Antiprogestins Mediate Differential Effects on Glucocorticoid Receptor Remodeling of Chromatin Structure*

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Christy J. Fryer‡§, Steven K. Nordeen¶, and Trevor K. Archer†‡

From the Departments of Obstetrics and Gynecology, Biochemistry and Oncology, The University of Western Ontario, London Regional Cancer Centre, London, Ontario N6A 4L6, Canada and the Department of Pathology, The University of Colorado Health Sciences Center, Denver, Colorado 80262

We examined the mechanism(s) by which the progesterone receptor (PR) is able to inhibit glucocorticoid receptor (GR) activation from the mouse mammary tumor virus (MMTV) promoter in vivo. Using specific hormone antagonists, we demonstrate that the PR complexed with a type II antiprogestin blocks glucocorticoid-induced activation of the MMTV promoter. However, when complexed with a type I antiprogestin the PR is unable to block glucocorticoid-induced activation. PR repression of GR activity results from the inhibition of the ability of the GR to remodel chromatin such that the antiprogestin-occupied/PR prevents the glucocorticoid induced assembly of a preinitiation complex at the MMTV promoter. These experiments suggest that the specific chromatin organization of the MMTV promoter provides a mechanism for regulating cross-talk between the GR and PR in vivo.

Steroid hormones act via a group of high affinity receptors that regulate transcription by binding to hormone response elements located within promoters of hormone-inducible genes (1, 2). Molecular cloning and analysis of the major steroid hormone receptors, estrogen, glucocorticoid, progesterone, mineralocorticoid, androgen, vitamin D, thyroid hormone, and retinoic acid receptors, as well as a large number of related proteins, have led to their grouping into a superfamiliy of nuclear hormone receptors (3). This family is distinguished by the fact that the receptors share a similar structure consisting of a variable length amino-terminal region that modulates the transcriptional activity of the receptor, a conserved DNA-binding domain, and a multifunctional carboxyl-terminal hormone or ligand-binding domain. Within the steroid receptor superfamily, the progesterone receptor (PR), glucocorticoid receptor (GR), androgen receptor, and mineralocorticoid receptor represent a distinct subgroup (4). These receptors share regions of high homology, particularly within the DNA-binding domain, and they bind to and enhance transcription from a common consensus sequence, originally designated as the glucocorticoid response element (5, 6).

Hormone activation of responsive genes can be envisioned as a series of discrete steps that include: binding of hormone to receptor, dissociation of heat shock proteins from the receptor, dimerization of receptors, binding of receptor to DNA, and activation of transcription (7). Antagonists of steroid receptor action have been used extensively to dissect receptor mechanisms of action and may inhibit agonist action at several of these steps. Classically antagonists may directly inhibit agonist action by competing for binding to the receptor. Two types of antiprogestins (AP) have been defined by their differential ability to allow binding of the PR to the HRE in vitro (8–10). Type I APs such as ZK98299 (ZK98) do not promote conversion of the PR to a form capable of binding to DNA (8–11). Type II APs, exemplified by RU486, ZK112993 (ZK112), and Org31710 (Org), have been shown to stimulate PR binding to DNA in vitro (8). As with APs, several distinct classes of antiestrogens differentially modulate estrogen receptor action and exhibit distinct biologies (12). Therefore the pathway leading to steroid receptor activation of target genes is amenable to manipulation by pharmacological intervention.

To dissect the mechanisms of steroid hormone action on chromatin templates, we used the steroid-responsive mouse mammary tumor virus (MMTV) promoter. When stably introduced into cells, the MMTV promoter is reproducibly assembled into a phased array of six nucleosomes (13, 14). The second nucleosome (nuc-B) is positioned directly over the region containing the binding site for nuclear factor 1 (NF1) and the multiple binding sites for the GR. It is adjacent to those for the octamer transcription factor and TFIID complex. Although NF1 is present in the nucleus prior to hormonal stimulation, assembly of the promoter into chromatin excludes NF1 binding (15). Glucocorticoid administration does not result in increased levels or modifications of NF1, but alters the chromatin structure to permit NF1 binding, suggesting that the prior assembly of the promoter into chromatin prevents NF1 binding (14).

