The Mouse Mammary Tumor Virus Promoter Adopts
Distinct Chromatin Structures in Human Breast
Cancer Cells with and without Glucocorticoid Receptor*

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Steroid receptors represent a class of transcription regulators that act in part by overcoming the often repressive nature of chromatin to modulate gene activity. The mouse mammary tumor virus (MMTV) promoter is a useful model for studying transcriptional regulation by steroid hormone receptors in the context of chromatin. The chromatin architecture of the promoter prevents the assembly of basal transcription machinery and binding of ubiquitous transcription factors. However, in human breast carcinoma T47D cells lacking the glucocorticoid receptor (GR), but expressing the progesterone receptor (PR), nucleosome B (nuc B) assumes a constitutively hypersensitive chromatin structure. This correlation led us to test the hypothesis that the chromatin structure of nuc B was dependent on GR expression in T47D cells. To examine this possibility, we stably cotransfected the MMTV promoter and the GR into T47D cells that lacked both the GR and the PR. We found that in T47D cells that lack both the GR and the PR or express only the GR, nuc B assumes a constitutively "open" chromatin structure, which allows hormone-independent access by restriction endonucleases and transcription factors. These results suggest that in GR−/− T47D cells, the MMTV chromatin structure permits GR transcriptional activation, independent of chromatin remodeling.

In eukaryotic cells, the packaging of DNA and histone proteins as chromatin allows DNA sequences to be compacted efficiently and economically (1). Several lines of evidence suggest that compact DNA regions are transcriptionally silent (2, 3). Biochemical experiments suggest that chromatin imposes constraints to the recognition of specific sequences by transcription factors, and this may limit the transcription process (4, 5). In addition, regions of decondensed chromatin are often correlated with binding of transcription factors and increased gene activity. Consequently, transcriptional activators that counteract the repressive nature of chromatin are critical for regulating gene expression (6, 7).

Steroid receptors interact with the repressive chromatin structure and recruit necessary activities that result in changes in chromatin remodeling (5–10). A role for steroid receptors in chromatin remodeling and gene expression is exemplified by the glucocorticoid receptor (GR)-mediated changes in the chromatin structure of the mouse mammary tumor virus (MMTV) promoter. When stably integrated into mammalian cells, the MMTV promoter assumes a phased array of six positioned nucleosomes, A–F (11). Nucleosome B (nuc B) contains the glucocorticoid response elements and a binding site for nuclear factor-1 (NF1). Adjacent to nuc B are binding sites for the octamer transcription factors and the TFIID complex, which includes the TATA-binding protein (12, 13).

In rodent cells that have not been exposed to hormone, the chromatin structure of the second nucleosome (nuc B) is repressed or "closed" (11, 14, 15). In response to glucocorticoids, the GR disrupts the chromatin structure of nuc B, creating a hypersensitive region, which permits binding of transcription factors such as NF1 and assembly of the transcription initiation complex (12, 14). The change in chromatin structure is concomitant with transcription from the stably integrated MMTV promoter (12, 14–16).

Studies of chromatin structure and transcriptional activation of the MMTV promoter in human T47D cells have presented a more complicated picture than that observed with rodent cells (17). In T47D/A1-2 cells, which express comparable amounts of the GR and the progesterone receptor (PR), GR activation of MMTV is similar to that observed in rodent cells (18, 19). However, in T47D/2963.1 cells (2963.1) cells, which express the PR but not the GR, the chromatin organization of nuc B is nonrepressed or "open," and transcription factors are constitutively bound onto the promoter (20). In response to progestins, the PR activates transcription from the MMTV organized as a stable open template in 2963.1 cells, but not a stable closed template in A1-2 cells (19, 20). These studies demonstrated that in the presence of the GR in both mouse (GR−/−) and human (GR+/PR+) cell lines, the MMTV promoter is organized in a repressive state and requires the GR for activation (17).

The correlation of GR expression and a closed chromatin structure led us to consider whether expression of the GR may

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be a prerequisite for and or related to the establishment of the repressive MMTV chromatin organization. To examine this hypothesis, we characterized T47D cell lines that were devoid of the GR or expressing varying amounts of the GR. Our results show that in both the GR-negative (M10) and the GR-positive (GR2 and GR4) cell lines, nucleotide sequences of the MMTV promoter assume an open chromatin structure. Consistent with the open chromatin structure, NF1 is constitutively bound onto the MMTV promoter independent of GR expression. However, while the MMTV promoter is transcriptionally inactive in the M10 cells, in the GR-positive cells the GR recruits co-activator proteins to activate transcription in a hormone-dependent manner.

