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Differential activation of the IκBα and mouse mammary tumor virus promoters by progesterone and glucocorticoid receptors

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Abstract

The glucocorticoid and progesterone receptors (GR and PR) are structurally homologous and bind a common hormone response element (HRE). The mechanisms by which receptors activate specific promoters when multiple steroids are present in a cell is a critical question in endocrinology. To investigate how co-existing steroid receptors regulate gene transcription, we have compared two hormone-responsive promoters in T47D/A1–2 human breast cancer cells expressing both the GR and PR. The promoters chosen were those for the mouse mammary tumor virus (MMTV) and the gene for IκBα, the inhibitor of the ubiquitous transcription factor, nuclear factor kappa B (NFκB). Several differences between glucocorticoid and progestin activation of the IκBα and MMTV promoters were revealed. Both steroids activated the endogenous IκBα promoter, while only glucocorticoids activated a stably integrated MMTV promoter. In combination, progestins enhanced glucocorticoid activation of IκBα, but antagonized glucocorticoid activation of MMTV. These differences in steroid receptor competition were further demonstrated when levels of the PR were reduced by prolonged treatment with progestin. Under these conditions, the PR no longer competes effectively with the GR for activation of the MMTV promoter. However, on the IκBα promoter, the GR and PR still activate the promoter in a cooperative fashion. Another difference between the two promoters is their chromatin structure. In this cell line, the MMTV promoter chromatin is "closed" and insensitive to restriction enzyme cleavage, while the IκBα promoter is "open." Using PR antagonists, we demonstrate that at least one cofactor complex, the BRG-1 chromatin remodeling complex, differentially contributes to activation of both promoters.
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1. Introduction

Steroid hormone receptors (SHRs) are ligand-activated transcription factors that regulate the expression of genes involved in development, homeostatic mechanisms, and cellular differentiation [1]. SHRs repress or activate transcription by interacting with cofactors, chromatin remodeling complexes, and the basal transcriptional machinery [2]. A subfamily consisting of the receptors for androgens, glucocorticoids, progestins, and mineralocorticoids share regions of high homology and bind a common hormone response element (HRE) [3]. Despite their structural similarity and common HRE, these receptors achieve different physiological consequences by targeting gene promoters in a receptor-specific manner.

Tissue-specific expression of receptors is one mechanism by which receptor-selective gene expression is accomplished [4]. However, in many cell types, steroid receptors co-exist. Consequently, co-expressed receptors must exploit additional mechanisms to specifically regulate transcription. These mechanisms include tissue-specific steroid hormone metabolism, regulation by other proximal-bound transcription factors, selective binding to HRE sequence variants, and competition for receptor-specific transcriptional coactivators. For example, early squelching studies with steroid receptors indicated that SHRs compete for factors that were not part of the basal transcriptional machinery [5]. Since then, numerous transcriptional coactivators have been identified through genetic and biochemical approaches [6,7]. Many of these coactivators modulate steroid-activated transcription by disrupting chromatin structure [7–9].

In the eukaryotic nucleus, DNA is wrapped around histone proteins, forming chromatin. Chromatin structure of promoters is one barrier to transcription that SHRs must

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overcome [10]. Highly compact regions of chromatin are associated with low transcriptional activity, while less compact regions show higher transcriptional activity [11]. Two main coactivator families that disrupt chromatin include chromatin remodeling complexes and histone acetyltransferases (for recent reviews, see [7,9]). Steroid receptors compete for members from both of these families of coactivators. For example, the PR competes with the GR for binding to BRG-1, a component of the SWI/SNF chromatin remodeling complex in cells co-expressing these receptors [12]. ER squelching of PR transactivation is reversed by overexpression of SRC-1 or p300, both histone acetyltransferases and SHR coactivators [13,14]. Thus, the differential ability of SHRs to disrupt chromatin structure may result from their ability to recruit chromatin remodeling complexes and/or compete for chromatin remodeling coactivators.

To investigate the mechanisms by which multiple steroid receptors specifically activate steroid-responsive promoters within the same cell, we turned to the mouse mammary tumor virus (MMTV) and *IκBα* genes. The chromatin organization of both promoters has been previously defined [15,16]. When stably integrated into mammalian cells, the MMTV promoter acquires a phased array of six nucleosomes [17]. Glucocorticoid treatment induces chromatin remodeling, and transcription factor loading [18]. The MMTV LTR is activated by several SHRs, including those for glucocorticoids and progestins [19]. The IκBα promoter is also activated by both glucocorticoids and progestins in tissue culture cells [20,21]. Like the MMTV promoter, the IκBα promoter is assembled into a regular array of nucleosomes, and promoter activation may lead to transcription factor binding [15]. However, the chromatin of the IκBα promoter is in an "open" or remodeled state prior to hormone addition.

