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Progesterone Regulates Transcription of the p21^{WAF1} Cyclin-dependent Kinase Inhibitor Gene through Sp1 and CBP/p300*

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Progesterone has biphasic effects on proliferation of breast cancer cells; it stimulates growth in the first cell cycle, then arrests cells at G_1/S of the second cycle accompanied by up-regulation of the cyclin-dependent kinase inhibitor, p21. We now show that progesterone regulates transcription of the p21 promoter by an unusual mechanism. This promoter lacks a canonical progesterone response element. Instead, progesterone receptors (PRs) interact with the promoter through the transcription factor Sp1 at the third and fourth of six Sp1 binding sites located downstream of nucleotide 154. Mutation of Sp1 site 3 eliminates basal transcription, and mutation of sites 3 and 4 eliminates transcriptional up-regulation by progesterone. Progesterone-mediated transcription is further prevented by overexpression of E1A, suggesting that CBP/p300 is required. Indeed, in HeLa cells, Sp1 and CBP/p300 associate with stably integrated *flag*-tagged PRs in a multiprotein complex. Since many signals converge on p21, cross-talk between PRs and other factors co-localized on the p21 promoter, may explain how progesterone can be either proliferative or differentiative in different target cells.

Progesterone is a paradoxical hormone having either growth stimulatory effects or growth inhibitory and differentiative effects, depending on the tissue in question and the dose and treatment regimen (1, 2). In the uterus for example, progesterone inhibits epithelial growth and has differentiative effects (3). It is therefore used to counteract the proliferative and carcinogenic effects of unopposed estrogens in women prescribed hormone replacement therapy (4). In the breast, the role of progesterone is more complex. The hormone is required for terminal growth and differentiation of the mammary gland (2). Therefore, mice lacking progesterone receptors (PRs)[1] exhibit incomplete mammary gland ductal branching and failure of lobulo-alveolar development (5). In animal models of mammary carcinogenesis, progesterone, depending on the regimen used, can either inhibit or promote tumor formation (2). On the other hand, in animals with established PR-positive mammary tumors, progesterone is usually proliferative, and progesterone antagonists inhibit tumor growth (6). Despite this, in humans, second-line high dose progestin therapy effectively suppresses the growth of hormone-dependent PR- and estrogen receptor-positive breast cancers that have acquired resistance to the antiestrogen tamoxifen (6).

How can these contradictory effects of progesterone be reconciled? Recent studies have dealt with the effects of progesterone on mitosis and key cell cycle regulatory proteins in cultured human breast cancer cells (1, 7–9). Treatment of such cells with progestins produces biphasic effects. Studies focusing on the initial growth stimulatory component show that progestin-induced entry of cells into S-phase is accompanied by transient increases of cyclin D1 and cyclin-dependent kinase 4 activity (1, 7). Indeed, cyclin D1 is a critical component of the mitogenic response to progestins, and its overexpression in transgenic mice produces lobulo-alveolar changes in the mammary gland usually associated with progestational effects in pregnancy (8). Growth stimulation by progestins is restricted to one cycle, however, and is followed by growth arrest at the G_1/S boundary of the second cycle (1, 7, 9). Cells then enter a period of resistance to growth regulatory effects of additional progesterone, accompanied by hypophosphorylation and profound down-regulation of pRb, loss of cyclins D, A, and B, and sequential increases first, in the levels of the cyclin-dependent kinase inhibitor p21 (p21$^{Waf1,\ Cip1,\ Kip1,\ Sdi1}$) followed by increases in the cyclin-dependent kinase inhibitor p27 (1).

The control of proliferation and differentiation by many hormones and growth factors is linked by events that occur at the G_1/S boundary of the cell cycle (10, 11). Recent studies implicate growth arrest accompanied by up-regulation of p21 not only in inhibiting proliferation but in promoting differentiation (12). Elevated p21 levels are associated with phorbol ester-, retinoic acid-, and vitamin D-induced differentiation in the myelomonocytic cell line U937, with butyrate-induced differentiation in WiDr cells, MyoD-induced differentiation of myoblasts, and thrombopoietin-induced differentiation of the megakaryoblastic leukemia cell line, CMK (12–18). In each case, the differentiating agents were also shown to induce transcription in a p53-independent manner from the p21 promoter fused to a reporter gene.