**Experimental Procedures**

**Cell Lines—**M10 human breast cancer cells were derived from T47D-Y cells that lack the PR and GR by stable transfection with a MMTV reporter plasmid (22). The GR cell lines were engineered by stably co-transfecting a GR expression vector pRSGV (21) and the pGKpuro construct carrying the puromycin gene into M10 cells (22, 23). T47D/AI-2 cells were stably transfected with a MMTV promoter reporter and a GR expression (pGRNeo) plasmid (18, 21). All cells were grown in RPMI 1640 with 2 mM glutamine, 100 μg/ml penicillin/streptomycin, 10 mM HEPES, and 10% fetal bovine serum and maintained with 1 μg/ml puromycin (GR2 and GR4) or 160 μg/ml G418 (AI-2). All tissue culture reagents were purchased from Life Technologies, Inc.

**Determination of MMTV Copy Number—**Genomic DNA was digested to completion using SstI and analyzed by Taq DNA polymerase amplification and a 32P-labeled oligonucleotide primer specific for the MMTV (5'-GGT TTA AAT TAA TTT ATG GTT ATG ACA AAC TG-3'). A series of standards consisting of SstI-digested MMTV-CAT plasmid expressed in the M10-GR cell lines were subjected to similar PCR analysis as described previously (18). PCR products were separated on 8% denaturing polyacrylamide gels and quantified by PhosphorImager and ImageQuant Analysis software (Molecular Dynamics, Inc., Sunnyvale, CA).

**Immunoprecipitation and Western Blotting—**Cells expressing the GR were identified by SDS-PAGE and Western blotting. Cells were treated with dexamethasone (10−5 M) for 1 h. Nuclear extracts were immunoprecipitated with anti-GR antibody, and the immunocomplex was separated on 10% SDS-polyacrylamide gel electrophoresis. The GR was analyzed by Western blotting. A1-2 (lanes 1 and 5), GR2 (lane 2), M10 (lane 3), and GR4 (lane 4). B, to confirm the integration of the MMTV promoter, genomic DNA purified from two clones expressing the GR, GR2 (lanes 5 and 6), GR4 (lanes 9 and 10) and the parent cell line M10 (lanes 7 and 8) was sequenced. M10-GR cell lines were subjected to similar PCR analysis. PCR products were analyzed using 8% polyacrylamide denaturing gels and exposed to Kodak film or PhosphorImager screens.

**In Vivo Chromatin Analysis and λ Footprinting—**Cells were left untreated or treated with hormones for 1 h or as indicated in the figure legends. Nuclei were isolated as described previously (28, 29) and subjected to limited digestion using various restriction endonucleases per 100 μl of nuclei volume: SstI (5 or 25 units), EcoR1 (25 units), and EcoRV (100 units) per λ. λ exonuclease footprinting, SstI (300 units/100 μl) was used as the in vitro enzyme entry, and λ exonuclease (40 units/100 μl) was used to detect 5′ boundaries of transcription factors. After in vivo digestion, DNA was purified by phenol/chloroform extraction and ethanol precipitation. Purified DNA samples were digested to completion using EcoR1 (100 units, GR2) or BamHI (100 units, A1-2) to provide an internal standard for the in vivo cleavage. Purified DNA (20 μg) was amplified using reiterative primer extension and Taq DNA polymerase and 32P-labeled specific oligonucleotides complementary to MMTV sequences. MMTV sequences of the oligonucleotide primers used for the in vivo chromatin and footprinting analysis were 5′-TTA GCC TCT TTA CCT GAA AAT-3′ (base pairs +18 to +42) and 5′-CCT TCT TCT ATG TTT TGG GCG-3′ (base pairs +168 to +192) generated a 150-base pair PCR fragment. The 5′ primer was end-labeled with T4 polynucleotide kinase (New England Biolabs, Beverly, MA). Human β2-microglobulin was amplified using primer sequences, 5′-ACC CCC ACT GAA AAA GAT-3′, and 5′-ACC CCC ACT GAA AAA GAT-3′. PCR amplification with these primers generated a 120-base pair fragment (19). PCR products were analyzed on 8% polyacrylamide denaturing gels and exposed to Kodak reflection film at −80 °C or PhosphorImager screens for analysis.