We compared steroid activation from the endogenous human IκBα promoter and a stably integrated MMTV promoter in T47D/A1–2 breast cancer cells, which express comparable levels of PR and GR. Although, both promoters were activated by glucocorticoids, only the IκBα promoter was activated by progestin. Secondly, when both steroids were used in combination, progestin enhanced glucocorticoid activation of IκBα, but antagonized glucocorticoid activation of MMTV. Thus, although the PR and GR compete for a limiting cofactor on the MMTV promoter, this competition does not occur on the IκBα promoter. When PR levels are reduced by prolonged progestin treatment, competition with the GR is lost on the MMTV promoter, but the receptors maintain their additive effect on the IκBα promoter. The antiprogestin, ORG31710, which inhibits glucocorticoid induction of MMTV by sequestering the chromatin remodeling complex, BRG-1, also inhibits IκBα transcription, but not to the same extent. These results suggest that while transcriptional activation of the IκBα and MMTV promoter may share common cofactors, glucocorticoid activation of each promoter may require distinct coactivator complexes.

2. Materials and methods

2.1. Cells

T47D/A1–2 cells were derived from T47D breast cancer cells by stable transfection with the plasmids pGRneo and pHHLuc as described previously [22]. The plasmid pHHLuc contains MMTV sequences from *Hae*III (−224) to *Hpa*II (+100). T47D/A1–2 cells were grown at 37 °C with 5% CO_2 in modified Eagle's medium containing 10% fetal bovine serum and 0.16 mg/ml Geneticin (Invitrogen Corporation, Carlsbad, CA).

2.2. Preparation of nuclear extracts

The protocol for preparing nuclear and cytoplasmic extracts was carried out as described previously [23]. Briefly, A1–2 cells were plated on 100 mm dishes and grown until 80% confluent. The cells were resuspended in Buffer E containing 0.3% NP40, incubated for 5 min on ice, then centrifuged on a benchtop centrifuge at 2500 rpm for 5 min at 4 °C. The supernatant was removed as cytoplasmic extract, and the pellet of nuclei resuspended in Buffer E without NP40, then centrifuged again at 2500 rpm for 5 min. The pellet was resuspended in Buffer C, and the NaCl concentration adjusted to 0.4 M. After incubation at 4 °C with gentle rotation, the mixture was centrifuged at 12,500 rpm for 10 min at 4 °C and the supernatant removed as nuclear extract.

2.3. Gel mobility shift assay

Gel shift assays were carried out by pre-incubating 10 μg of nuclear extract and 1 μl of poly dI/dC (1 μg/ml) in binding buffer (10 mM Tris–HCl (pH 7.5), 50 mM NaCl, 3 mM DTT, 10% glycerol, 0.05% NP40, and 0.1 mM $ZnCl_2$) at room temperature for 10 min [24]. Double-stranded oligonucleotides for the SP1 (Geneka Biotechnologies, Montréal, Canada) and IL-2 promoter nuclear factor kappa B (NFκB) consensus sequences were end labeled with γ^{32}P ATP and T4 polynucleotide kinase, then incubated with the extract for 20 min at room temperature. The mixture was then electrophoresed on a 5% non-denaturing polyacrylamide gel in 1× Tris–borate–EDTA buffer. The gels were dried and exposed to film.

2.4. Isolation of RNA: Northern blots and RT-PCR

Cells were left untreated or treated as previously described. Cells were plated on 100 mm dishes and grown until 80% confluent. The cells were washed with PBS, then scraped from the dishes with PBS, and briefly pelleted by centrifugation in 5 ml polypropylene tubes. Total cellular RNA was isolated using TRIZOL (Invitrogen Corporation, Carlsbad, CA) according to the manufacturer's instructions.

2.4.1. RT-PCR

The cDNA was synthesized as described previously [25]. For PCR of the cDNA, two separate reactions were run for each experimental condition. The 300 ng RNA was combined with MMTV primers, and 100 ng RNA for β2-microglobulin primers to serve as a control. The RNA was combined with 5 U of Taq DNA polymerase in a final volume of 50 μl. The PCR mixture contained 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 100 μM each dNTP, and 5 pmol of each primer MMTV-619 and MMTV-618 (end labeled with T4-polynucleotide kinase to generate a ^{32}P-labeled single-stranded primer). Human β2-microglobulin was similarly amplified using sequences previously described [25]. PCR products were analyzed on 8% polyacrylamide gels and exposed to Molecular Dynamics Phosphorimager (Amersham Biosciences Inc., Piscataway, NJ) screens or autoradiography film for analysis.

