Glucocorticoid and progestin receptors are differently involved in the cooperation with a structural element of the mouse mammary tumor virus promoter.

S. Le Ricousse, F. Gouilleux, D. Fortin, V. Joulin, H. Richard-Foy

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 5072–5077, May 1996
Biochemistry

Glucocorticoid and progestin receptors are differently involved in the cooperation with a structural element of the mouse mammary tumor virus promoter

S. Le Ricousse*, F. Gouilleux†, D. Fortin, V. Joulin‡, and H. Richard-Foy§¶

Unité de Recherche sur les Communications Hormonales de l'Institut National de la Santé et de la Recherche Médicale, 80 Avenue du Général Leclerc, 94276 Bicêtre Cedex, France

Communicated by Etienne-Emile Baulieu, Institut National de la Santé et de la Recherche Médicale, Bicêtre, France, October 17, 1995

ABSTRACT **We have previously characterized a regulatory element located between −294 and −200 within the mouse mammary tumor virus (MMTV) long terminal repeat (LTR). This element termed *AA* element cooperates with the glucocorticoid response elements (GREs) for glucocorticoid activation. Here we show that in a MMTV LTR wild type context, the deletion of this element significantly reduces both glucocorticoid and progestin activation of the promoter. Deletion of the two most distal GREs forces the glucocorticoid receptor (GR) and the progestin receptor (PR) to bind the same response elements and results in a dramatic decrease in the inducibility of the MMTV promoter by the two hormones. The simultaneous deletion of the two distal GREs and of the *AA* element abolishes completely the glucocorticoid-induced activation of the promoter. In contrast it restores a significant level of progestin-induced activation. This different effect of the double deletion on glucocorticoid- and progestin-induced MMTV promoter activation is not cell specific because it is also observed, and is even stronger, when either GR or PR is expressed in the same cell line (NIH 3T3). This is the first description of a mutated MMTV promoter that, although retaining GREs, is activated by progestins and not by glucocorticoids. This suggests a different functional cooperation between protein(s) interacting with the *AA* element and GR or PR. Cotransfections with constructs containing wild-type or mutated MMTV LTR with either PR lacking its C-terminal domain or GR/PR chimeras in which the N-terminal domains have been exchanged demonstrate that the N-terminal domains of the receptors specify the different behavior of GR and PR regarding the *AA* element.**

Steroid receptors are ligand-dependent trans-acting transcription factors. They exert their effect by binding to a specific cis-acting DNA sequence termed hormone response element (HRE). The activity of the glucocorticoid receptor (GR) (1–3), progestin receptor (PR) (4), androgen receptor (AR) (5, 6), and mineralocorticoid receptor (MR) (7, 8) is mediated by the same cis-acting element termed glucocorticoid response element/progestin response element (GRE/PRE). This palindromic element interacts with a hormone-receptor homodimer; it confers hormone inducibility to an adjacent promoter (9). Glucocorticoids, progestins, androgens, and mineralocorticoids are involved in distinct biological pathways and thus modulate the transcription of distinct sets of genes. This raises the question of how hormone receptor complexes that recognize identical cis-acting elements can modulate the expression of only a subset of the genes containing it. Sometimes the specificity is achieved through the different distribution of receptors in different tissues (10). Frequently, however, two receptor types are expressed simultaneously in the same cell; this often occurs with GR, which is present in almost every cell type. In such a situation, the specificity of the steroid-hormone response can be achieved via mechanisms such as (*i*) the metabolism of one of the hormones in a limited number of cell types, resulting locally in either its activation (11) or inactivation (12); or (*ii*) the binding of proteins to DNA in the vicinity of the HREs, which may restrict or facilitate the access of a receptor to its cognate target.