**FIG. 1. Stable expression of the GR and MMTV promoter sequences in transfected T47D cells.** A, cell lines were treated with DEX (10−7 M) for 1 h. Nuclear extracts were immunoprecipitated with anti-GR antibody, and the immunocomplex was separated on 10% SDS-polyacrylamide gel electrophoresis. The GR was analyzed by Western blotting. A1-2 (lanes 1 and 5), GR2 (lane 2), M10 (lane 3), and GR4 (lane 4). B, to confirm the integration of the MMTV promoter, genomic DNA purified from two clones expressing the GR, GR2 (lanes 5 and 6), GR4 (lanes 9 and 10) and the parent cell line M10 (lanes 7 and 8) was sequenced. M10-GR cell lines were subjected to similar PCR analysis. PCR products were analyzed using 8% polyacrylamide denaturing gels and exposed to Kodak film or PhosphorImager screens.
RESULTS

Stable Introduction of the GR into M10 Cells—To examine the impact of GR expression on MMTV promoter chromatin structure in T47D cells, M10 cells were stably transfected with a GR expression vector. From the pool of transfected cells, two clones designated GR2 and GR4 were selected and analyzed for expression of the GR by Western blotting (Fig. 1A). T47D-derived A1-2 cells, which express approximately 100,000 copies of GR per cell (21) served as a positive control. As seen on the Western blot, both the GR2 and the GR4 cells express the GR (Fig. 1A, lanes 2 and 4) but at much lower levels than the A1-2 cells (cf. lanes 1 and 5 with lanes 2 and 4). Western blot analysis indicated that GR2 cells contained ~10,000 GR copies/cell, 10 times less GR than A1-2 cells, while the GR4 contained ~2,000 GR copies/cell, 50 times less GR than the A1-2 cells (21). As expected, the parental cell line M10 did not express any GR (lane 3).

We next determined the MMTV copy number in the M10, GR2, and GR4 cells to establish that introduction of the GR did not affect the number of MMTV templates in the cell lines. The number of copies of the MMTV-CAT construct integrated into the cell lines was determined by PCR as described previously (18). The M10, GR2, and GR4 cell lines each express one copy of the MMTV-CAT construct per cell (Fig. 1B).

Glucocorticoids Activate MMTV Expression in GR2 and GR4 Cells—We examined the effect of dexamethasone and anti-glucocorticoids RU43044 and RU486, alone and in combination, on GR-mediated MMTV expression. Cells were left untreated (Fig. 2, lane 1); treated with dexamethasone (lane 2), RU43044 (lane 3), and RU486 (lane 5); or treated with either anti-glucocorticoid plus dexamethasone (lanes 4 and 6) prior to harvest and CAT assay. In GR2 cells, MMTV-CAT activity was induced 8-fold by the addition of dexamethasone (Fig. 2, lane 2) and slightly less than 3-fold in the GR4 cells, consistent with the lower GR levels seen in these cells (Fig. 1A). M10 cells that...
lack the GR showed basal CAT activity under all conditions. Anti-glucocorticoids blocked the dexamethasone-induced CAT activity in GR2 cells, with RU344 decreasing activity by ~75% and RU486 by ~70% (Fig. 2, lanes 4 and 6). Results with the GR4 cells were similar but less pronounced, with RU344 showing a ~45% inhibition and RU486 inhibiting the GR by only ~20% (Fig. 2, lanes 4 and 6). Because the GR2 cells exhibited a more robust glucocorticoid response, compared with GR4 cells, we selected the GR2 cells for subsequent experiments.