A mechanistically distinct picture emerges when one examines hormone activation of MMTV plasmids introduced into cells by transient transfection (16). The transient templates are not organized in a phased array of nucleosomes and have NF1 constitutively bound both in the presence and absence of hormone. Thus, the chromatin transition and loading of NF1 induced by the GR is not required for transient templates (16). The MMTV long terminal repeat also responds to progestins (17–20) and detailed analysis of the PR and GR interactions with the HRE suggest a similar sequence specificity (5, 21). Experiments have suggested that the organization of the MMTV promoter into a specific chromatin structure may regulate the activity of these hormone receptors. These differences
between chromatin and transiently transfected MMTV templates are mirrored by the ability of the PR to activate the transiently transfected template, but not the chromatin template when transfected into mouse C127 cells which normally lack the PR (22, 23). These studies demonstrated that the deficiency in PR activation of the chromatin template is a defect in chromatin remodeling (24). However, upon stable introduction of the PR it acquired the ability to activate the chromatin template (22). Indeed these and other studies suggest that progesterin regulation of MMTV transcription may be more complex than that seen with glucocorticoids and dependent on chromatin structure, promoter length, cell line, receptor complement, and reporter gene copy number (22, 23, 25).

The concept that the assembly of the MMTV promoter into a specific chromatin structure may provide for selective activation was substantiated by experiments in the cell line T47D/A1-2, which expresses both the PR and GR. In this cell line, both receptors are able to activate transcription from transiently introduced MMTV templates, but only the glucocorticoid receptor is able to efficiently induce transcription from a stable MMTV chromatin template (26). This functional data correlated with the superior ability of glucocorticoids to remodel promoter chromatin and induce transcription factor loading at the MMTV promoter (26). However, even though the PR is unable to efficiently activate this reporter organized as chromatin, upon co-administration of glucocorticoids and progesterins, it can inhibit glucocorticoid-induced activation of transcription. The activated PR inhibited the ability of the activated GR to remodel chromatin structure at the MMTV promoter as assessed by the inhibition of glucocorticoid-induced nuclease hypersensitivity at promoter sequences (26).

In this paper we have examined the mechanism by which the PR is able to inhibit GR-mediated activation of transcription of the MMTV promoter assembled as chromatin. Through the use of two types of PR antagonists, that were originally defined by their differential ability to allow DNA binding by the PR, we established that the PR need not be transcriptionally competent to repress GR activation of the MMTV promoter. Our experiments demonstrate that the PR bound to a type II AP is necessary and sufficient for repression of GR activity. This repression of GR activity is observed both at the level of chromatin remodeling and the assembly of a preinitiation complex by in vivo footprinting experiments. In contrast, experiments that make use of a type I AP the PR is unable to block GR activation. These results suggest a mechanism, where the antagonist-bound PR competes with agonist-bound GR to inhibit the ability of the GR to remodel chromatin structure and thereby prevents the GR-mediated increase in transcription.

MATERIALS AND METHODS

Cell Lines—T47D/A1-2 cells were derived from T47D breast cancer cells by stable transfection with plasmids pGRneo and pHHLuc as described previously (27, 65). The plasmid pHHLuc (66) contains MMTV sequences from Homo sapiens (100). T47D/A1-2 cells were grown at 37 °C with 5% CO2 in modified Eagle’s medium containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl2, 10 m M dithiothreitol, 500 μ M each dNTP, and 20 units of Superscript reverse transcriptase (Life Technologies, Inc.). After 1 h at 37 °C and 10 min at 75 °C the cDNA was stored at −80 °C until use. PCR was carried out with cDNA derived from 100 ng (β-actin primers) or 300 ng (MMTV primers) of RNA, 5 units of Taq DNA polymerase in a final volume of 50 μl. The reaction solution contained 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl2, 100 μ M each dNTP, and 5 pmol of each primer. MMTV-619 primer, CAGACTCTCTTCCCAGAATCT (18 to +42, and MMTV-22, TCTGAGAA-AGTAGGATA-AGTGCAGCA (+60 to +85), were used to generate a product of 60-base pairs upon PCR amplification. The MMTV-22 primer was end labeled with T4 polynucleotide kinase to generate a 32P-labeled single-stranded primer. The primers used for amplification of β-actin and MMTV sequences were 5′ ACCCCCACTGAAAAGATGA (residues 1524–1563, sense strand) and ATCTCTTAAACTTCCCATGATG (residues 2253–2282 and 3508–3517, antisense strand) (68). PCR using these primers yield a 120-base pair product. The β-actin primer was end labeled with T4 polynucleotide kinase to generate a 32P-labeled single-stranded primer. Each cycle of PCR included 2 min of denaturation at 94 °C, 2 min of primer annealing at 55 °C, and 3 min of extension/synthesis at 72 °C. PCR products were analyzed on 7% polyacrylamide denaturing gels and exposed to DuPont Reflection film at room temperature.