2.4.2. Northern analysis

The 10 μg of RNA was separated on 1% agarose gels containing formaldehyde and MOPS (3-(*N*-morpholino) propane-sulfonic acid) buffer, and the RNA blotted to Zeta Probe nylon membrane (Bio-Rad Laboratories, Hercules, CA) in 10× SSC overnight at room temperature. The membrane was hybridized overnight with a ^{32}P-labeled IκBα cDNA *Pst*I–*Pst*I fragment corresponding to +272/+455 of the IκBα cDNA sequence, which was cut and purified from a CMV-IκBα plasmid (kindly donated by Dr. A. Israel). This fragment was labeled with ^{32}P by random priming (Ready-to-Go beads, Amersham Biosciences Inc., Piscataway, NJ) and the membrane hybridized overnight according to the manufacturer's 'standard protocol' instructions. As a loading control, the same membrane was similarly hybridized with either a rat cyclophilin cDNA fragment (kindly donated by Dr. G. DiMattia) or a cyclophilin 40-mer oligonucleotide (Geneka Biotechnologies, Montréal, Canada). The cyclophilin cDNA was labeled by random priming and hybridized as described for the IκBα cDNA fragment. The cyclophilin oligonucleotide was end labeled with ^{32}P using polynucleotide kinase, and incubated according to the Zeta Probe membrane "oligonucleotide" protocol. After hybridization and washing, the membrane was visualized and quantified by the Molecular Dynamics Phosphorimager (Amersham Biosciences Inc., Piscataway, NJ).

2.5. Restriction enzyme hypersensitivity analysis

Cells were either untreated, or treated as described in the figure legends. Nuclei were digested in vivo with 30 U of *Ava*I or *Sst*I as described previously [17]. After purification of genomic DNA, samples were recut with *Dpn*II or *Bam*HI. DNA fragments were analyzed using linear Taq polymerase amplification with a ^{32}P-labeled single-strand primer corresponding to the +56 to +73 region of the IκBα coding region [26] or the +60 to +84 region of the MMTV coding region (MMTV-22). Purified extended products were analyzed on 8% polyacrylamide denaturing gels and quantified using the Molecular Dynamics Phosphorimager (Amersham Biosciences Inc., Piscataway, NJ).

2.6. Western blot analysis

Cells were plated on 100 mm dishes and grown until approximately 80% confluence before preparation of extracts. Whole cell extracts were prepared using "single-detergent lysis buffer" [27]. The 100 μg of whole cell extract was separated by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) on NOVEX Tris–glycine gels, transferred to PVDF membrane, and immunoblotted with the following antibodies: GR (BD Biosciences Clontech, Palo Alto, CA) or PR (AB-52; Santa Cruz Biotechnology, Inc., Santa Cruz, CA).

3. Results

3.1. Both glucocorticoids and progestins repress NFκB activity in A1–2 cells

NFκB is maintained in an inactive form in the cytoplasm, bound by the IκB family of inhibitors. Of this family, IκBα has been the most thoroughly characterized. As an indirect measure of IκB levels, we first determined if glucocorticoid or progestin treatment would repress NFκB activity in these cells using a gel mobility shift assay. We found that pretreatment with the synthetic glucocorticoid, dexamethasone (dex), or the synthetic progestin, R5020, repressed activation of NFκB by the phorbol ester, PMA (Fig. 1A, compare lane 2 with lanes 5–6). Both steroids repressed NFκB to comparable levels, and in combination repressed NFκB even more than either steroid alone (Fig. 1A, lane 7). PMA treatment was maintained for the remainder of the studies unless otherwise indicated, to ensure that the same treatment conditions were used to study the DNA binding activity of NFκB in Fig. 1A.

3.2. Progestins enhance the glucocorticoid-induced increase in IκBα RNA, but antagonize this increase in MMTV RNA

We next determined if the repression of NFκB was due, at least in part, to an increase in IκBα expression by dex or R5020. A1–2 cells were treated with dex and R5020 as in the gel shift experiments. Both steroids increased IκBα RNA levels three- to four-fold (Fig. 1B, lanes 3–4), and in combination, had an additive effect on RNA levels (compare lanes 3 and 4 with 5). PMA at this dose had no significant effect on IκBα RNA levels (Fig. 1B, lane 2). In contrast, previous studies with the MMTV promoter in A1–2 cells have shown that although the endogenous PR activates transcription of MMTV much less efficiently than the GR, the PR inhibits the GR-dependent activation of MMTV when