Anti-glucocorticoids Inhibit Glucocorticoid-induced MMTV RNA Expression in GR2 and A1-2 Cells—To confirm the data obtained with CAT assays, we used primer extension analyses to examine glucocorticoid stimulation of MMTV mRNA expression in GR2 cells in the presence and absence of glucocorticoid antagonists. For comparison, we used the T47D/A1-2 cells in which we have previously shown a glucocorticoid-dependent increase in MMTV mRNA levels. Dexamethasone increased MMTV mRNA expression in GR2 cells 4-fold (Fig. 3A, lane 2). Both glucocorticoid antagonists RU43044 and RU486 inhibited glucocorticoid-induced MMTV mRNA expression to basal levels (Fig. 3A, cf. lanes 2, 4, and 6). Compared with GR2 cells, T47D/A1-2 cells, which have 10 times more GR, showed a more robust response to dexamethasone, a 35-fold increase in mRNA expression (Fig. 3B, lane 2). The anti-glucocorticoid RU486 inhibited dexamethasone-induced MMTV expression by ~80% (Fig. 3B, cf. lanes 2, 4, and 6). These experiments showed that transcription from the MMTV promoter in both GR2 and A1-2 cells was hormone-dependent (Fig. 3, A and B, cf. lanes 1 and 2), and the antagonist inhibited GR-induced MMTV mRNA expression (Fig. 3, A and B).

Constitutive Hypersensitivity of the MMTV Promoter in GR2 Cells—We have previously shown that transcription from the MMTV promoter coincides with alteration of the chromatin structure of nuc B in the presence of hormone in GR-positive human and rodent cell lines (16, 19). To examine if similar chromatin remodeling occurred in the GR2 cells, we determined the extent of restriction enzyme cleavage in the nuc B region in the presence and absence of hormone. The MMTV DNA in GR2 cells assumes a persistent open chromatin structure such that there are no differences in the extent of SstI cleavage between the untreated and hormone-treated cells (Fig. 4A, cf. lanes 3 and 4 and lanes 7 and 8). As a control, we examined the extent of cleavage on the MMTV DNA integrated into A1-2 cells in which nuc B assumes a closed chromatin structure in the absence of hormone. In contrast to the GR2 cells, the addition of hormone increased enzyme cleavage up to 4.5-fold (Fig. 4B) in the hormone-treated A1-2 cells relative to untreated cells (Fig. 4A, cf. lanes 1 and 2 and lanes 5 and 6). For both cell lines, the level of restriction enzyme digestion observed was independent of enzyme concentration (Fig. 4A, cf. lanes 1 and 2 versus lanes 5 and 6 and lanes 3 and 4 versus lanes 7 and 8). These results demonstrate that the GR-dependent nuc B chromatin remodeling seen in GR+/PR− A1-2 cells was not observed in GR2 cells, which express only the GR. Thus, the hormone-dependent transcriptional activation observed in GR2 cells occurs against a background of constitutive promoter hypersensitivity.

An interesting observation from chromatin analysis of the MMTV promoter in the GR2 cells was that SstI cleavage was high, 30–40% in the absence of hormone. Conversely, in A1-2 cells, which have a closed chromatin structure in the absence of hormone, SstI cleavage was minimal (1.5%) in the absence of hormone. The open chromatin structure observed at nuc B in the GR-positive GR2 cells is similar to that observed previously in PR−/gr− T47D/2963.1 cells and with transiently transfected MMTV templates (16, 20). However, in the PR−/gr−-positive cells, although nuc B was constitutively open, cleavage in the adjacent nuc A was limited (20). This is distinct from what is observed with transiently transfected MMTV promoter, where
cleavage at nuc A is equivalent to that seen at nuc B (16). Thus, we examined whether other sites within nuc B and adjacent sites within nuc A were equivalently hypersensitive to restriction endonucleases in the GR2 cells. As a control for the role of the GR in specifying the chromatin structure over nuc B and nuc A, we performed similar experiments with the parental M10 cells that lack the GR. To analyze cleavage in both nuc B and nuc A, we utilized restriction enzymes that cleave DNA both in nuc B and nuc A and anti-parallel primers specific for nuc B and nuc A in reiterative primer extension analyses of the same DNA sample (Fig. 5A) (20). A summary of these results is shown in Fig. 5B. As seen with experiments using SstI (Fig. 4, A and B), percentage of cleavage for both FoFl (150) and DdeI (105) was between 40 and 60% within nuc B in GR-negative M10 and GR-positive GR2 cells. Concurrent analysis from the same DNA sample in nuc A showed that cleavage in nuc A in both cell lines was less efficient (10–20%; Fig. 5B, NUC A panel). Thus, the architecture of nuc B and A is similar to that seen previously in the T47D/2963.1 cells (20). These results suggest that the chromatin organization of both nuc B and A in these cells was not dependent on the GR, since cells not expressing the GR (M10) exhibited enzyme cleavage similar to those cells expressing the GR (GR2).