RESULTS

PR Inhibition of Glucocorticoid Induction of Transcription—T47D/A1-2 human breast cancer cells express high levels of both the A and B isoforms of the PR (27). In these cells the endogenous PR is unable to induce transcription from the stably integrated MMTV promoter. Not only is the agonist-occupied PR unable to induce chromatin remodeling and transcription factor loading, but glucocorticoid activation was dramatically inhibited by co-administration of a progesterin (26).

In the experiments outlined below, we used the MMTV promoter to define the step in the activation pathway at which the PR inhibited GR action. To pursue this line of enquiry we made use of two types of APs, which were originally defined by their differential ability to allow binding of the PR to the HRE in vitro (8–10). In this approach we use the APs at 10-fold lower levels than glucocorticoid, to ensure that they will bind only the PR and not the GR (8, 9). In the first series of experiments, we found that ZK98, a type I AP that does not permit PR binding to DNA (8–11), to examine whether the PR binders yields a transcriptionally competent to inhibit glucocorticoid activation. T47D/A1-2 cells were transfected with ZK98 (10−8 M) for 18 h prior to dexamethasone (10−7 M) addition for 6 h and transcription was monitored by a reverse transcription-polymerase chain reaction assay (see Fig. 1). In the absence of hormone there is a very low level of transcription from the MMTV promoter (Fig. 1,
FIG. 1. Type II APs inhibit glucocorticoid induction of MMTV transcription. T47D/A1-2 cells were untreated (lanes 1 and 5), treated with dexamethasone (10⁻⁷ M) for 6 h (lanes 2 and 6), treated with AP (10⁻⁴ M) for 24 h (lanes 3 and 7), or pretreated with AP for 18 h prior to dexamethasone addition for 6 h (lane 4 and 8). The antiprogestin used in each panel is indicated. Three µg of total RNA was reverse transcribed to cDNA using an oligo(dT) primer and Superscript reverse transcriptase (Life Technologies, Inc.). One-tenth of the cDNA was analyzed by Taq polymerase amplification with ³²P-labeled single-stranded primers. PCR products were analyzed on 7% polyacrylamide denaturing gels and exposed to DuPont Reflection film at room temperature. Lane No DNA, PCR amplification with no cDNA template; lane φX, φX174 replicative-form DNA cut with HaeIII. Sizes are indicated in base pairs.

lane 1). However, when glucocorticoids are added there is a marked induction of transcription (Fig. 1, cf. lanes 1 and 2). Treatment with ZK98 on its own had no effect on transcription observed in the absence of exogeneously added ligand (Fig. 1, cf. lanes 1 and 3), nor did it significantly inhibit glucocorticoid induction of transcription (Fig. 1, cf. lanes 2 and 4). As a control, a β₂-microglobulin product was amplified and its levels were shown to be unaffected by any of the hormone treatments (Fig. 1, cf. lanes 5–8). In these experiments the APs were used at a concentration of 10⁻⁸ M as at this level, according to in vitro DNA binding studies (9), and Fig. 4, it should not compete significantly for dexamethasone binding to the GR. Thus in contrast to the agonist bound PR the type I AP-PR complex which is transcriptionally inert fails to inhibit the agonist bound GR (Fig. 1 and Ref. 26).