We therefore asked whether the up-regulation of p21 protein levels induced by progesterone involves transcriptional regulation of the p21 promoter. We find that this promoter lacks a canonical progesterone response element (PRE). Instead, progesterone activates the gene through a novel mechanism that involves interactions between PRs and CBP/p300, with the transcription factor Sp1, at Sp1 DNA binding sites lying just upstream of the TATA box. Since other agents, such as phorbol esters, butyrate, Ca^{2+}, BRCA-1, and TGF-β also induce p21

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[1] The abbreviations used are: PR, progesterone receptor; PRE, progesterone response element; TGF-β, transcription growth factor-β; MEM, Eagle's minimum essential medium with Earle's salts; nt, nucleotides; bp, base pair(s).

from this region of the promoter (13, 16, 19–21), this model may provide a means of analyzing cross-talk between steroid hormones and other signal transduction pathways.

MATERIALS AND METHODS

Cell Lines and Reagents—Wild-type PR-positive T47D$_{CO}$ breast cancer cells were previously described (22). HeLa cells stably expressing *flag*-tagged PR A or B will be described elsewhere.[2] Cells are routinely cultured in 75-cm^2 plastic flasks and incubated in 5% CO_2 at 37 °C in a humidified environment. The stock medium consists of Eagle's minimum essential medium with Earle's salts (MEM), containing L-glutamine (2 mM) buffered with sodium bicarbonate (4 mg/liter) and HEPES (4.8 mg/liter), insulin (6 ng/ml), and 5% fetal calf serum (HyClone, Logan, UT) without antibiotics. For routine subculturing, cells are diluted 1:20 into new flasks once per week, and the medium is replaced every 2–3 days. Cells are harvested by incubation in Hanks' solution-EDTA for 15 min at 37 °C.

Antibodies were obtained from the following sources. Anti-mouse polyclonal CBP-CT and anti-human monoclonal p300 were from Upstate Biotechnology, Lake Placid, NY; anti-p21 and anti-Sp1 were from Santa Cruz Biotechnology, Santa Cruz, CA; anti-PR AB-52 and B-30 were produced in our laboratories (23); and secondary antibodies were from Bio-Rad.

Various p21 promoter and expression vectors were obtained as follows: the wild-type −2320 promoter and serial 5′-truncated derivatives (13) were a gift of Andrew Kraft and Joseph Biggs (Div. of Oncology, University of Colorado Health Sciences Center, Denver). They were cloned by blunt-end ligation into the *Sma*I site of the luciferase reporter vector, PA3-LUC (24), a gift of William Wood (Div. of Endocrinology, UCHSC, Denver). The −93 to −33 p21P93-S wild-type construct and its mutants (21) were generously provided by Xiao-Fan Wang (Dept. of Pharmacology, Duke University Medical Center, NC). The wild-type E1A expression vector, and the 2–36 deletion mutant were also a gift from Andrew Kraft and Joseph Biggs. hPR1 and hPR2, the PR B and A expression vectors were gifts from Pierre Chambon (Strasbourg, France).

Transfection and Transcription—T47D breast cancer cells (1 million) were plated and grown in 100-mm cell culture plates in MEM supplemented with 5% fetal calf serum. Transfection of plasmid DNA by calcium phosphate coprecipitation was usually performed 24 h after plating using 3 μg of the luciferase-fused reporter construct (23), 3 μg of the β-galactosidase expression plasmid PCH110 (Amersham Pharmacia Biotech) to correct for transfection efficiency, and Bluescript™ carrier plasmid (Stratagene, La Jolla, CA) for a total of 20 μg of DNA as described previously (23). 16 h later, transfection was completed when the medium was aspirated, and the cells shocked at room temperature for 2 min with 2 ml of phosphate-buffered saline containing 20% glycerol. Cells were washed twice with serum-free MEM to remove the glycerol, then 10 ml of MEM containing 5% fetal calf serum were added per dish either with 30 nM progesterone or ethanol vehicle. Cells in duplicate plates were harvested 48 h later in 250 μl of lysis solution (Analytical Luminescence Laboratories, Ann Arbor, MI), and 100 μl of lysate was analyzed for luciferase activity using the Enhanced Luciferase Assay Kit and a Monolight 2010 Luminometer (Analytical Luminescence Laboratories, Ann Arbor, MI) as described by the manufacturer. A further 100 μl of lysate was assayed for β-galactosidase activity as described previously (25) to normalize for transfection efficiency. In Fig. 1, T47D cells were treated with progesterone or ethanol for 48 h prior to transfection, then re-treated with progesterone or ethanol for a second 48-h period as shown. HeLa cell transfection was carried out as above with the exception that 90,000 cells were plated in 60-mm dishes in 5% charcoal-stripped phenol red-free MEM. HeLa cells were not glycerol shocked and were harvested for analysis 24 h after hormone treatment.