To confirm that the extent of cleavage within nuc B in the GR2 cells was not dependent on the GR, we determined SstI cleavage in the presence of glucocorticoid antagonists. We predicted that in the presence of antagonist-bound GR, SstI cleavage would be inhibited if the GR was required for chromatin remodeling within nuc B. For control experiments, we also determined SstI cleavage in A1-2 cells, which display a hormone-dependent increase in enzyme cleavage (Fig. 4A). Cells were left untreated (Fig. 6, lane 1) or treated with dexamethasone (lane 2) or anti-glucocorticoid RU43044 or RU486 (lanes 3, 4, 5, and 6). As seen in Fig. 6, A and C, anti-glucocorticoids had no significant effect on SstI cleavage in GR2 cells (cf. lane 2, with lanes 3, 4, 5, and 6). In comparison, anti-glucocorticoids inhibited dexamethasone-induced SstI cleavage in the A1-2 cells (50 and 95% for RU43044 and RU486, respectively) (Fig. 6, B and C; cf. lanes 1 and 2 with lanes 3–6).

Constitutive NF1 Loading on the MMTV Promoter in M10 and GR2 Cells—Previous analysis of the MMTV promoter has shown that induction of the promoter upon hormone addition is accompanied by the appearance of the hypersensitive region within nuc B and the concomitant loading of transcription factors (15, 16, 30). However, NF1 was also shown to bind constitutively on transiently transfected MMTV DNA that is not organized into chromatin (16, 30), and the addition of glucocorticoids did not increase NF1 binding on transiently transfected DNA. This suggests that the GR may not be required for NF1 binding when the MMTV promoter is in an open conformation in the GR2 cells. To examine this possibility, we determined occupancy of the MMTV promoter by the transcription factor NF1 in M10 cells that lack the GR and the GR2 cells. The results from the GR2 cells demonstrate equal NF1 binding in the absence or presence of hormone (Fig. 7, cf. lanes 5 and 6). The lack of a role for the GR in NF1 binding in the GR2 cells is corroborated by the constitutive NF1 loading observed in the M10 cells (Fig. 7, cf. lanes 2 and 3).
Hormone-dependent Association of the GR with SRC-1/NCoA-1---The above studies establish that the open chromatin structure of nuc B, as well as the constitutive binding of NF1, occur independently of both the GR and dexamethasone. However, transcription from the MMTV promoter was hormone-inducible only in the GR2 cells, suggesting that the receptor was required for gene transactivation at a step independent of chromatin remodeling or NF1 binding (Figs. 2 and 3). Steroid receptors interact with a variety of steroid receptor co-activator proteins to regulate gene expression (8, 10, 31). We examined whether the ability of the GR to induce transcription from the MMTV promoter in GR2 cells was correlated with the ability of the receptor to associate with the prototypical co-activator protein SRC-1/NCoA-1 (32, 33). The GR differs from most members of the nuclear receptor superfamily in that in the absence of ligand it is localized within the cytoplasm. To capture the dynamic effects of hormone addition, we carried out co-immunoprecipitation experiments using fractionated nuclear and cytoplasmic extracts. As predicted, the GR resides in the cytoplasm prior to the addition of hormone and then moves to the nucleus in the presence of glucocorticoids (Fig. 8A, cf. lanes 2 and 3 with lanes 5 and 6). In contrast, SRC-1/NCoA-1 protein is exclusively nuclear, and treatment with hormone has no effect on the protein levels (Fig. 8B, cf. lanes 4 and 5). Consistent with the GR-induced changes in MMTV mRNA levels and CAT activity observed earlier (Figs. 2 and 3), the GR associates with the co-activator protein SRC-1/NCoA-1 in a hormone-dependent manner (Fig. 8A, cf. lanes 5 and 6).

**DISCUSSION**

The MMTV promoter has been used extensively to examine the role steroid receptors, particularly the GR, play in remodeling chromatin structure and gene activation (13, 34, 35). The organization of the MMTV promoter into chromatin limits the access of ubiquitous transcription factors to their cognate binding sites in the absence of hormone (12, 30). The GR facilitates transcription factor binding by remodeling the MMTV chromatin structure. This remodeling is highly localized and limited to the regions that encompass a single nucleosome. In addition, the remodeled state is transitory, since the chromatin structure reverts to the closed state and evicts the bound transcription factors from the promoter (16). The GR-mediated chromatin remodeling is accomplished by the human BRG1 chromatin remodeling complex, which shows a hormone-dependent association with GR (25). Delivery of this complex to the proximal promoter containing HREs by GR is the initiating step in the cascade of DNA-protein and protein-protein interactions that allow gene activation (10, 36).