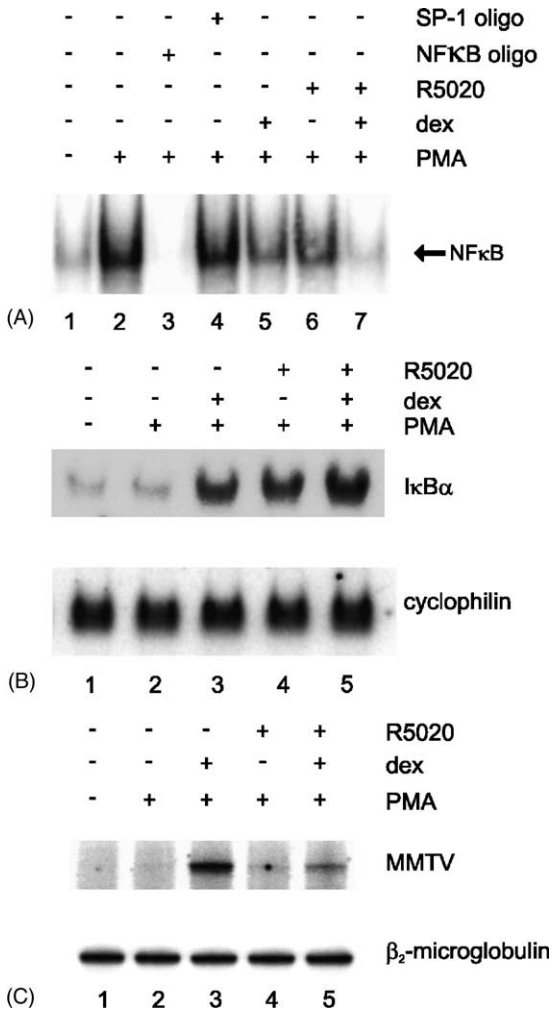

Fig. 1. Differential activation of the IκBα and MMTV promoters by glucocorticoids and progestins. (A) Gel shift assays were conducted using A1–2 cells that were either untreated (lane 1) or treated with PMA (40 ng/ml) for 45 min (lanes 2–4). Cells were pretreated with dexamethasone (dex; 10^{-7} M; lane 5) or R5020 (10^{-8} M; lane 6) or the combination (lane 7) for 4 h. Treatment with PMA (40 ng/ml) for 45 min followed all steroid treatments (lanes 2–7). Nuclear extracts were prepared, and analyzed by gel shift, using 10 μg of nuclear extract with a ^{32}P-labeled double-stranded oligonucleotide corresponding to the NFκB consensus sequence of the IL-2 promoter. An unlabeled NFκB oligonucleotide and an unlabeled SP1 oligonucleotide were added in 100-fold excess in lanes 3 and 4, respectively. The binding reactions were analyzed on a 5% non-denaturing polyacrylamide gel, followed by autoradiography. Note that free probe was run off the gel. (B) Cells were either untreated (lane 1) or treated with PMA (40 ng/ml) for 45 min (lane 2). Cells were pretreated with dex (10^{-7} M; lane 3) or R5020 (10^{-8} M; lane 4) or both steroids (lane 5) for 4 h. Following steroid treatment, the cells were treated with PMA (40 ng/ml) for 45 min (lanes 3–5). Total cellular RNA was isolated and analyzed by Northern blot. (C) Aliquots of the same RNA prepared in 'B' were analyzed for MMTV and β2-microglobulin RNA levels by RT-PCR.

glucocorticoids and progestins are used in combination [16]. Although, R5020 treatment enhanced the dex increase in IκBα RNA levels, R5020 had the opposite effect on the dex increase in MMTV RNA levels (Fig. 1C, compare lanes 3–5). These results suggest that the PR may either cooperate or interfere with GR-mediated transcription in the same cell, depending on the promoter context.

3.3. Loss of progesterone receptor eliminates antagonism of the GR

To further demonstrate the differential activation of the two promoters by the PR and GR, we reduced PR protein levels by prolonged treatment with progestin before addition of glucocorticoid. PR down regulation by prolonged progestin exposure has previously been reported in T47D cells [28]. We predicted that if the PR antagonizes GR transactivation of the MMTV promoter, then reduction of PR levels should inhibit this antagonistic effect. However, on the IκBα promoter, a reduction in PR levels should simply reduce the additive effect of the two receptors. Progestin treatment for 4 h partially reduced levels of both PR_A and PR_B isoforms (Fig. 2A, upper panel, lanes 3 and 4). However, 20 h R5020 treatment reduced PR levels dramatically (Fig. 2A, lanes 5 and 6). Dex treatment for 4 h had no effect on PR levels in the presence or absence of R5020 (Fig. 2A, lanes 2, 4, and 6). GR levels were unaffected by R5020 treatment, while dex treatment partially reduced GR levels (Fig. 2A, lower panel).

We then examined RNA levels under these conditions of prolonged progestin exposure. As expected by the lower PR levels, the increase in IκBα RNA due to progestin treatment was now reduced compared to 4 h R5020 treatment (compare Fig. 1B, lane 4 with Fig. 2B, lane 4) and also lower than the glucocorticoid-mediated increase. In combination, the two steroids maintained their additive effect (Fig. 2B, lane 5). In contrast, dex activation of MMTV was no longer antagonized by co-treatment with progestin (Fig. 2C, lane 5), suggesting that less PR was available to compete for activation of the MMTV promoter.

