when this 140-amino acid “repressor” domain is placed onto chicken PR-A, the activity of the latter changes from a transcriptional activator to a repressor. Interestingly, however, this “repressor domain” is necessary, but not sufficient, for trans-repression as it is inactive when it is tethered to a heterologous protein. This suggests that the trans-repression function is comprised not only of the repressor domain of hPR-A but also requires the context of the receptor to function. The identification of a discrete inhibitory region within hPR-A which is transferable to another receptor implies that this region interacts with a set of transcription factors or adaptors that are distinct from those recognized by hPR-B, the identification of which will be required to define the mechanism by which hPR-A modulates steroid hormone receptor transcriptional activity. Thus, although chickens and humans both produce two very similar forms of the progesterone receptor, it is clear from these studies that the mechanism of action of progesterone in these two systems is quite different.

The progesterone receptor (PR) belongs to the superfamily of intracellular receptors that mediate the nuclear effects of steroid hormones, thyroid hormone, and the non-nutritional vitamins A and D (1). The mechanism of action of PR is similar to that of the other steroid receptors. In the absence of ligand the receptor is transcriptionally inactive and remains sequestered in a large complex of heat shock proteins (HSPs) as follows: HSP-90, HSP-70, and P59 (2–4). Upon ligand binding, the receptor undergoes a distinct change in conformation (5) that results in the dissociation of a monomeric receptor from the heat shock complex (5, 6). Ligated receptors then spontaneously dimerize and bind to DNA via specific progesterone response elements (PREs) located within the regulatory regions of target genes (7). The binding of either an agonist, or of most antagonists, converts the receptor into a DNA binding competent form (8). However, only agonist-bound PR receptors are capable of enhancing transcriptional activation when bound to PREs.

The progesterone receptor structure is similar to that of other steroid receptors in that it contains a highly conserved DNA-binding domain (DBD), a hormone-binding domain (HBD) (conserved among the related steroid receptors such as PR and glucocorticoid receptor (GR)), and an N-terminal domain which is the most variable region among the family members (9). The regions responsible for receptor dimerization and interaction with heat shock proteins are also located at the C terminus within the HBD of PR (10). More importantly, the HBD also contains one of the transcriptional activation domains AF-2 (11). The other transcriptional activation domain, AF-1, is located in the N terminus upstream of the DBD (9, 12).

The human PR is unique in that it exists as two isoforms hPR-B (114 kDa) and hPR-A (94 kDa) (13). The human PR-A is a truncated form of hPR-B lacking the first 164 N-terminal amino acids. These two isoforms are transcribed from distinct estrogen-inducible promoters within a single copy PR gene (14). Both isoforms have been identified in most species, with the exception of the rabbit where PR exists only as the B isoform (15). The biochemical properties of the two PR isoforms have been analyzed extensively in vitro. Both forms have similar DNA and ligand binding affinities (16). However, work done using reconstituted progesterone-responsive transcription sys...
tems in various mammalian cells revealed that hPR-A and hPR-B are not functionally identical (9, 14, 17). Specifically, hPR-B functions as a transcriptional activator in most contexts, whereas in most cells hPR-A does not activate transcription but functions as a strong trans-dominant repressor of hPR-B, glucocorticoid receptor (hGR), androgen receptor, mineralocorticoid receptor, and estrogen receptor (hER) transcriptional activity (17–19).

Unlike the receptor, both A and B isoforms of the chicken PR act as potent activators of progesterone-responsive genes in transfected mammalian cells (20–22). Thus, although the primary sequences of the cPR-A and hPR-A are quite similar, they are functionally quite different. A comparison of the amino acid sequence revealed that cPR-A and hPR-A are very homologous over most of the length of the protein; however, they are divergent in their N termini. Consequently, we hypothesized that the key sequences responsible for the different activities of cPR-A and hPR-A lie within the N terminus. We anticipated that by creating and analyzing chimeras between the two proteins that the sequences within hPR-A required for trans-repression could be defined.

In this study, we show that the N-terminal 140-amino acid region of hPR-A is necessary, but not sufficient, for trans-repression of ER transcriptional activity. In addition, our findings indicate that this repressor region of hPR-A requires other domains within the receptor to form the structures necessary for trans-repression. It is possible that this structure is required to sequester a co-factor required for proper hER transcriptional activity.