A significantly different result was obtained in experiments when cells were treated with dexamethasone in the presence of a type II AP. Type II APs, such as Org and ZK112, have been shown to allow PR binding to DNA in vitro, but do not induce transcription (8). T47D/A1-2 cells were pretreated with Org (10⁻⁸ M) prior to dexamethasone (10⁻⁷ M) addition and the effect of DNA binding of antagonist-bound PR on glucocorticoid induction of transcription was examined (see Fig. 1). In contrast to the type I AP, Org inhibited glucocorticoid induction of MMTV transcription by approximately 70% (Fig. 1, cf. lanes 2 and 4). Similar results were observed for a second type II AP, ZK112 where 80% inhibition by the antagonist-bound PR was observed in the presence of dexamethasone (Fig. 1, cf. lanes 2 and 4). For ZK112 less than 5% of the AP is bound or competes with tritiated dexamethasone for binding to the GR.² Again, under these conditions no change in β₂-microglobulin transcription was observed (Fig. 1, cf. lanes 5–8). Transcription was also monitored by luciferase assay at 10-fold excess AP (10⁻⁶ M) and 10-fold lower AP (10⁻⁸ M) than dexamethasone (Fig. 2). Consistent with the reverse transcriptase-PCR data, at 10⁻⁸ M AP only the type II APs (Org and ZK112) inhibited dexamethasone-induced transcription. However, at 10-fold excess of AP, which competes significantly with the GR for dexamethasone binding (11), both type I and type II APs inhibited glucocorticoid induction. The cells were also treated with APs at both concentrations for 24 h but no significant induction of transcription (above baseline) from the MMTV-luciferase reporter was detected (data not shown). Thus, the data from these studies demonstrate that type II but not type I APs inhibit glucocorticoid-induced transcription, and that the PR need not be transcriptionally competent for inhibition.

Type II APs Inhibit GR-mediated Hypersensitivity in GR⁺/PR⁺ T47D/A1-2 Cells—We have established that between binding to DNA and transactivation of the MMTV promoter, there are additional distinct and separable steps which include remodeling of chromatin and recruitment of a transcription preinitiation complex (30). Induction of transcription from the MMTV promoter has been mechanistically linked to the appearance of a hypersensitive region within the proximal promoter localized to nuc-B (31). To determine the effect of APs on GR-mediated hypersensitivity, we used restriction enzymes that cleave at sites within the region occupied by nuc-B (see Fig. 3). Consistent with previous experiments, there is inefficient cleavage by restriction enzymes, in the region of nuc-B, in the absence of hormone (26). The efficiency of cleavage by SstI was unaffected by treatment of A1-2 cells with ZK98 (Fig. 3A, cf. lanes 1 and 3). Upon addition of dexamethasone there was a

²S. Nordeen, unpublished data.
marked induction in cleavage by SstI (Fig. 3A, cf. lanes 1 and 2), while pretreatment of T47D/A1-2 cells with ZK98 prior to dexamethasone addition did not significantly alter the ability of dexamethasone to induce hypersensitivity to SstI (Fig. 3A, cf. lanes 2 and 4). Because ZK98 has a lower binding affinity for the PR than the agonist R5020 or the two type II APs used in these studies, we questioned whether the failure of ZK98 to inhibit glucocorticoid activation might be due to an inefficient interaction of ZK98 with the PR in vivo. To rule out this possibility an experiment was performed identical to that shown in Fig. 3 except that ZK98 was used at 10^-7 m and dexamethasone at 10^-6 m. No inhibition of GR-mediated hypersensitivity to restriction enzymes was detected (data not shown). Previous experiments have shown that this concentration of ZK98 effectively blocked R5020 induced activation of a MMTV-CAT reporter by 50% in a PR^-/^- T47D-derived cell line (11). Therefore, the inability of type I APs to inhibit glucocorticoid induction of transcription is in agreement with its inability to prevent GR-mediated hypersensitivity.

In the next series of experiments we examined the consequences of type II AP administration on GR-mediated chromatin reorganization. Untreated cells or cells treated with the type II AP Org alone exhibited inefficient cleavage by restriction enzyme, SstI (Fig. 3B, cf. lanes 1 with 3). Upon dexamethasone addition there was an 8–9-fold induction in cleavage by these restriction enzymes (Fig. 3B, cf. lanes 1 with 2). When cells were treated with Org prior to dexamethasone addition there was a 70–80% reduction in the cleavage by SstI as compared with dexamethasone alone (Fig. 3B, cf. lanes 2 with 4). The cleavage by the restriction enzymes was quantified by a Molecular Dynamics PhosphorImager and the fold induction was calculated by comparison with the extent of cleavage by restriction enzymes in the samples from untreated cells. Similar inhibition of GR-mediated hypersensitivity was obtained with a second type II AP, ZK112 (Fig. 3C). In this experiment two concentrations of dexamethasone (40 and 100 nM) were used. The antiprogestin efficiently inhibited in the presence of 4–10-fold excess dexamethasone (Fig. 3C, cf. lane 2 with 5 and 3 with 6). Therefore, the inhibition of transcription by type II APs can be correlated with the inability of the GR to remodel local chromatin structure in the presence of type II AP-PR complexes.