3.4. Differences in promoter chromatin structure of the IκBα and MMTV promoters

To compare these two promoters at another level of gene regulation, we investigated the chromatin structure of the proximal promoters using a restriction enzyme hypersensitivity assay (Fig. 3). In this assay, a change in restriction enzyme sensitivity of DNA wrapped around a nucleosome indicates a change in chromatin structure, also known as 'chromatin remodeling.' We have previously shown in the A1–2 cell line that in the absence of steroid the IκBα promoter chromatin is "open" and sensitivity to restriction enzymes which cut within nucleosomes does not change significantly upon hormone treatment [15]. In contrast, the MMTV promoter within the same cell line is resistant to enzymatic cleavage within nucleosome-B (nuc-B); 1 h of dex treatment renders nuc-B hypersensitive, while progestin treatment has little effect on hypersensitivity. In combination, progestins antagonize this dex-induced hypersensitivity [16]. Here, we investigated the effect of both

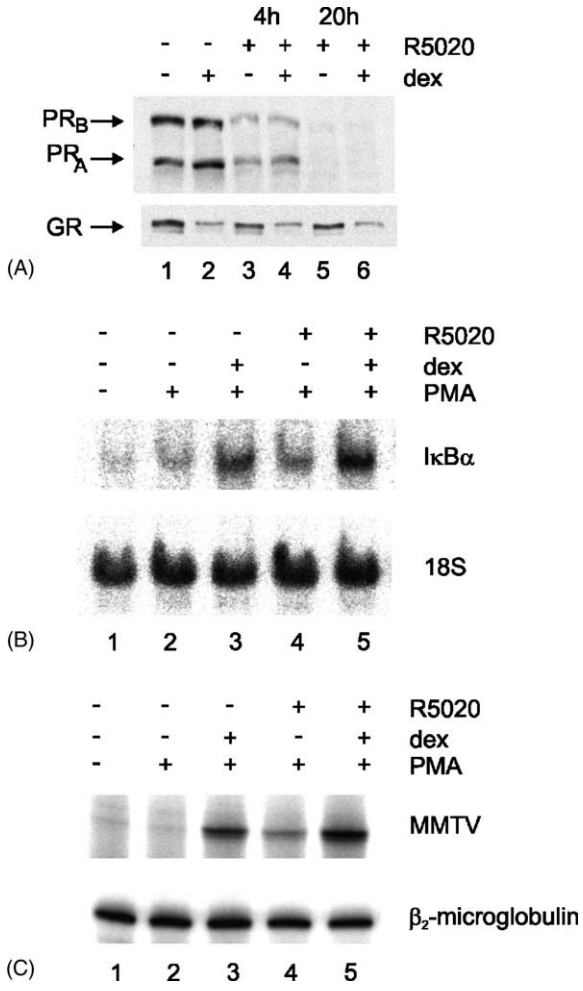

Fig. 2. Loss of PR compromises PR–GR antagonism on the MMTV promoter. (A) Cells were either untreated (lane 1), treated with dex (10^{-7} M) for 4 h (lanes 2, 4, 6) either in combination with R5020 (10^{-8} M) for 4 h (lanes 3 and 4) or pretreated with R5020 (10^{-8} M) for 16 h (lanes 5 and 6) prior to addition of dex for 4 h. Whole cell extracts were prepared and separated by SDS-PAGE, transferred to PVDF membrane, and immunoblotted with antibodies to the PR (upper panel) or GR (lower panel). (B) Cells were either untreated (lane 1) or treated with PMA (40 ng/ml) for 45 min (lane 2), treated with dex (10^{-7} M) for 4 h (lane 3) or R5020 (10^{-8} M) for 20 h (lane 4), or R5020 for 16 h after which dex was added for 4 h (lane 5). Following steroid treatment, the cells were treated with PMA (40 ng/ml) for 45 min (lanes 3–5). Total cellular RNA was isolated and analyzed by Northern blot. (C) Aliquots of the same RNA prepared in 'B' were analyzed for MMTV and β2-microglobulin RNA levels by RT-PCR.

short (1 h) and prolonged (17 h) progestin treatment on the PRs ability to antagonize GR-mediated MMTV hypersensitivity. Treatment with dex increased nuc-B sensitivity to *Sst*I (Fig. 3A, lane 3). The short-term (1 h) treatment with R5020 alone had little effect on hypersensitivity (lane 4), but as shown previously, R5020 co-treatment antagonized the dex-mediated increase in hypersensitivity (lane 5). In contrast, the ability of R5020 to antagonize the dex-mediated increase in *Sst*I sensitivity was lost after prolonged treatment (Fig. 3A, lane 7). On the IκBα promoter, neither dex nor R5020 altered promoter hypersensitivity. The combination of dex and R5020 were also without effect. Thus, in contrast to MMTV, IκBα promoter enzyme sensitivity was generally unaffected by hormone treatment (Fig. 3B, lanes