**EXPERIMENTAL PROCEDURES**

**Materials**—DNA restriction and modification enzymes were obtained from Promega (Madison, WI), Boehringer Mannheim, or New England Biolabs (Beverly, MA). PCR reagents were obtained from Perkin-Elmer or Promega Corp. (Madison, WI). Progesterone and 17b-estradiol were purchased from Sigma. R5020 (promegestone) was purchased from NEN Life Science Products. Secondary antibodies, Hybond-C Extra (nitrocellulose) transfer membrane, and developing film were obtained from Amersham Corp. PR22 primary monoclonal antibody was a gift from David Teo (Mayo Clinic, Rochester, MN). Polyclonal antibody raised against hPR-A was a gift from Nancy Weigel (Baylor College of Medicine, Houston).

**Plasmids**—The expression plasmid CMV-hPR-B was constructed as follows: YePlhPR-B (23) was digested with XhoI and KpnI, and the fragment containing the coding sequence for hPR-B was ligated into pBK-CMV mammalian expression vector, previously digested with XhoI and XbaI. La Jolla, CA). YePlhPR-A (23) was constructed as follows: YePlhPR-A was digested with XhoI and KpnI, and the fragment containing the coding sequence for hPR-A only was ligated into pBK-CMV as described above. YePlhPR-A was constructed as follows: YePlE2 (24) vector was digested with NotI and KpnI and subsequent cloning of a PCR-generated fragment from YePlhPR-B. The sequences of the oligonucleotides for PCR were 5'-CCGGAATTCATGTCGACCCTGGAGTGCATCCTG (forward) and 5'-CCCTCTAGATTACCTCAGGTAGTTGAGATAGGGCGG (reverse). The plasmid pBK-hPR-B was constructed as described above.

The plasmid pBK-CMV-hPR-B was constructed by digesting pBK-CMV with EcoRI and KpnI and subsequent cloning of a PCR-generated fragment from YePlhPR-B. A control pBCK-CMV-A plasmid was constructed as follows: YePlaPR-A was digested with XhoI and KpnI, and the fragment containing the coding sequence for a new translation start site. All deletion mutants were verified by sequencing to ensure the fidelity of the resulting constructs.

RESULTS

**Differential Transcriptional Activities of hPR-B, hPR-A, and cPR-A**—To compare the transcriptional activities of hPR-B, hPR-A, and cPR-A, we used the expression vectors pBKChPR-B, pBK-hPR-A, and pBK-cPR-A which specifically encode either hPR-B, hPR-A, or cPR-A. The expression constructs were transiently transfected into HeLa (human cervical carci-
noma) cells (Fig. 1A) or HepG2 (human hepatoma) cells (Fig. 1B) together with a progesterone-responsive luciferase reporter (PRE3-TK-LUC). Western immunoblot analysis using a human PR-specific polyclonal antibody (B13-TK) and a chicken PR-specific monoclonal antibody (PR22) confirmed that the receptors expressed in an intact form at approximately the same level (data not shown).

HeLa cells and HepG2 cells contain no endogenous PRs. As a result, there was no significant hormone-dependent activation of the PRE3-TK promoter in the absence of transfected receptor.
Transfections were normalized for efficiency using an internal standard, under given experimental conditions. The average coefficient of variation at each hormone concentration was independently calculated for each data point. Each data point represents the average of triplicate determinations of the transcriptional activity of PRE3-TK promoter by hPR-A was observed. The influence of HepG2 cells no significant progesterone-induced activation titrates out a limiting factor (29). In contrast, in HeLa cells due to self-squelching where overexpression of the receptor increases in the transcriptional activity of hPR-B. This is likely higher plasmid concentrations, however, we observed a decrease in the transcriptional activity of hPR-B. This is likely due to self-squelching where overexpression of the receptor titrates out a limiting factor (29). In contrast, in HeLa cells but not HepG2 cells no significant progesterone-induced activation of PRE3-TK promoter by hPR-A was observed. The influence of cell type on the human PR subtype-specific activation of progesterone-responsive promoters has been documented previously (17).