One possible explanation of the AP inhibition of glucocorticoid activation is that during the time period of the hormone treatments there is a reduction in GR levels. To address this question we examined the GR levels under the hormone regimes used in our studies by Western blotting. The level of GR did not significantly change under the treatment conditions employed (data not shown). Therefore the inhibition that we observe upon antiprogestin treatment is not mediated through a change in receptors levels but is linked to the ability of the PR to inhibit GR action.

Type II APs Do Not Affect Glucocorticoid-mediated Hypersensitivity in GR^-/^- Cells—The type II AP Org is structurally similar to RU486 but was designed to exhibit lower antiguocorticoid activity than RU486 (8, 28, 29). At higher concentrations (10^-7 m), APs such as RU486 bind the GR with high affinity (32). To ensure that the effects of type II APs were acting via the PR and not by binding to the GR, a mouse breast cancer cell line (2305) which expresses the GR but not the PR was examined. Using identical AP concentrations and pretreatment times as the T47D/A1-2 experiments, we monitored changes in chromatin hypersensitivity to determine the effect of the APs on GR action in the absence of the PR. At 10^-6 m Org and 10^-7 m dexamethasone, there was no change in cleavage by SstI as compared with dexamethasone alone (Fig. 4A, cf. lanes 2 and 4). Similarly at these concentrations the AP was unable to block transcription as measured by expression and activity of the MMTV-LUC reporter present in these cells (Fig. 2 and Ref. 32). ZK98 is also selective for the PR and does not inhibit GR-mediated hypersensitivity induced by dexamethasone in 2305 cells (Fig. 4B, cf. lanes 2 and 4). Thus, the inhibition of GR activity observed in T47D/A1-2 cells by type II APs is mediated via the PR.

Antagonist-mediated Inhibition of Transcription Factor Loading on the MMTV Promoter—The chromatin remodeling of the MMTV promoter induced by glucocorticoids is concomitant with the acquisition of transcription factors to form a preinitiation complex competent for transcription initiation (15, 16). To ascertain if the failure to modify chromatin was reflected in transcription factor occupancy on the MMTV promoter after treatment with APs and glucocorticoids, we made use of an in vivo exonuclease III footprinting assay. The addition of dexamethasone resulted in a clear induction of nuclear factor I (NF1) loading onto the MMTV chromatin template, while addition of ZK98 alone did not induce NF1 loading (Fig. 5B, cf. lanes 1 and 3). Pretreatment of cells with ZK98 prior to dexamethasone addition did not significantly alter the loading of NF1 onto the promoter (Fig. 5B, cf. lanes 2 and 4).

Next, we examined the ability of type II AP-PR complexes to inhibit dexamethasone-induced transcription factor loading (Fig. 5C). Treatment of cells with the type II AP Org alone failed to induce loading of NF1 (Fig. 5C, cf. lanes 1 and 3), whereas NF1 loading was induced upon the addition of dexamethasone (Fig. 5C, cf. lanes 1 and 2). In contrast to type I APs, pretreatment with Org, substantially reduced the level of NF1 loading as compared with the dexamethasone-induced level (Fig. 5C, cf. lanes 2 and 4). Thus, the significant reduction in transcription factor loading in the presence of type II APs and dexamethasone is consistent with the decreased chromatin hypersensitivity and transcription observed under these conditions. Taken together, these results demonstrate that the PR, bound specifically to a type II but not a type I AP prevents assembly of a transcription initiation complex and thereby prevents GR-mediated activation of MMTV transcription.
PR need not be transcriptionally active (i.e., bound to an agonist such as R5020) to inhibit glucocorticoid activation.

**DISCUSSION**

The MMTV promoter has proved to be a valuable experimental system to examine the role of chromatin structure in regulating transcriptional induction by steroid receptors (14, 33, 34). While glucocorticoids, progestins, mineralocorticoids, and androgens all act, via specific receptors, to stimulate transcription, it is the GR that has been most extensively studied with respect to chromatin (34, 35). Support for a direct role of chromatin in regulating MMTV transcription has evolved from both *in vivo* and *in vitro* studies (16, 36). The *in vitro* assembly of the MMTV promoter sequences either as mononucleosomes or dinucleosomes demonstrates that this organization is sufficient to prevent the binding of NF1 to the promoter, while allowing interaction with the GR (36–38). A clear *in vivo* role for chromatin structure was established with experiments which demonstrated that the GR is able to initiate a cascade of events that result in the disruption of the local chromatin structure at the promoter and the assembly of an active transcription initiation complex (13, 16, 31, 39).