In Vivo Association among PRs, Sp1, and CBP/p300—HeLa cells were stably transfected with *flag* epitope-tagged PR B (*f*:PR$_B$) or A (*f*:PR$_A$) or the wild-type PR isoforms. In the presence of transiently transfected PRE$_2$-TATA$_{tk}$-chloramphenicol acetyltransferase reporter and the synthetic progestin R5020, the *flag*-tagged and wild-type receptors produce equal chloramphenicol acetyltransferase activity (not shown). For co-immunoprecipitation assays, nuclear extracts were prepared from HeLa *f*:PR cells or PR-negative HeLa cells treated with or without 100 nM R5020 for 1 h. The PRs were immunoprecipitated using an anti-*flag* M2 affinity gel (Eastman Kodak Co.), washed twice with TEDG (10 mM Tris, pH 7.4, 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol), twice with 0.3 M KCl in TEDG, twice with 0.1% Nonidet P-40 in TEDG and then eluted from the gel using 0.2 mg/ml *flag* peptide (Kodak). The PRs and associated eluted proteins were resolved by SDS-polyacrylamide gel electrophoresis on 7.5% gels, transferred to nitrocellulose, and immunoblotted with the antibodies against PRs, Sp1, or CBP/p300 described above. The protein bands were detected by enhanced chemiluminescence (Amersham Corp.).

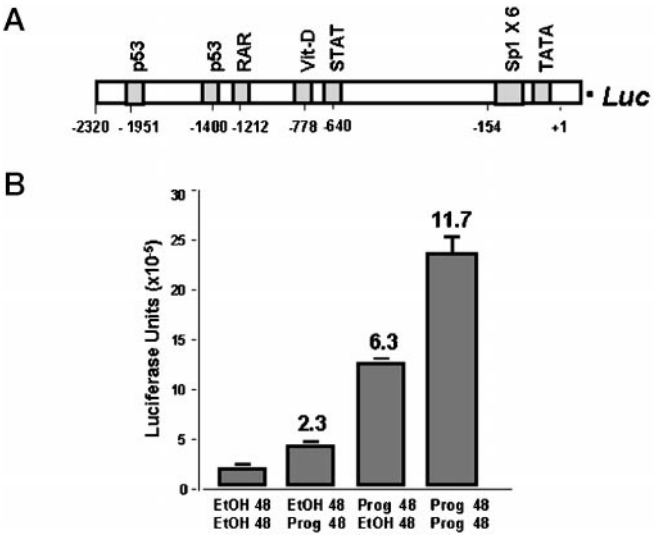

FIG. 1. **Progesterone regulates the transcriptional activity of the p21 promoter.** *A*, the proximal 2320-bp p21^{WAF1} promoter showing key regulatory regions and factor binding sites. *RAR*, retinoic acid receptor; *Vit-D*, vitamin D receptor. *B*, the 2320-bp p21 promoter fused to the luciferase gene was transfected into B receptor-expressing T47D-YB cells that had been pretreated with either progesterone (30 nM) or ethanol vehicle for 48 h. Cells were then treated for a further 48 h as shown. Relative luciferase activity is normalized to cell number.

RESULTS

Progesterone Activates the p21 Promoter through an Sp1 Site—We recently demonstrated that in PR-positive T47D breast cancer cells, a pulse of progesterone induces one round of cell division. The cells then enter a period of prolonged growth arrest, during which they are resistant to the proliferative effects of a second dose of progesterone (1). The cells are not resistant to other mitogenic signals, however, because they can reenter the mitotic cell cycle in response to epidermal growth factor. Furthermore, during the arrested state the cells express functional PRs, which following treatment with a second dose of progesterone can activate transcription of a synthetic promoter/reporter (1) and can promote cellular differentiation and accumulation of lipid vesicles (26). Cells arrested by one dose of progesterone express high levels of p21, and the levels rise further following a second dose of progesterone given 48 h after the first (1).