The most striking result, however, was observed when we compared the transcriptional activities of hPR-A and cPR-A. In HeLa cells, as we had observed in the past, hPR-A was only marginally active as a transcriptional activator, at any expression level tested. However, cPR-A demonstrated an activity that was equivalent to hPR-B. Furthermore, at higher receptor concentrations, cPR-A displayed increased ligand-independent activity which was not observed with either isoform of human PR (Fig. 1A). This clearly demonstrates a functional difference between the A-form of PR from the two species. Interestingly, this difference was not manifested in HepG2 cells where all three receptor isoforms tested were transcriptionally active. These results confirmed and expanded our previous studies showing that hPR-A and hPR-B were functionally distinct. However, more importantly they indicated that the PR-A isoforms from chicken and human are not functionally equivalent as activators of transcription.

Trans-dominant Repressor Effect of hPR-A but Not hPR-B or cPR-A on hER Transcriptional Activity—Previously, it has been shown that hPR-A but not hPR-B is capable of trans-dominant repression of steroid receptor activity in contexts where it has no independent positive transcriptional activity (17, 19, 30). To determine whether cPR-A is also capable of trans-dominant repression of heterologous steroid receptor action, we transiently transfected into HeLa cells the constructs expressing either hPR-B, hPR-A, or cPR-A together with an estrogen-responsive luciferase reporter (ERE3-TATA-LUC) and an expression vector for hER (pRST7-ER) (Fig. 2). The experiments were performed using concentrations of hPR-B, hPR-A, and cPR-A which gave the maximal ligand-dependent transcriptional activation (Fig. 1A). Estradiol-dependent activation of the ERE3-TATA promoter in HeLa cells expressing hER together with control plasmid was not affected by coaddition of R5020 at any concentration (ranging from 10⁻¹¹ to 10⁻⁶ M). However, HeLa cells cotransfected with hPR-B inhibited hER-mediated transcriptional activity by 78% at 10⁻¹¹ M R5020 which increased to >80% with increasing concentrations of R5020 (Fig 2). In contrast, HeLa cells cotransfected with hPR-B or cPR-A showed little or no trans-dominant repression of hER transcriptional activity. At higher concentrations of R5020 (10⁻⁶ M) hER activity could be repressed by 45% in the presence of hPR-B but not cPR-A. Importantly, in these experiments cPR-A has no effect on ER transcriptional activity. These data suggest a selective role for hPR-A, but not hPR-B, or cPR-A in the negative regulation of steroid receptor transcriptional activity.

The observation that the structurally related cPR-A and hPR-A proteins have completely different functions suggests to us that by constructing receptor chimeras we would be able to define the regions within hPR-A responsible for trans-dominant repression.

Structural Differences between hPR-B, hPR-A, and cPR-
A—It has been postulated that the 164-amino acid B-upstream segment (BUS), unique to hPR-B, is in part responsible for the functional differences between the two isoforms of human PR (29). However, this cannot be the complete answer as our data, and those of others (21, 31), show that unlike its human counterpart the cPR-A is a strong activator of progesterone-responsive promoters, yet it lacks the BUS activating function present in hPR-B. These observations imply that it is some other regulatory element present in hPR-A that is responsible for the differential activities observed with the two isoforms of human PR. We compared the primary structures of hPR-A and cPR-A (Fig. 3) to identify sequences present in hPR-A that may be responsible for its unique inhibitory action. From this comparison we concluded that the most extensive differences in the primary structures of the chicken and human PR-As are found in the N-terminal domains, upstream of a unique PmLI restriction site present in both receptors. These N-terminal domains of the receptors share only 55% similarity and 30% identity compared with 90% similarity and >72% identity shared between the C-terminal regions of the receptors. Based on this observation we hypothesized that the unique trans-dominant activities of hPR-A were determined by the extreme N-terminal 140 amino acids.