A mechanistically distinct picture emerged when activation by the PR was examined in a human breast cancer cell line that stably expresses comparable levels of the PR and GR and maintains an integrated MMTV-linked reporter (27). In this case the PR is unable to remodel chromatin and is an ineffective inducer of transcription from MMTV templates assembled as chromatin (26). Furthermore, co-stimulation of the GR and PR resulted in a progesterone-mediated down-regulation of glucocorticoid-induced transcriptional activity (26). This observation suggested that the ligand occupied PR, while being an ineffective inducer of MMTV chromatin, is, however, capable of blocking the more efficient GR-mediated transactivation from this promoter. In the present work, we used APs that were defined by their differential ability to promote PR binding to DNA, to dissect the mechanism by which PR inhibition of GR activation is mediated *in vivo*. We have shown that type II AP (Org and ZK112) but not type I AP (ZK98) bound PR inhibits glucocorticoid activation. This observation extends our previous results with the synthetic agonist R5020, by demonstrating that the inhibition of glucocorticoid-induced response requires that the PR be bound to a type II AP but not necessarily competent to induce transactivation (26).

A number of mechanisms can be envisioned by which the GR activation could be inhibited by the PR. These could include direct receptor-receptor interaction, receptor competition for binding to target elements, and receptor competition for members of the chromatin remodeling machinery required to activate MMTV transcription. Recent experiments have demonstrated that PR inhibition of GR activation is mediated *in vivo*. The MMTV promoter has proved to be a valuable experimental system to examine the role of chromatin structure in regulating transcriptional induction by steroid receptors (14, 33, 34). While glucocorticoids, progestins, mineralocorticoids, and androgens all act, via specific receptors, to stimulate transcription, it is the GR that has been most extensively studied with respect to chromatin (34, 35). Support for a direct role of chromatin in regulating MMTV transcription has evolved from both *in vivo* and *in vitro* studies (16, 36). The *in vitro* assembly of the MMTV promoter sequences either as mononucleosomes or dinucleosomes demonstrates that this organization is sufficient to prevent the binding of NF1 to the promoter, while allowing interaction with the GR (36–38). A clear *in vivo* role for chromatin structure was established with experiments which demonstrated that the GR is able to initiate a cascade of events that result in the disruption of the local chromatin structure at the promoter and the assembly of an active transcription initiation complex (13, 16, 31, 39).

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**Fig. 3. Type II APs inhibit GR-mediated hypersensitivity.** A, T47D/A1-2 cells were untreated (lane 1), treated with dexamethasone (10⁻⁷ M) for 6 h (lane 2), treated with ZK98 (10⁻⁸ M) for 24 h (lane 3), or pretreated with ZK98 (10⁻⁸ M) for 18 h prior to dexamethasone (10⁻⁷ M) addition for 6 h (lane 4). Nuclei were isolated and digested with restriction endonuclease *Sst*I for 15 min at 30 °C. After purification, genomic DNA was digested with *Bam*HI to provide an internal standard and 10 mg of each sample was amplified using *Taq* polymerase and a 3²P-labeled single-stranded primer specific to the MMTV promoter. Purified extension products were analyzed on 5% polyacrylamide denaturing gels and exposed to DuPont Reflection film at room temperature.

**B**

Dex (10⁻¹ M) Org31710 (10⁻⁴ M) 

Dex (10⁻¹ M) Org31710 (10⁻⁴ M) 

**C**

Dex ZK112993 (10⁻⁴ M) 

Dex ZK112993 (10⁻⁴ M)
strated that the mineralocorticoid receptor and GR as well as the androgen receptor and GR are able to physically associate to form heterodimers in vitro (40, 41). This heterodimerization takes place via contacts between the carboxyl-terminal zinc fingers in both proteins. In transfection experiments the co-expressed mineralocorticoid receptor and GR are able to heterodimerize on a shared HRE in vivo leading to the inhibition of GR-mediated induction by mineralocorticoid (41). In our experiments we have failed to detect PR and GR heterodimers in gel shift experiments using nuclear extracts from cells maintained under the drug regimens described above. Under these conditions antibodies for either the GR or the PR demonstrate that only homodimers of either the PR or GR are detected.3