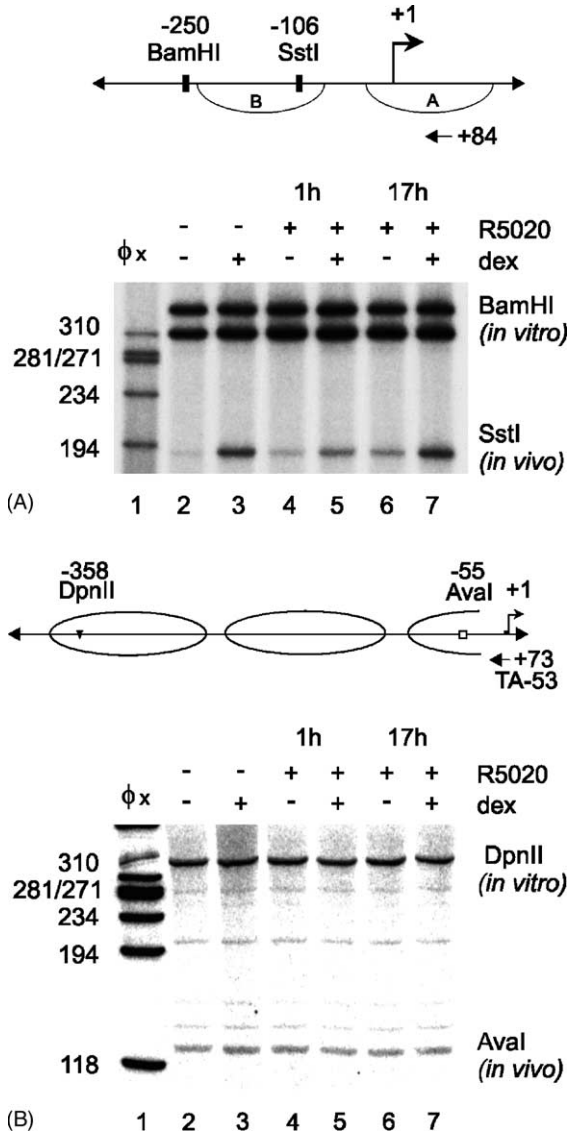

Fig. 3. The IκBα and MMTV promoters adopt distinct chromatin structures in the absence and presence of hormone. (A) Upper panel: schematic of nucleosome position, restriction enzyme sites, and probe locations for analysis of the MMTV promoter. Both short-term (1 h) and long-term (17 h) progestin treatment was carried out. For combination treatments, dex was co-treated with R5020 for 1 h, or added for 1 h after long-term R5020 treatment. Specifically, A1–2 cells were either untreated (lane 2), treated for 1 h with dex (10^{-7} M; lane 3), 1 h with R5020 (10^{-8} M; lane 4), 1 h with both dex and R5020 (lane 5), 17 h with R5020 (10^{-8} M; lane 6), or with R5020 (10^{-8} M) for 16 h followed by 1 h of dex (10^{-7} M; lane 7). Nuclei were isolated, digested in vivo with *Sst*I and *Bam*HI, and analyzed by reiterative primer extension and denaturing polyacrylamide gel electrophoresis. (B) Upper panel: schematic of nucleosome position, restriction enzyme sites, and probe locations for analysis of the IκBα promoter. A1–2 cells were treated as in 'A'. Nuclei were isolated, digested in vivo with *Ava*I and in vitro with *Dpn*II, and analyzed by reiterative primer extension and denaturing polyacrylamide gel electrophoresis.

3–7). PMA treatment had no significant effect on chromatin restriction enzyme hypersensitivity in either the absence or presence of hormone on both promoters (data not shown). Therefore, within the same cell, the IκBα and MMTV promoters differ in chromatin structure in both the absence and presence of progestin and/or glucocorticoid.

3.5. Antiprogestin similarly affects glucocorticoid activation of both promoters

To compare cofactors required for activation of the IκBα and MMTV promoters, we took advantage of the antiprogestin, ORG31710 (ORG). The PR bound to ORG antagonizes glucocorticoid activation of the MMTV promoter by sequestering the BRG-1 chromatin remodeling complex away from the GR, thus, inhibiting GR-mediated transactivation [12,25]. We therefore, wanted to determine if this complex was also required for activation of the IκBα promoter, given the differences in chromatin structure that we observed. For both promoters, 4 h of dex treatment increased RNA levels (Fig. 4A and B, lane 3). ORG alone had little effect on RNA levels of either IκBα or MMTV (lane 4). However, 18 h pre-treatment with ORG inhibited dex activation of IκBα by 42% (Fig. 4A, lane 5) and MMTV activation by 65% (Fig. 4B, lane 5). This suggests that in contrast to progestin treatment, antiprogestin treatment similarly affects glucocorticoid activation of both promoters (Fig. 4C).