The N-terminal 140-Amino Acid Region of hPR-A Is Necessary for Trans-repression of Heterologous Steroid Receptor Transcriptional Activity—Previous work has mapped a major activation function within hPR to a 90-amino acid region (AF-1), contained within both hPR-A and hPR-B. These studies revealed also that another region wholly contained within the BUS region of hPR-B was required for maximal AF-1 activity (29). This suggested to us that a major role of BUS was to overcome a repressive activity of the N-terminal of hPR-A on AF-1. To address this hypothesis we created a series of deletion mutants that lacked the first 140-amino acids in the human (ΔhPR-A) and the corresponding 90-amino acids in the chicken (ΔcPR-A) upstream of the unique PmLI site (Fig. 4A). We observed that unlike full-length hPR-A, ΔhPR-A acquired the ability to activate progesterone-responsive promoters (Fig. 4B). Western immunobots confirmed equal expression of hPR-A and ΔhPR-A (data not shown). On the contrary, deletion of the first 90 amino acids from cPR-A did not affect its ability to activate progesterone-responsive promoters (Fig. 4B). Overall, these results suggest that the inability of hPR-A to function as a transcriptional activator is not due to a loss of an activation sequence in BUS but due to the active inhibitory actions of the N-terminal 140 amino acids of hPR-A.

If the inability of hPR-A to activate transcription is due solely to the inhibitory activity of N-terminal 140 amino acids, then we predicted that hPR-A mutants lacking this activity would be unable to act as trans-dominant repressors. Therefore, to determine the ability of the deletion mutants to trans-repress heterologous steroid receptor activity, we tested the ability of the individual mutants to repress hER transcriptional activity. Vectors expressing hER and either ΔhPR-A or ΔcPR-A, respectively, were cotransfected into HeLa cells (Fig. 4C) together with the ERE7-TATA-LUC reporter. hER transcriptional activity in the presence of 10^{-7} M 17β-E2 alone or in the presence of 10^{-7} M 17β-E2 and 10^{-7} M R5020 in combination was measured after 24 h. As shown in Fig. 4C wild type hPR-A repressed 17β-E2-dependent transcription by hER by 80%, whereas ΔhPR-A was unable to repress hER activity under the same conditions. Both cPR-A and the 90-amino acid truncated form of this receptor, ΔcPR-A, displayed no trans-repressive effect on hER transcriptional activity. From these data we concluded that the N-terminal 140 amino acids of hPR-A contain a specific “inhibitory domain” and that this is necessary for trans-dominant repression of hER. Similarly, we also showed that the ability of hPR-A to repress the transcriptional activity of either the human glucocorticoid receptor (hGR) or that of hPR-B required the 140-amino acid hPR-A inhibitory domain (data not shown). We conclude, therefore, that hPR-A-mediated repression of steroid receptor transcriptional activity occurs through a similar mechanism.

To characterize further hPR-A’s inhibitory domains and to see whether it was transferable, we swapped the 140 amino acids of hPR-A with the 90 amino acids from cPR-A, to create the chimeras HC-PR-A and CH-PR-A, respectively (Fig. 5A). Both chimeric receptors were subcloned into pBK-CMV mammalian expression vectors and were shown by Western immunoblot to be expressed at the same level as their wild type counterparts (data not shown). As expected, when testing the ability of these chimeric fusions to activate progesterone-responsive promoters (PRE7-TK-LUC), we noticed that unlike wild type hPR-A, HC-PR-A had no positive transcriptional activity on this promoter, whereas wild type cPR-A was capable of 12-fold activation under these conditions (Fig. 5B). These results strongly suggested that the repressor effect observed with hPR-A is transferred along with the N-terminal 140 amino acids of the receptor since deletion of the 90 amino acids of cPR-A has no effect on the ability to transactivate. The chi-
FIG. 4. ΔhPR-A is unable to repress hER transcriptional activity. A, the DNA sequences of hPR-A and cPR-A were obtained from GenBank. These represent the full-length sequences of the two receptors. ΔhPR-A and ΔcPR-A, subcloned into pBK-CMV mammalian expression vector, were generated by deleting the 140 amino acids of hPR-A upstream of the PmL1 restriction site and the corresponding 90 amino acids of cPR-A, respectively. B, HeLa cells were transiently transfected with vectors expressing hPR-A, ΔhPR-A, cPR-A, or ΔcPR-A, respectively. The transcriptional activity was measured following the addition of $10^{-7}$ M R5020. A control vector (pBK-RevTUP1) was used to assess the basal level of transcription of the PRE−TK-LUC reporter. Transfections were normalized for efficiency using the internal pBK-βgal control plasmid. The data are represented as Fold Induction, a measure of ligand induced activity divided by basal (no hormone) activity, for each data point. C, HeLa cells were transiently transfected with vectors expressing the human estrogen receptor, hER, alone or in combination with a vector expressing hPR-A, ΔhPR-A, cPR-A, or ΔcPR-A, respectively. The transcriptional activity was measured following the addition of $10^{-7}$ M $17\beta$-estradiol and $10^{-7}$ M R5020 alone or in combination. In these experiments estrogen receptor transcriptional activity was assayed on an ERE−TATA-LUC promoter.
meric fusion CH-PR-A was as active as wild type CPR-A, and both were similarly active to wild type hPR-B (Fig. 5B).