To determine whether progesterone regulates p21 transcriptionally, a luciferase reporter gene driven by 2320 nt of the p21 promoter (Fig. 1*A*) was transfected into PR-positive T47D$_{CO}$ cells that had been pre-treated with ethanol or 30 nM progesterone for 48 h, then re-treated with ethanol or progesterone for another 48 h (Fig. 1*B*). Luciferase activity increased 2.3-fold in cells harvested 48 h after progesterone treatment, and 11.7-fold in cells receiving two sequential 48 h progesterone treatments. If progesterone is removed after 48 h, but the cells are not harvested for another 48 h, luciferase transcription continues and rises to 6.3-fold above controls. Since the half-life of progesterone is 3–4 h (27) and of luciferase is 90 min (28), we speculate that secondary signals prolong p21 transcription.

Similarly, in PR-negative HeLa cells transfected with an expression vector encoding PR A, 24 h of progesterone increases luciferase activity driven by the proximal 2320 nt of the

[2] J. K. Richer, manuscript in preparation.

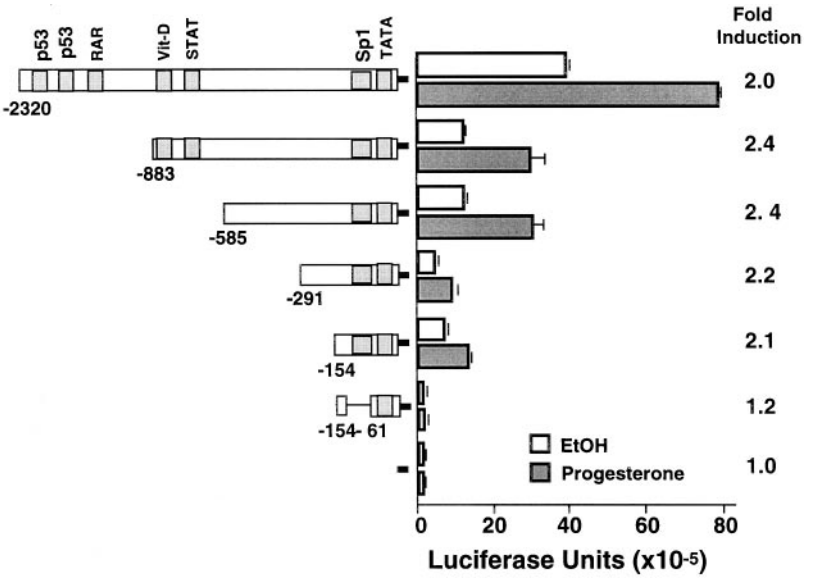

FIG. 2. **Progesterone regulation of the p21^{WAF1} promoter maps to Sp1 binding sites.** Sequential 5′-truncations of the 2320-bp p21^{WAF1} promoter linked to luciferase were cotransfected into HeLa cells together with an expression vector for PR A, and the cells were treated with either progesterone (100 nM) or ethanol vehicle for 24 h. Relative luciferase units were corrected for transfection efficiency based on β-galactosidase activity.

p21 promoter, 2–3-fold (Fig. 2). To map the progesterone-responsive region, a set of sequentially truncated promoter constructs driving the luciferase gene was transfected into HeLa cells transiently expressing PR A and treated with progesterone for 24 h (Fig. 2). Progressive 5′-truncation led to a decrease in basal activity, but the 2–3-fold induction by progesterone was maintained through a construct that retained only 154 nt at the 3′-end of the promoter. However, when sequences between −154 and −61 were deleted, the progesterone response was abolished, suggesting that PR effects are mediated from this region.