One mechanism by which the PR could block GR activation would be via a direct competition for binding to the HREs in the promoter. As indicated previously, both receptors are able to interact with the HREs and simulate transcription when their respective agonists are independently supplied (27). Thus the requirement for PR to be complexed to a type II AP to inhibit the GR may suggest that the PR forms a non-productive complex with the MMTV promoter to prevent GR activation. Al-

3 C. J. Fryer and T. K. Archer, unpublished data.
though the type II AP/PR has been shown to bind to DNA in vitro (8–11) whether this occurs in vivo and in this system has not been explicitly demonstrated. Consistent with these in vitro studies we have detected PR binding to an HRE in the presence of a type II AP but not upon treatment with the type I AP (data not shown). While competition between the receptors for DNA binding is a possible explanation we do not feel it is likely given that the inhibition is specific for the integrated promoter assembled as chromatin and not detected on transiently introduced MMTV templates.

Further support for this idea comes from transient transfection experiments utilizing overexpression of both individual receptors, receptor isoforms, and target templates. These studies have demonstrated that the PR-A isoform is able to act as a potent trans-dominant repressor of hPR-B-mediated transcription within certain promoter and cell contexts where hPR-A fails to activate transcription (42, 43). Furthermore, the PR-A isoform was shown to be responsible for the progesterone-mediated repression of glucocorticoid, estrogen, androgen, and mineralocorticoid receptor-mediated gene transcription by a mechanism that did not require DNA binding (42, 44). We have not been able to ascertain if the same holds true in our experiments as both PR-A and -B isoforms are expressed in these cells at comparable levels (27).
Although type I and type II APs were initially distinguished by their differential ability to induce DNA binding by the PR in vitro they also exhibit differences in several other properties. Type II APs have been shown to acquire agonist activity and decreased antagonist activity upon stimulation of cAMP signaling pathways, whereas type I APs do not exhibit positive functional cooperation with cAMP (45, 46). The two types of APs have also been shown to have distinct effects on site-specific PR phosphorylation (47) and PR carboxyl-terminal tail conformation (48). We have also detected a slower mobility of the PR on SDS gels upon treatment with the progestin agonist R5020, or the type II AP, Org, but not upon treatment with the type I AP, ZK98.4 A similar mobility shift of the PR in T47D cells has been attributed to phosphorylation of Ser345 (49). The differential protein phosphorylation conformation induced by type I and type II antiprogestins may influence protein-protein interaction between the steroid receptor and factors required for steroid hormone activation of gene transcription resulting in distinct biological effects of these antiprogestins. This concept has recently been validated for steroid receptors with the SWI/SNF complex and consequently sequester it from the GR, thereby inhibiting its activity.

Our results suggest a model in which the ability of the PR to inhibit the GR-mediated activation of transcription is critically dependent on the class AP to which the PR is bound. Furthermore, it reveals the PR need not be transcriptionally competent, to prevent the disruption of chromatin intrinsic to the activation process (Fig. 6). In the absence of hormone, the MMTV promoter is maintained in a closed chromatin conformation that restricts transcription factor access, resulting in a low basal level of transcription. Upon glucocorticoid addition the chromatin organization of the promoter is remodeled with the concurrent formation of the transcription preinitiation complex and induction of transcription. Pretreatment with type II, but not type I APs results in a reduced GR transcriptional response suggesting that the PR must be bound by a specific type of antiprogestin to inhibit the GR. In the GR-mediated activation of transcription from the MMTV promoter the binding of the receptor is inseparable from the chromatin disruption that follows (22, 31, 39). Consequently our results would be consistent with either: 1) a mechanism by which the PR competed directly with the GR for binding to the HREs or 2) acted to prevent the remodeling of the MMTV chromatin. As we have no evidence for PR binding to MMTV chromatin in these cells and observe the inhibition of chromatin remodeling, we favor the second mechanism to explain our observations. Consistent with this idea, we do not observe this inhibition when analyzing transiently introduced MMTV reporters.4 This is particularly relevant as we have shown previously that, under these conditions where chromatin remodeling is not required for transactivation (16), the PR is able to activate transcription from transient templates (26). Thus, our results argue for a direct role for the assembly of these sequences into a specific chromatin structure in the control of glucocorticoid and progestin transcriptional activation.
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