We next examined the ability of the chimeric fusions to repress estradiol-dependent ER-transcriptional activity. Vectors expressing hER (pRST7-ER), HC-PR-A (pBK-CCH-PR-A), CH-PR-A (pBK-CH-PR-A), hPR-A (pBK-hPR-A), or CPR-A (pBK-CPR-A), respectively, were cotransfected into HeLa cells (Fig. 5C) together with the ERE<sub>T</sub>-TATA-LUC reporter. hER-mediated transcriptional activity was measured after 24 h in the presence of 10<sup>-7</sup> M 17β-E<sub>2</sub> alone or in the presence of 10<sup>-7</sup> M 17β-E<sub>2</sub> and 10<sup>-7</sup> M R5020. As shown in Fig. 5C, HC-PR-A repressed hER-mediated transcriptional activity by 78%, and CH-PR-A repressed hER activity by only 30%. Once again wild type hPR-A was the strongest trans-repressor of hER activity, repressing hER activity by as much as 88%. Repression by CPR-A was only 18% in this experiment. Together these data suggest that the trans-repressor function of hPR-A is localized within the first 140 amino acids of the protein. Moreover, these data show that this 140-amino acid region is necessary for trans-repression of heterologous steroid receptor activity.

The N-terminal Repressor Region of hPR-A Is Not Sufficient for Trans-repression of hER Transcriptional Activity—To determine whether this 140-amino acid inhibitory region was necessary and sufficient for trans-dominant repression of heterologous steroid receptor activity, we transferred this region to the DNA binding domain of GAL4 (DBD-140NhPR-A) and assessed its ability to repress hER transcriptional activity (Fig. 6B). A similar GAL4-DBD fusion construct containing the N-terminal 90 amino acids of CPR-A was also made and used as a control. Western immunoblot analysis using the polyclonal hPR-A-specific antibody (B13-TK) and the monoclonal CPR-A-specific antibody (PR22) confirmed that these proteins were expressed intact and at similar levels to their cognate wild type receptors (data not shown). Expression vectors pBK-DBD-140NhPR-A, pBK-DBD-90NhPR-A, or pBK-DBD (empty control plasmid) (Fig. 6A) were cotransfected into HeLa cells with an ERE<sub>T</sub>-TATA-LUC reporter and the pRST7-ER expression vector. The cells were incubated with estradiol alone or estradiol and R5020 as before. It was observed that the GAL4-DBD fusions of the N terminus of the chicken and the human A isoforms of PR were unable to trans-repress hER activity under these conditions, whereas wild type hPR-A was capable of repressing hER activity by 80%. These observations suggest that the N-terminal repressor region of hPR-A acts only in the context of the full-length PR (either chicken or human) and that this region is necessary but not sufficient for trans-repression of hER transcriptional activity. More importantly, these results suggest that regions of the receptor other than the N terminus are required for trans-repression.