The region between −154 and the transcription start site lacks PREs or AP-1 binding sites, through which progesterone responses could in theory be transmitted (29). On the other hand, this region contains four consensus Sp1 binding sites, and downstream of −61 there are two more Sp1 sites and a TATA box (Fig. 3). Since PR responsiveness maps downstream of −154, this region was analyzed in detail using a series of 60-bp constructs, spanning nt −93 to −33 of the promoter (p21P93-S, WT), in which 10-bp blocks were progressively mutated (Fig. 3; *p21P93-S, mut 1–6*) (16). The wild-type construct retains progesterone sensitivity narrowing the PR-responsive region to the 32 nt lying between −93 and −61 (Fig. 3). Mutations that destroy the Sp1-3 and Sp1-4 binding sites within this region (p21P93-S, mut 2 and 3) abolish progesterone induction. Mutation of Sp1-3 (p21P-S, mut 2) appears to produce a general loss of basal transcriptional activity. Therefore this site, coupled with Sp1-4 (p21P-S, mut 3) located between nt −70 and −65, encompass the PR-responsive element. Interestingly, neither mutation of the Sp1-5 or Sp1-6 sites further downstream nor mutation of the TATA box, eliminate the progesterone response. TATA box-independent transcription of the p21 promoter has been previously reported (21). We find, however, that fusion of nt −117 to −61, which contains the progesterone-responsive region, directly upstream of the luciferase transcription start site, produces an inactive promoter (not shown), suggesting that proper spacing between the upstream Sp1 sites and the transcription start site must be maintained, as shown also by Prowse *et al.* (19), and/or that along with the Sp1 sites, the proximal 34 nt of the promoter are necessary for PR function.

Involvement of CBP/p300 in PR Regulation of the p21 Promoter—Recently, Missero *et al.* (30) demonstrated that keratinocyte differentiation associated with an increase in p21 levels is abolished by overexpression of the E1A oncoprotein. E1A

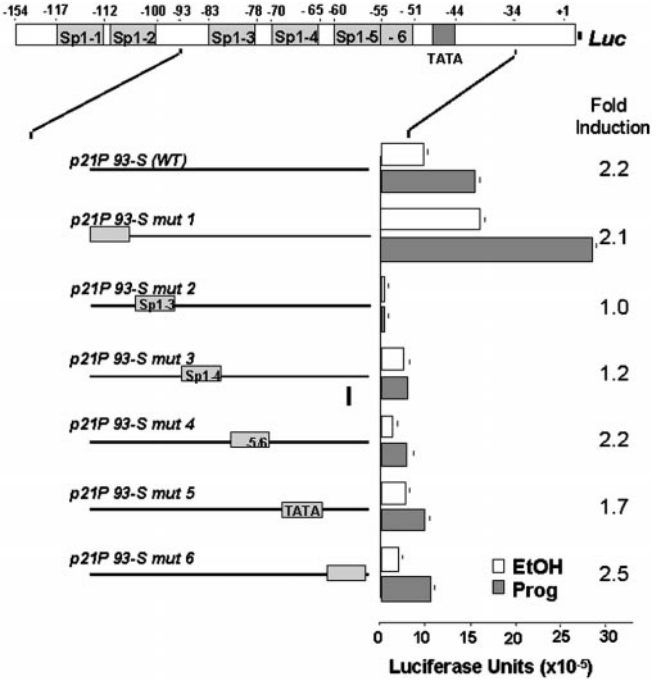

FIG. 3. **Progesterone regulation of the p21^{WAF1} promoter maps to Sp1-3 and Sp1-4.** p21P 93S (WT) containing the wild-type −93 to −34 nt of the p21 promoter and mut 1–6 in which consecutive 10-bp regions were mutated as shown by the gray boxes were cotransfected into HeLa cells together with the PR A expression vector, and the cells were treated with either progesterone (100 nM) or ethanol vehicle for 24 h. Relative luciferase units were corrected for transfection efficiency using β-galactosidase activity.

binds CBP/p300, therefore titration of CBP/p300 into the keratinocytes restored p21 expression (30). To determine whether CBP/p300 is important in the induction of p21 by progesterone, HeLa cells were cotransfected with empty-, wild-type E1A-, or mutant E1A-containing expression vectors, the −2320 bp or the truncated −154 bp p21 promoter, and the PR_A expression vector, and the cells were treated with vehicle or progesterone (Fig. 4). In the absence of excess E1A, progesterone induces luciferase transcription, but when wild-type E1A is overexpressed, progesterone-regulated transcription is abolished from either promoter. In contrast, an E1A mutant (E1A del 2–36; Ref. 30) lacking the N-terminal region required to bind CBP/p300, cannot block progesterone-regulated transcription