We have described two distinct chromatin structures that are adopted by the MMTV promoter in human and rodent cells (36). The first state is the archetypal closed chromatin state originally described in mouse cells and more recently in human cells expressing the GR (12, 16, 19). Promoters in this state require an obligatory chromatin-remodeling step to initiate transcription. The second state is observed in a subset of human breast cancer cells that express PR but not GR (20). In these cells, the nuc B region is constitutively remodeled into an open conformation, and transcription factors are bound in the absence of exogenous hormone. However, the nucleosomes adjacent to nuc B have a closed conformation (20). These observations led us to consider the possibility that GR status might contribute to the chromatin conformation that the promoter adopts in these cells (17).

A caveat in the above comparisons is that the experiments represent a variety of cell lines that differ in the receptors expressed, the number of MMTV templates in each cell, and the specific MMTV reporter constructs present in each cell line (17). To address this potential difficulty, we characterized T47D/M10 cells, stably transfected with an MMTV reporter, that did not express either the GR or the PR. These M10 cells were then used to derive cell lines that expressed the GR, designated as GR2 and GR4 cells. Both GR2 and GR4 cells exhibit a classical hormone transcriptional response that is inhibited by specific antagonists to the GR. However, in contrast to previous rodent and human GR-expressing cell lines, analysis of the chromatin structure in M10 and GR2 cells demonstrated that nuc B is constitutively open and accessible to restriction endonucleases and transcription factor NF1. These results suggest that the presence of the GR does not always result in the imposition of a closed chromatin structure seen previously (17, 37). They also allow one to consider that the open conformation seen in M10 and GR2 cells may be the default state adopted by the promoter in a T47D background. Indeed, the chromatin organization of the MMTV promoter in the M10 and GR2 cells is similar to that observed in T47D/
2963.1 (PR/βgr) cells, in which the proximal promoter chromatin assumes an open structure (20). In 2963.1 cells, the novel chromatin organization is intimately linked to the occupancy of the promoter by the PR and NF1 in the absence of hormone (20). On the other hand, the open chromatin organization in the M10 and GR2 cells cannot be attributed to promoter occupancy by the GR for a number of reasons. The GR in the absence of hormone is predominantly cytoplasmic and therefore would not interact with the HREs. Second, even in the presence of hormone, the GR appears to have a very transient interaction with the HREs (38–40). Finally a similar, if not identical, pattern of hypersensitivity and promoter-bound NF1 is observed in the M10 parental line, which lacks the GR. This observation suggests that the chromatin organization of the proximal MMTV promoter in these cells is not dependent on the GR.

The GR-independent binding of NF1 to the promoter has been explored previously using transient transfection experiments (16). While NF1 binding is hormone-dependent for a stable MMTV template in GR-positive rodent cells, NF1 is bound to the promoter in the absence of hormone in transiently transfected DNA, suggesting that the binding is not directly dependent on the GR (16, 30). The stably transfected MMTV promoter in the GR-negative M10 cells confirms that NF1 binding to the promoter, as for transiently transfected DNA, is independent of the GR. However, constitutive NF1 binding or rearrangement of the chromatin structure alone is not sufficient for transcriptional-activation (30). Rather, efficient transcription requires recruitment by the GR of other co-regulatory factors to the promoter (36). Indeed, we find that activation of the MMTV template in GR2 cells involves at least the hormone-dependent interaction between SRC-1/NCoA-1 and GR.

Two categories of inducible genes can be envisioned depending on their chromatin structure: those genes that exhibit a preset structure and those that require chromatin remodeling prior to activation (41). Our experiments with M10 and GR2 cells suggest that the chromatin organization of the MMTV promoter is not dependent on the receptor status of the cell. Rather, our studies imply that constitutive binding of other factors to the promoter (36). Indeed, we find that activation of the MMTV template in GR2 cells involves at least the hormone-dependent interaction between SRC-1/NCoA-1 and GR.

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