One of the most studied hormone-responsive promoters is the mouse mammary tumor virus (MMTV) promoter located within the viral long terminal repeat (LTR) (13, 14). Promoter activity is induced by glucocorticoids, progestin, androgens, and, to a lesser extent, mineralocorticoids (1–8). The promoter contains four GRE/PREs, one distal palindromic site (located between positions −190 and −160) and three proximal hemipalindromes (located between positions −128 and −78). Both GR and PR bind to these GRE/PREs, thereby activating transcription (15, 16). However, clear differences in the interaction of GR and PR with the HRE region and cooperation between DNA-bound receptor molecules have been observed (15). Mutational analysis of the HREs revealed that the proximal GREs are more important than the distal one for progestin activation, while the distal one is more important for glucocorticoid activation (6, 17).

The mechanisms by which the binding of the hormone–receptor complex is converted into transcriptional activation involve the possible interaction of the steroid receptor with the basal transcription machinery and/or with nuclear proteins binding in the vicinity of the GRE/PREs. A number of groups have identified transcription factors that modulate both the glucocorticoid and progestin responses of the MMTV LTR promoter (reviewed in ref. 18). The role of nuclear factors NF-I and OTF-1 in the hormone-induced activation of the MMTV promoter has been well documented.

NF-I binds immediately downstream of the most proximal GRE (between positions −76 to −63). *In vivo*, this binding is glucocorticoid-dependent (19), while *in vitro* GR does not facilitate the binding of NF-I to its adjacent target but competes for its binding (20). Transient and stable transfection experiments and *in vitro* transcription experiments (21) reveal

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MMTV, mouse mammary tumor virus; LTR, long terminal repeat; GRE, glucocorticoid response element; PRE, progestin response element; HRE, hormone response element; GR, glucocorticoid receptor; PR, progestin receptor.

*Present address: Laboratoire de Biotechnologie des Anticorps, Institut Curie, 26 rue d'Ulm, 75231 Paris Cedex 05, France.
†Present address: Laboratoire d'Oncologie Cellulaire et Moléculaire de l'Institut National, de la Santé et de la Recherche Médicale, Hôpital Cochin, Batiment Gustave Roussy, 27, rue du Faubourg St Jacques 75014 Paris, France.
‡Present address: Laboratoire de Génétique et Pathologie Expérimentales de l'Institut National de la Santé et de la Recherche Médicale, Institut Cochin de Génétique Moléculaire, 22 rue Mechain, 75014 Paris, France.
§To whom reprint requests should be addressed.
¶Present address: Laboratoire de Biologie Moléculaire des Eucaryotes du Centre National de la Recherche Scientifique, 118 route de Narbonne, 31062 Toulouse, France.

a clear difference in the requirements of progestins or glucocorticoids for NF-I binding (6, 17). Glucocorticoid-dependent activation requires NF-I binding, while progestin-dependent activation does not.

Two degenerate octamer motifs are located between positions −56 and −37 upstream of the CAP site. *In vitro* PR and GR bound to the HRE facilitate binding of OTF-1 to the two motifs (22). However, only the promoter-distal octamer motif plays a role in glucocorticoid- and progestin-induced MMTV transcription.

DNA sequences, located outside the HRE, also play a role in modulating the responsiveness of the MMTV promoter to steroids. A point mutation at position −200 significantly decreases glucocorticoid and androgen, but not progestin, activation of the promoter (17).

We have previously characterized a regulatory element located between −294 and −200, termed *AA* element, that cooperates with the HREs for glucocorticoid activation (23). Here we report data on the role of this *AA* element in both glucocorticoid and progestin activation of the MMTV promoter. We present evidence for a different functional cooperation between protein(s) interacting with the *AA* element and either GR or PR and show that the receptors' N-terminal domains are involved in this cooperation.