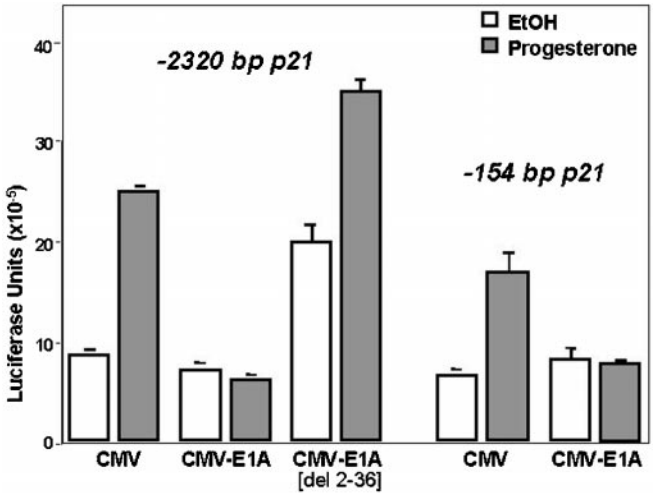

FIG. 4. **Progesterone induction of the p21^{WAF1} promoter requires CBP/p300.** HeLa cells were cotransfected with the −2320-bp p21^{WAF1} promoter or the −154-bp p21 promoter constructs fused to luciferase, with the PR A expression vector and with an empty-, wild-type E1A-, or mutant E1A-encoding CMV expression vector. Cells were treated with either progesterone (100 nM) or ethanol vehicle for 24 h. Relative luciferase units were corrected to cell number.

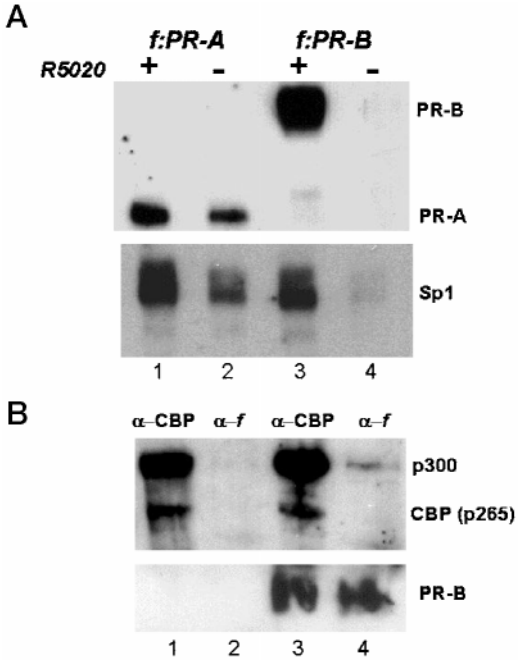

FIG. 5. **Sp1 and CBP/p300 co-immunoprecipitate with PRs.** *A*, HeLa cells were stably transfected with *flag*-tagged PR A or PR B expression vectors. Cells were treated for 1 h with ethanol vehicle (−) or with 20 nM of the synthetic progestin R5020 (+) and then harvested, and nuclear extracts were prepared. Nuclear extracts were immunoprecipitated with an anti-*flag* antibody covalently attached to agarose. The PRs and coprecipitated proteins were eluted from the resin with a *flag* peptide and detected by SDS-polyacrylamide gel electrophoresis and immunoblotting using the anti-PR A- plus B-specific antibody, AB-52, or the anti-Sp1-specific antibody, PEP 2. *B*, wild-type HeLa cells (*lanes 1* and *2*) and HeLa cells stably expressing *flag*-tagged PR B (*lanes 3* and *4*) were treated with R5020 for 1 h, and nuclear extracts were prepared. The extracts were immunoprecipitated with the anti-*flag* antibody linked to agarose (α-*f*) or the anti-CBP/p300 polyclonal antibody CBP-CT (α-*CBP*). The *flag* peptide-eluted or CBP/p300-coprecipitated proteins were resolved by SDS-polyacrylamide gel electrophoresis and detected by immunoblotting with the anti-PR antibody, AB-52, or an anti-human p300 monoclonal antibody.

from the −2320 bp promoter. This mutant also increases basal transcription of the p21 promoter, which is similar to its effects on p15 promoter activity previously described (31). Interestingly, overexpression of wild-type E1A also abolishes progesterone regulation of the PRE_2-$TATA_{tk}$ promoter, yet fails to alter progesterone-regulated transcription of the Sp1-Luc (32) promoter (not shown). This suggests that the ability of E1A overexpression to block progesterone-induced transcription is promoter specific and not necessarily Sp1-dependent.