The B-upstream Sequence (BUS) of hPR-B Suppresses the Negative Effects of the N-terminal Repressor Region on AF-1 Function—One of the most interesting aspects of the experiments thus far is that we have mapped the inhibitory region of hPR-A to a domain that is wholly contained within hPR-A and hPR-B. Since the activation function in the N terminus, AF-1, is also contained within hPR-A, it suggests possibly that the "A" inhibitory region is dominant over the activation function contained within AF-1. Furthermore, it suggests that the role of BUS is to suppress the activity of the inhibitory region and permit AF-1 activity to be manifested. To test this hypothesis we created a series of fusion constructs by transferring various domains of hPR-B, hPR-A, and CPR-A to GAL4-DBD. These constructs are outlined in Fig. 7A. Western immunoblot analysis using B13-TK and PR22 confirmed the relative expression levels of these constructs (data not shown). Expression vectors pBK-DBD-140NhPR-A, pBK-DBD-90NhPR-A, pBK-DBD-NhPR-B, pBK-DBD-NhPR-A, pBK-DBD-AF-1, pBK-DBD-HBD, and pBK-DBD (containing the 140-amino acid repressor region of hPR-A, the N-terminal 90-amino acids of CPR-A, the N-terminal region up to its DBD of hPR-B, the N-terminal region up to its DBD of hPR-A, the 90-amino acid AF-1 region present in both isoforms of human PR, the C-terminal hormone binding domain (HBD) region also present in both receptors, and the GAL4-DBD domain, respectively) were cotransfected into HeLa and HepG2 cells. To access the transcriptional activity of these GAL4-DBD fusion constructs, we cotransfected them into mammalian cells with a GAL4<sub>T</sub>-TATA-LUC reporter. After transfection the cells were induced for 24 h with two hormonal stimuli, no hormone and 10<sup>-7</sup> M R5020, and then assayed for luciferase activity.

The results shown in Fig. 7B indicate that the 140-amino acid repressor region of hPR-A, the entire N-terminal region of hPR-A, and the 90-amino acid region of CPR-A have no activity on a GAL4-responsive promoter in either HeLa or HepG2 cells, whereas the whole N terminus of hPR-B, as well as the AF-1 and the HBD domains, displayed a significant increase in transcriptional activity (Fig. 7, B and C). AF-1 is more transcriptionally active in HepG2 (Fig. 7C), an AF-1 dominant cell line, than in HeLa cells (45- versus 5-fold). In contrast, the HBD of PR has greater activity in HeLa cells (Fig. 7B), an AF-2 dominant cell line (8- versus 1.8-fold) (30). From these observations we concluded that when tethered to AF-1, the 140-amino acid region of hPR-A (see NhPR-A fusion) is capable of repressing AF-1 activity (Fig. 7, B and C). Interestingly, by tethering the BUS region unique to hPR-B onto A-N (see NhPR-B fusion), it is possible to rescue the repressive effect of the 140-amino acid repressor region on AF-1 thus resulting in >20–30-fold activation of the GAL4-responsive promoter, in both cell lines. The observation that the transcriptional activity of the entire N terminus of B is more active than AF-1 alone suggests that in addition to overcoming the "A" repressive domain, the BUS region contains sequences that contribute to AF-1 activity (29).

**DISCUSSION**

The ability of progesterone to oppose estrogen action in vivo has been extensively documented. Progesterone abrogates estrogen induction by down-regulating ER protein concentration, decreasing the circulating estrogen levels (reviewed in Ref. 32), and antagonizing ER action at the molecular level. The mechanism of progesterone action on ER was first described in the mammalian uterus. These studies showed that uterine ER levels, of estrogen-treated rats, were decreased upon progesterone administration (33). Furthermore, it was reported that endometrial ER levels, in women undergoing curettage during the follicular phase of the menstrual cycle, could be decreased by administering medroxyprogesterone (a synthetic progesterin) (34). More recently, studies done in breast cancer cells (35, 36) described a progesterone-mediated decrease in ER protein concentration due to decreased cellular ER mRNA levels, a direct result of inhibition of transcription of the ER gene.