MATERIALS AND METHODS

Cell Lines and Transfection. NIH 3T3 (mouse fibroblasts) and 34i (mouse mammary epithelial carcinoma) cells were grown as described (23). T47D cells (human mammary carcinoma) were grown under the same conditions, except that the medium was supplemented with 0.6 μg of bovine insulin per ml. For transfection experiments, cells were plated to a density of 10^6 cells per 10-cm dish. Each dish was transfected with 5 μg of the plasmid to be tested (wild-type or mutated MMTV driving luciferase); 5 μg of control plasmid pCH110; when indicated, 5 μg of a vector expressing either GR, PR, or PR/GR chimeras; and 5 or 10 μg of pSP72 to adjust the total DNA to 20 μg (final amount). Transfections were performed as previously described (23) by the standard calcium phosphate method (24).

Luciferase and β-Galactosidase Assays. Cell extracts for luciferase and β-galactosidase assays were prepared as described by Nguyen *et al*. (25) with the previously described modifications (23). The luciferase data were normalized to the β-galactosidase data in each individual sample. Normalized results are expressed in arbitrary units and represent the ratio: relative luciferase units (RLU) (integration time, 30 sec; 50-μl sample)/β-galactosidase activity (OD_{420}, reaction time, 2 hr; 200-μl sample)/1000.

Plasmids. Plasmids pFC31luc and pC3ΔAAluc (pC3DAAluc in ref. 23) have been described (23). The other constructs were pFC31luc with the following deletions within the MMTV LTR: pC3ΔPAluc (from the 5′-end to −201), pC3ΔPSsluc (from the 5′-end to −110), pC3ΔGREluc (−210 to −110), and pC3ΔAAΔGREluc (−294 to −110). Plasmid pSG5hPR was a gift of H. Loosfeldt and E. Milgrom (INSERM, Bicêtre, France). It contained a cDNA encoding the human progesterone receptor cDNA inserted in the pSG5 vector. Plasmid pSG5hGR was obtained by inserting the human GR coding sequences from pRSVhGRα (26) in pSG5. hPR5 and hPR3 (27), obtained from H. Gronemeyer and P. Chambon, IGBMC, Strasbourg, France, are expression vectors encoding PR lacking either the C-terminal (hPR5) or the N-terminal (hPR3) domains. PR/GR chimeras, in which the N-terminal domains of the two receptors were exchanged, were constructed by PCR site-directed mutagenesis with Vent DNA polymerase. Construct pSG5hPRmut was obtained by generating point mutations in pSG5hPR (Amersham mutagenesis kit) with the following oligonucleotide: GTTTAgTCTGTcGaGATGAAGCtagcGGCTGTCATTATGG (lowercase letters indicate a mismatch with the wild-type sequence). These mutations created a *Nhe* I site and generated a two-amino-acid change in the mutated PR. The ability of this mutant to transactivate transcription was tested in transient transfection experiments in parallel with wild-type PR. Its transactivation activity was found to be identical to that of PR. To generate the construct PRN/GRC, which expresses a chimera that contains amino acids 1–566 of human PR and amino acid 421 to the end of human GR, the *Nhe* I/*Xba* I fragment that encodes the C-terminal domain of PR was replaced in pSG5hPRmut by the GR *Nhe* I/*Xba* I fragment, which was amplified by PCR by using pSG5hGR as a template and oligonucleotides GRC1 (GATGAAGCTagcGGATGTCATTATGGAGTC) and GRPRC2 (GAAGCGGAAGAGTCTAGAGTCGACCAG) as primers. Construct GRN/PRC was obtained by inserting first the pSG5hGR *Eco*RI/*Nhe* I fragment, amplified by PCR by using oligonucleotides GRN1 (ATCCgctAGCTTCATCAccgCaGATCAGGCAGAGTTTGGG) and GRN2 (TAGGGCGAATTCCCGGGTACCTCCTGC) as primers in vector pSG5PL (pSG5PL is the pSG5 vector in which a polylinker was introduced) between *Eco*RI and *Nhe* I, generating pSG5GRN. The pSG5PR *Nhe* I/*Xba* I fragment, encoding the C-terminal part of PR, was amplified by PCR with oligonucleotides PRC1 (GATGAAGCtagcGGCTGTCATTATGGTGTC) and GRPRC2 as primers. The *Nhe* I/*Xba* I fragment was subsequently inserted into construct pSG5GRN between the *Nhe* I and *Xba* I sites, generating construct GRN/PRC, which encodes a chimera containing amino acids 1–420 of the human GR and amino acids 567 to the end of the human PR. The structure of the recombined region of the two constructs encoding the chimeras (limit between the N-terminal and DNA-binding domains) (see Fig. 4*A*) was verified by sequencing.