PR Bind Sp1 and p300—Since the PR-dependent induction of p21 involves Sp1 and CBP/p300 in a region of the p21 promoter lacking a PRE, we postulated that PRs are tethered to the promoter through Sp1 and/or CBP/p300. To test this hypothesis, HeLa cells were stably transfected with expression vectors encoding *flag*-tagged PR A or B. The cells were treated or not with the progestin R5020, and nuclear extracts were prepared. PRs and associated proteins were immunoprecipitated from nuclear extracts with an anti-*flag* M2 affinity gel, then eluted from the resin with a *flag* peptide, and detected by immunoblotting. Fig. 5*A* (*top*) shows expression of the PR A- or B-isoforms in nuclear extracts from untreated or R5020-treated PR-positive HeLa cells. Low levels of A receptors can be immunopurified from nuclear extracts with anti-*flag* resin even in the absence of ligand (*lane 2*), and the levels increase further after ligand addition (*lane 1*). In the absence of ligand, no B receptors can be precipitated from nuclear extracts (*lane 4*), but high levels are purified following ligand treatment (*lane 3*). Fig. 5*A* (*bottom*) shows that Sp1 co-immunoprecipitates with the anti-*flag* antibody and is eluted by the *flag* peptide only from extracts that contain PRs. Similarly, no Sp1 is present in immunoprecipitates of nuclear extracts from PR-negative HeLa cells (not shown).

Interactions between PRs and CBP/p300 are shown in Fig. 5*B* using R5020-treated PR-negative HeLa cells, or HeLa cells expressing *flag*-tagged PR B. Nuclear extracts were immunoprecipitated with an anti-CBP/p300 antibody or the anti-*flag* resin-bound antibody, and copurified proteins were detected with anti-CBP/p300 or anti-PR antibodies. CBP (p265) and p300 can be immunoprecipitated with the anti-CBP/p300 antibody from PR-negative HeLa cells, but PR B are absent (*lane 1*), and no nonspecific binding is seen with the anti-*flag* antibody control (*lane 2*). However, in HeLa cells expressing *flag*-tagged PR B, high levels of B receptors co-immunoprecipitate with the anti-CBP/p300 antibody (*lane 3*) and in the reciprocal assay, p300 is co-immunoprecipitated with B receptors using the anti-*flag* antibody (*lane 4*).

DISCUSSION

In breast cancer cells, a transient pulse of progesterone has biphasic effects; it accelerates proliferation in the first cell cycle then arrests cells at the G_1/S boundary of the second cycle (1, 9). A second dose of progesterone prolongs the growth arrest. We have proposed that the arrested cells are poised to enter a proliferative or differentiative pathway, depending on the nature of other cellular signals. This model calls for the convergence and integration of signals from the cell surface and cytoplasm with those in the nucleus. Since the progesterone-induced growth arrest is accompanied by a 10–15-fold increase in the levels of p21 (1) and this protein is a key player in determining cell fate (12), we speculated that integration of divergent signals occurs at the p21 promoter. To that end we sought to understand the mechanisms by which progesterone regulates the levels of p21.

We show here that a transient pulse of progesterone increases transcription from the p21 promoter 2–3-fold (Figs. 1 and 2), which rises to approximately 12-fold in cells receiving a second hormone dose (Fig. 1). The progestin-responsive element maps to the Sp1-3 and Sp1-4 sites located between −84 and −65 nt. This suggests that progesterone induction is p53-

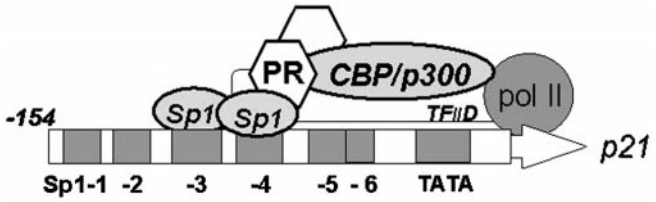

FIG. 6. **Progesterone regulation of the p21 promoter; PRs bind through Sp1 and CBP/p300.** The data are consistent with a model in which PRs are indirectly tethered to the promoter through Sp1 protein(s) bound to Sp1 sites 3 and/or 4 in a multiprotein complex that also includes CBP/p300. Binding of these proteins to the TATA box is not required.