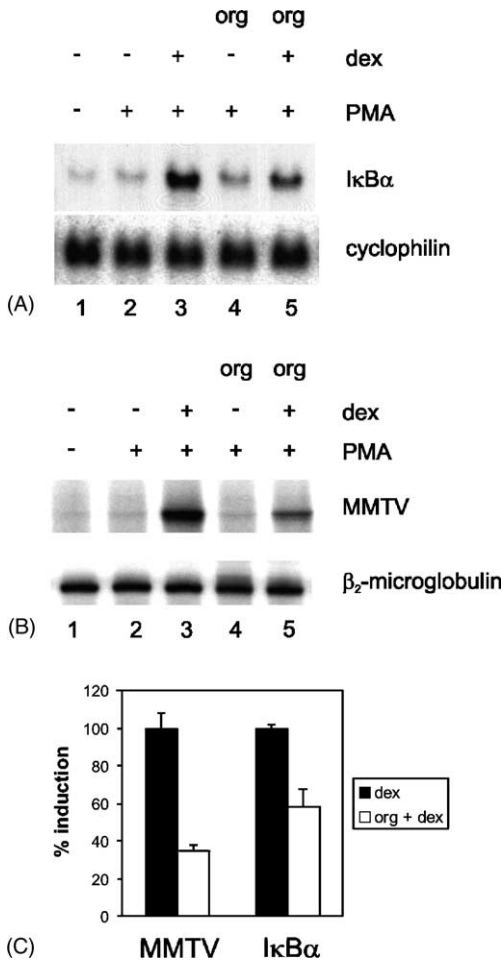

Fig. 4. The antiprogestin ORG31710 inhibits glucocorticoid activation of both the IκBα and MMTV promoters. (A) Total cellular RNA was isolated from cells and analyzed by Northern blot. Lane 1, no treatment, Lane 2, PMA (40 ng/ml) for 45 min, lane 3, dexamethasone (10^{-7} M) 4 h. In addition, cells were treated for 22 h with ORG31710 alone—ORG31710 (10^{-8} M; lane 4), or pretreated with antiprogestin for 18 h, followed by dexamethasone (10^{-7} M) for 4 h (lane 5). In all cases, steroid treatment was followed by PMA treatment (40 ng/ml) for 45 min (lanes 3–5). (B) ORG31710 inhibits glucocorticoid activation of the MMTV promoter. Aliquots of the same RNA prepared in 'A' were analyzed for MMTV and β2-microglobulin RNA levels by RT-PCR as described previously. (C) Summary chart of data from A and B. Both experiments were conducted three times, and averages and standard deviation determined from these experiments.

4. Discussion

The receptors for progesterone, glucocorticoids, androgens, and mineralocorticoids form a subset of the SHRs based on structural similarity and sequence-binding specificity [3]. Several mechanisms exist that explain how multiple receptors with similar or identical sequence specificity can differentially activate genes when co-expressed in tissues or cells. Examples of these mechanisms include regulation by other proximal-bound transcription factors, selective binding to HRE sequence variants, and competition for receptor-specific transcriptional coactivators [12,29,30]. In addition, ability to disrupt chromatin structure by recruiting necessary transcriptional cofactors is another mechanism of receptor-specific gene activation.

To investigate differential steroid activation of two integrated promoters within the same cell type, we took advantage of the T47D/A1–2 human breast cancer cell line [16]. A1–2 cells contain comparable levels of the PR and GR and an integrated MMTV-luciferase reporter gene. In these cells, the integrated MMTV promoter acquires a 'closed' chromatin configuration and requires glucocorticoid treatment to 'open' the promoter and to activate transcription. However, progestin treatment does not significantly induce chromatin remodeling or activate transcription of the integrated promoter.

We sought to expand these previous observations by investigating steroid activation of an endogenous, hormone-responsive promoter within A1–2 cells. The effects of both progestins and glucocorticoids on NFκB activity and RNA of the endogenous IκBα promoter were investigated, and compared with the MMTV promoter under the identical conditions. We found that both glucocorticoids and progestins repressed NFκB activity in A1–2 cells, and this repression correlated with increased transcription of the IκBα promoter. Both glucocorticoids and progestins have been shown to inhibit NFκB activation and increase IκBα RNA levels in several cell types [20,21,31]. The effect of the steroids in combination was cooperative with respect to

NFκB DNA binding activity and IκBα RNA levels. Therefore, in contrast to MMTV, progestin treatment enhanced glucocorticoid activation of the IκBα promoter. In addition to the IκBα promoter, there are other examples in which SHRs cooperatively activate promoters. A human mineralocorticoid receptor splice variant enhances GR-mediated transactivation of a transiently introduced MMTV promoter, and in mouse fibroblast cell lines containing an integrated MMTV promoter, co-treatment of dihydroxytestosterone enhances dexamethasone-mediated transactivation [32,33].