Dnase I Footprint. Nuclear extracts were prepared from 34i and T47D cells, treated or not with 0.5 μM dexamethasone (34i) or 0.1 μM R5020 (T47D) as described by Dingman *et al*. (28). The nuclear extracts were concentrated by ammonium sulfate precipitation, the pellets were redissolved in buffer D (20 mM Hepes, pH 7.9/20% (vol/vol) glycerol/0.1 M KCl/0.2 mM EDTA/0.5 mM dithiothreitol/0.2 mM phenylmethylsulfonyl fluoride), and the solution was dialyzed overnight against 20 volumes of the same buffer. The proteins were assayed (they ranged from 3 to 20 mg/ml), and the extracts were aliquoted and stored frozen at −80°C. The probe was generated by PCR with 1 pg of plasmid pFC31luc as a template, 100 ng of primer [SO2 end-labeled with T4 kinase (TAAGTGACGAGCGGAGACGGGATGG) and VM (GGGACAGTGGCTGGACTAATAGAAC)], 5 mM dNTPs, and 2.5 units of *Taq* polymerase in a 100-μl final volume. This generated a 368-bp probe, which was purified by electrophoresis on a 6% nondenaturing acrylamide gel. The nuclear extracts (30 μg of protein, diluted to 10 μl in buffer D) were incubated for 15 min at room temperature with the labeled probe (1 μl, ≈50,000 cpm) and poly(dI-dC) (1 μg) in 20 mM Hepes, pH 7.9/8% glycerol/60 mM KCl/0.2 mM phenylmethylsulfonyl fluoride/1.5 mM dithiothreitol/0.08 mM EDTA/0.08 mM $MgCl_2$ in a final volume of 25 μl. Samples were digested with DNase I (0.25–2 μg in 1 μl of 10 mM Tris, pH 8) for 2 min at 0°C. After DNA purification and precipitation with ethanol, the samples were resuspended in 4 μl of loading buffer and run on a 6% denaturing acrylamide gel.

RESULTS

Deletion of the *AA* Element Causes a Decrease of Both Glucocorticoid- and Progestin-Induced MMTV Promoter Activity. In previous studies we demonstrated that an element located between positions −294 and −200 of the LTR cooperates with glucocorticoids for MMTV promoter activation. In Fig. 1 are comparisons of the effects of various deletions