Interestingly, progesterone is also capable of antagonizing ER-mediated regulatory events, although the molecular mech-
FIG. 5. HC-PR-A is a potent repressor of hER transcriptional activity in HeLa cells. A. The human/chicken chimeric constructs, HC-PR-A and CH-PR-A, were generated by swapping the N-terminal regions (upstream of the unique PmlI restriction site, present in both receptors) of the human and the chicken receptors. B. HeLa cells were transiently transfected with vectors expressing hPR-A, cPR-A, HC-PR-A, and CH-PR-A, respectively. The transcriptional activity of these chimeric constructs was assayed on the PRE3-TK, progesterone-responsive promoter. The activity was measured after 24 h induction with $10^{-7}$ M R5020. Fold Induction represents the normalized luciferase activity divided by basal (no hormone) activity, for each receptor-type after induction with ligand. C. HeLa cells were transiently transfected with vectors expressing the human estrogen receptor alone or in combination with a vector expressing hPR-A, cPR-A, HC-PR-A, and CH-PR-A, respectively. The transcriptional activity was
anism of this antagonism is not completely understood. Various groups, including ours, have suggested that PR can antagonize ER transcriptional activity by sequestering a transcription factor necessary for proper ER action (18, 30, 37, 38). Specifically, we reported (18) that hPR-A but not hPR-B, in the presence of either progesterone or anti-progestins, inhibited ER-mediated transcriptional activity in transfected HeLa, CV-1, and HS578T cells but not in the HepG2 cell line. PR-A was also capable of antagonizing endogenous ER transcriptional activity when cotransfected with a simple estrogen-responsive promoter in MCF-7 breast cancer cells in the presence of RU486 (30). Others, however, have observed that hPR-B but not hPR-A was capable of repressing ER activity on a complex estrogen-responsive promoter (estrogen-responsive region on the pS2 gene) when transfected in MCF-7 cells (38). These noted discrepancies result most likely from differences in the

![Diagram A](image1.png)

**FIG. 6.** The N-terminal repressor domain of hPR-A is not capable of autonomous repression of hER transcriptional activity. A, the GAL4 DNA-binding domain (GAL4-DBD) fusion constructs were made by transferring various N-terminal regions of hPR-B, hPR-A, and cPR-A onto GAL4-DBD. GAL4-DBD is depicted as a solid black box at the N terminus of these fusion constructs. B, HeLa cells were transiently transfected with vectors expressing the human estrogen receptor alone or in combination with a GAL4-DBD vector expressing GAL4-DBD fusions with various N-terminal regions of hPR-B (DBD-NhPRB), hPR-A (DBD-NhPRA and DBD-140NhPRA), or cPR-A (DBD-90NcPRA). The transcriptional activity was measured following the addition of 10⁻⁷ M 17β-estradiol and 10⁻⁷ M R5020 alone or in combination. A control was done in the absence of ligands. In these experiments estrogen receptor transcriptional activity was assayed on an ERE-TATA-LUC promoter. Transfections were normalized for efficiency using an internal β-galactosidase control plasmid. The data are presented as % activation where 100% represents a measure of 17β-estradiol-dependent transactivation by hER in the absence of R5020 for each data point. This value is independently calculated for each data point. Each data point represents the average of triplicate determinations of the transcriptional activity under given experimental conditions. The average coefficient of variation was <13%.

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FIG. 7. The N-terminal repressor region of hPR-A represses AF-1 activity and is itself antagonized by BUS. A, the GAL4 DNA-binding domain (GAL4-DBD) fusion constructs were made by transferring various domains of hPR-B, hPR-A, and cPR-A onto GAL4-DBD. GAL4-DBD is depicted as a solid black box at the N terminus of these fusion constructs. HeLa cells (B) and HepG2 cells (C) were transiently transfected with expression vectors pBK-DBD-140NhPR-A, pBK-DBD-90NcPR-A, pBK-DBD-NhPR-B, pBK-DBD-NhPR-A, pBK-DBD-AF-1, pBK-DBD-HBD, or pBK-DBD together with a GAL4-responsive reporter plasmid, GAL4-TATA-LUC. The transcriptional activity was measured following the addition of \(10^{-7}\) M R5020. The data are represented as Fold Induction in the presence of ligand versus absence of ligand for each triplicate data point. The average coefficient of variation was <12% for both experiments.
cell and promoter contexts used for analysis and in the relative expression of transcriptional co-factors and co-repressors.