To confirm that the PR and GR cooperatively activated the IκBα promoter but were antagonistic on the MMTV promoter, we reduced PR levels by prolonged (20 h) exposure of the synthetic progestin R5020 prior to glucocorticoid activation. This reduction in PR levels reduced R5020 activation of the IκBα promoter compared to a 4 h treatment. As expected, the cooperative effect of the two receptors was unchanged on the IκBα promoter. In contrast, reduction of available PR compromised the ability of the PR to antagonize GR-mediated MMTV activation. As has previously been observed in other cell lines, dex treatment partially reduced GR levels [34]. We conclude that the IκBα promoter and MMTV promoters are differentially regulated by the PR and GR. Whether steroid receptors compete or cooperate to activate transcription depends, among other factors, on the promoter and cellular context.

To investigate differences in promoter architecture that might contribute to this differential steroid activation, we compared the chromatin structure of the promoters before and after treatment with glucocorticoids and/or progestins using the restriction enzyme hypersensitivity assay. In this cell line, the IκBα promoter maintains an open chromatin structure in the absence of hormone, while the MMTV promoter is closed and requires hormone to be opened [15,16]. As predicted from the RNA results, prolonged treatment with progestin compromised the PR's ability to antagonize GR-mediated chromatin remodeling. On the IκBα promoter, no significant change in hypersensitivity was observed by either glucocorticoid or progestin, alone or in combination. Therefore, in the absence of hormone these promoters within the same cell acquire distinct chromatin structures. Upon hormone addition, the MMTV promoter chromatin is substantially remodeled, while the IκBα promoter chromatin is largely unaffected.

Progestin antagonism of GR-mediated transcription from MMTV has been shown to be due to competition for the BRG-1 chromatin remodeling complex [12]. Thus, on MMTV, the PR and GR compete for a limiting cofactor required for full activation. However, on IκBα, although the PR and GR may require similar cofactors to activate transcription, this factor cannot be limiting, since the two steroids in combination additively enhanced transcription compared to either steroid alone. These results suggest that the GR and PR may require distinct coactivator complexes to activate these two promoters.

To further determine if steroid activation of these promoters required common coactivators, we took advantage of antiprogestin compounds previously used to examine the mechanisms of PR–GR antagonism on MMTV. These studies showed that the antiprogestin ORG31710 bound to the PR blocked GR from activating the MMTV promoter in the presence of glucocorticoids [25]. This inhibition resulted from the ability of PR to compete with the GR for binding to BRG-1 and was specific for the MMTV promoter assembled as chromatin [12]. Interestingly, although the SWI/SNF complex was sequestered by the PR, the GR remained bound to coactivators such as p300 and SRC-1 [12]. To determine if the PR and GR might compete for BRG-1 on the *IκBα* gene, we investigated the effect of ORG31710 on glucocorticoid activation of IκBα. Similar to MMTV, ORG31710 inhibited glucocorticoid-induced activation of the IκBα promoter. However, although ORG31710 antagonized the glucocorticoid-induced increase in IκBα RNA levels, this inhibition was not as great as for MMTV. These results suggest that although the SWI/SNF complex may be required for full activation of IκBα transcription, it may play a lesser role than on the MMTV promoter. It is possible that the remodeled state of the IκBα promoter is actively maintained, at least in part, via the BRG-1 complex. Correspondingly, these results suggest a potentially greater role for the SRC-1 and p300 coactivators, that can still associate with the GR, in the activation of IκBα transcription seen in the presence of dex and ORG31710 [12]. The BRG-1 complex may also have other transactivational activities in addition to chromatin remodeling, because BRG-1 expression enhances glucocorticoid activation of a transiently transfected MMTV template in SW-13 cells, which lack endogenous BRG-1 [35]. Transiently transfected MMTV templates do not acquire the same phased nucleosomal array characteristic of the integrated MMTV template [18]. Alternatively, the ORG31710-bound PR may sequester another limiting coactivator required for IκBα transcription that has not yet been identified, that may or may not be part of the BRG-1 chromatin remodeling complex. The antagonist-bound PR may also sequester another transcription factor required for IκBα transcription, such as SP-1 or NFκB, that are required for activation of IκBα by PMA [36].

We have compared the effect of steroids on the RNA levels and chromatin structure of two different promoters in the same cell line under identical conditions. In particular, we have compared the differential ability of the PR and GR to activate promoters in cells where these two receptors are co-expressed. Our work comparing steroid activation of the MMTV and IκBα promoters suggests that multiple steroids within the same cell can differentially activate heterologous promoters. This differential activation may be due to the recruitment of distinct coactivator complexes to each promoter, which may be additionally influenced by differences in promoter chromatin structure.

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