independent, since the two p53 binding sites are located much further upstream at −1.4 and −2.3 kilobases (33). This also rules out involvement of sites for two other nuclear receptors in the progestin response, the retinoid-sensitive region, which maps to −1.2 kilobases (14) and the vitamin D site at −770 nt (15). The proximal p21 promoter contains six Sp1 binding sites and a TATA box. The region comprising Sp1-1 and Sp1-2 is involved in 12-*O*-tetradecanoylphorbol-13-acetate-mediated p21 induction in U937 cells (13) and in BRCA-1-mediated p21 induction in SW480 colon cancer cells (20). The region comprising Sp1-3 is required for p21 induction by TGF-β (21), butyrate (16), and Ca^{2+} (19). Butyrate sensitivity also requires Sp1-5 and Sp1-6 in conjunction with Sp1-3, with Sp1-3 being critical. We have mapped the progesterone-responsive region to 20 nt comprising Sp1-3 and Sp1-4 and show, furthermore, that Sp1-3 is required for basal promoter activity in HeLa cells. This explains the data of Biggs *et al.* (13) who demonstrated that deletion of −120 to −61 (which includes Sp1-3) from the full-length promoter abolishes 12-*O*-tetradecanoylphorbol-13-acetate- and p53-mediated induction. This would be expected if basal promoter function is destroyed. We conclude that if Sp1-3 is required for basal promoter activity, then progesterone response specificity requires Sp1-4, but clearly both sites are important. To our knowledge, no other p21 regulator has been mapped to Sp1-4.

The mechanisms underlying Sp1-mediated induction of p21 transcription are unknown. Phosphorylation of the protein is unaffected by 12-*O*-tetradecanoylphorbol-13-acetate or butyrate treatment (13), and footprinting studies demonstrate that Sp1 is constitutively bound to the DNA (13). We find that deletion of the TATA box does not abolish the response to progesterone, an observation also reported by Dotto *et al.* (21) in mapping the TGF-β-responsive element. Sp1 is known to interact with the N terminus of the TATA-binding protein-associated factor $dTAF_{110}$ in the TFIID complex, and this interaction may account for the ability of Sp1 to activate transcription from TATA-less promoters (34–36). Interestingly, the DNA binding domain of PRs interacts with $dTAF_{110}$ at its C terminus (37). Thus, PRs and Sp1 may bind $dTAF_{110}$ concurrently as shown in Fig. 6 and suggests a mechanism for PR regulation of the p21 promoter.

CBP/p300 may be a similar coregulator. It is necessary for p21-dependent differentiation of keratinocytes (30, 31) and p21-dependent cell cycle arrest and differentiation of muscle cells induced by MyoD (17). Although CBP/p300 was originally identified as a cAMP-response element binding protein, recent reports have demonstrated that many transcription factors including steroid receptors bind this protein (38). In this study we demonstrate that PRs can bind CBP/p300 and that, in conjunction with Sp1, CBP/p300 is required for induction of the p21 promoter by progesterone. This suggests that CBP/p300 is an integral component of the basal transcription machinery of this promoter and/or that PRs recruit CBP/p300 to this transcription complex (Fig. 6). The former is more likely since p21 promoter regulation by TGF-β in keratinocytes is also CBP/p300-dependent (31).

In sum, we have demonstrated a novel mechanism by which PRs can regulate gene transcription through a promoter that lacks canonical PREs. Instead, we suggest that PRs are brought to the DNA by binding to Sp1. It is possible that other Sp1-responsive genes (39, 40) can be similarly regulated. Furthermore, we have demonstrated this unusual regulation using the promoter of the p21 gene. This cyclin-dependent kinase inhibitor is a key intermediary protein in the pathway that determines whether cells proliferate or differentiate (10, 11), and it is therefore a target of regulation by multiple signaling molecules. We speculate that the proliferative *versus* differentiative fate of progesterone target cells is controlled by interactions between PRs and other transcription factors colocalized on the p21 promoter.

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