Interestingly, hPR-A has also been reported to function as a strong trans-repressor of other steroid hormone receptor activity (17–19); however, the physiological importance of these observations remains to be determined. Furthermore, this dominant inhibitory action of hPR-A appears to be restricted to steroid hormone receptor-activated transcription as hPR-A is unable to antagonize vitamin D receptor activity and unable to modulate heterologous viral promoter activity (i.e., SV40, Rous sarcoma virus, and CMV) (17). Both PR and ER are involved in the maintenance and development of female reproductive tissues and more importantly are involved in the progression of hormone-dependent tumors of the breast (32). In addition, the co-expression of hER, hPR-A, and hPR-B in these tissues suggests that the mechanisms of action of these receptors might be linked. Thus there is a need to understand the precise molecular mechanism behind PR-mediated repression of ER transcriptional activity. We have previously proposed (30) that hPR-A may facilitate the cross-talk between progesterone and estrogen signaling pathways in progesterone and estrogen-responsive tissues. In support of our original hypothesis we showed that it is possible to antagonize endogenous ER transcriptional activity in MCF-7 cells by co-expression of hPR-A. In addition, the PR antagonist RU486 is capable of functioning as an antagonist of ER only in the presence of hPR-A. These actions of RU486 do not require the physical interaction with hER and are likely mediated by a non-competitive mechanism of action of RU486. It is possible then that the clinical importance of RU486 in the treatment of endometriosis, uterine fibroids, brain meningiomas, and hormone-dependent breast cancers may well be a result of its ability to function as an anti-progestin as well as an anti-estrogen.

This study defines the structural differences between hPR-A and hPR-B that confer to the A isoform the ability to trans-repress hER transcriptional activity. Previously, it has been postulated that the differences in the transcriptional activities of the two isoforms of the human PRs were due to unique sequences present in hPR-B (29). However, the observation that cPR-A is also an activator of progesterone-responsive promoters but lacks the activating B-specific sequences suggested to us that something unique to hPR-A is responsible for the differences in the transcriptional activities of the two human receptors. Sequence analysis of the human and chicken A receptors revealed that the proteins differed in their N termini. It follows that the structural difference between the human and the chicken A isoform of PR confers to the human A receptor the ability to trans-repress steroid hormone receptor transcriptional activity. Here we show that only hPR-A but not hPR-B or cPR-A is capable of opposing ER-mediated transcriptional activity and that the N-terminal 140-amino acid region of hPR-A is responsible for this repressor activity.

Furthermore, our observation that the repressor region of hPR-A is necessary but not sufficient for trans-repression of heterologous steroid receptor activity suggests that regions of the receptor other than the N terminus of hPR-A are required for trans-repression. In support of this hypothesis it has recently been shown that the N terminus of hPR-A and its C-terminal hinge region interact when assayed in vitro (39). Thus, it is possible that sequences within the N terminus and the C terminus of hPR-B together form a surface that allows the receptor to interact with required transcription co-factors. Given this information, and that presented in this paper, it would appear that the simplest model to explain the differential activity of hPR-A and hPR-B is that both receptors compete for a limiting pool of co-factors and that the complex formed with hPR-A is transcriptionally inactive but represses transcription by sequestering a transcription factor required by hPR-B. However, this simple model is unlikely to be completely correct. In previous work, we demonstrated that the ability of hPR-A to inhibit hER transcriptional activity in a hormone-dependent manner occurred independently of the relative expression of the two receptors and was dependent on the absolute level of hPR-A. This would seem to rule out a classical squelching model. It suggests instead that the inhibitory activity of hPR-A occurs through a totally independent pathway. Our working model at the current time is that the interaction between sequences within the hPR-B BUS region permit PR-AF-1 to interact with cellular transcription factors within the cell which are different from those that interact with hPR-A. Specifically, we propose that in the presence of hormone hPR-B can interact with the co-factors required for transcriptional activity. On the other hand hPR-A may interact with a different subset of proteins and form a complex that can interfere with ligand-dependent transcriptional activity of all the steroid receptors. Although this model can only be tested upon the isolation of the PR-A- and hPR-B-associated proteins, the observation that the inhibitory activity of hPR-A occurs in a cell-restricted manner supports this model.

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