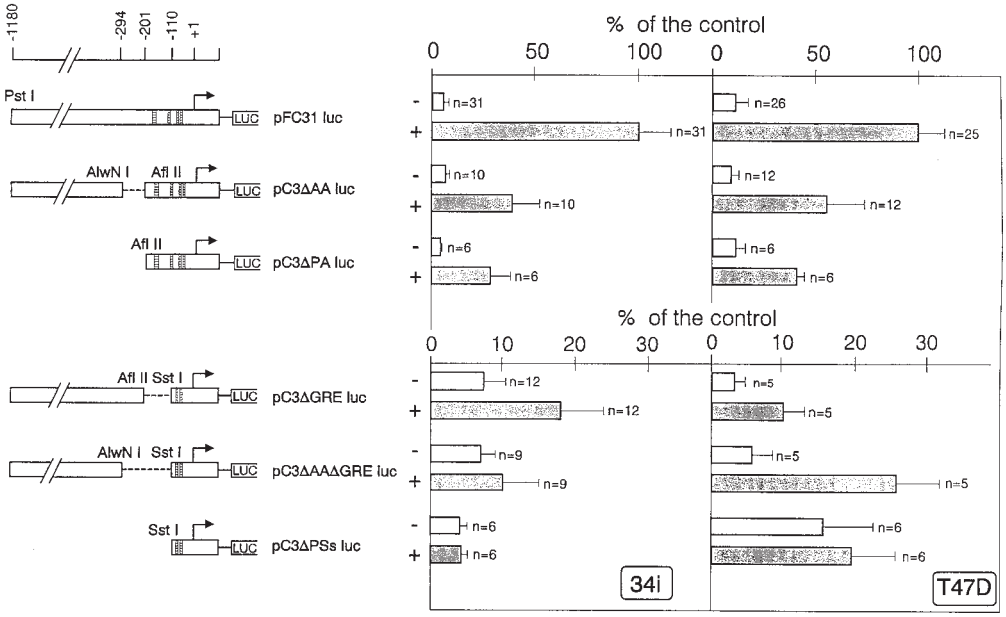

FIG. 1. Influence of deletions within the MMTV LTR on glucocorticoid- and progestin-induced luciferase activity. 34i or T47D cells (*Right*) were transfected with the constructs shown (*Left*) which contain a wild-type or mutated MMTV LTR driving the luciferase gene. Cells were treated or not with hormone (34i and T47D cells with dexamethasone and R5020, respectively), and the luciferase activity was measured in a transient expression assay. Open bars, untreated cells; grey bars, cells treated with hormone. Results presented in all the panels are expressed as the percentage of the luciferase activity measured in hormone-treated cells after transfection of the control plasmid pFC31luc; *n* is the number of independent transfections. The scale has been enlarged in *Right Lower.*

encompassing this region on activation of the MMTV promoter by either glucocorticoids in cell line 34i (that expresses GR) or progestin in cell line T47D (that expresses PR). Deletion of the region −294 to −200 (pC3ΔAAluc) led in both cases to a decrease of the steroid-induced expression of the reporter gene, when compared to the full-length MMTV LTR (pFC31luc). An even greater decrease was observed when the entire 5′ end of the LTR up to −200 was deleted (pC3ΔPAluc), suggesting that this effect is not due to placement of the proximal promoter closer to upstream cryptic elements.

Glucocorticoid- and Progestin-induced MMTV Promoter Activation Is Affected Differently by the Deletion of the *AA* Element if GR and PR Are Forced to Bind to the Same GREs. As discussed previously, the four GREs of the MMTV do not share the same properties in terms of binding and transcriptional transactivation by GR and PR. The MMTV promoter that contains 4 GREs is relatively complex; it is impossible to ascertain which GRE is interacting with GR or PR in transient transfection experiments. To force GR and PR to bind to the same GREs, we deleted the two distal GREs (pC3ΔGREluc). This should allow investigation of the contribution of the *AA* element in GR and PR induction of luciferase expression. In both cases, deletion of the two distal GREs resulted, as expected, in a strong reduction of hormone induction levels (Table 1) and, as a consequence, in a decreased hormone-induced luciferase expression (Fig. 1). The basal luciferase level, measured in the absence of hormone, was unchanged in 34i cells (5.91 ± 2.13% of the control for pFC31luc and 7.29 ± 3.11% of the control for pC3ΔGREluc), while it was apparently decreased in T47D cells (11.22 ± 6.26% of the control for pFC31luc and 3.27 ± 1.46% of the control for pC3ΔGREluc). Contrasting with this result, the simultaneous deletion of the *AA* element and of the two distal GREs (pC3ΔAAΔGREluc) resulted in the loss of induction in luciferase expression by glucocorticoids in 34i cells and in a partial restoration of induction in luciferase expression by progestins in T47D cells (Table 1 and Fig. 1).

Table 1. Hormonal induction of the wild-type and mutated MMTV promoter

	Induction in cell lines			
			NIH 3T3	
Construct	34i	T47D	GR	PR
pFC31luc	16.9	8.9	48.7	74.4
pC3ΔAAluc	5.6	6.1	35.2	55.5
pC3ΔGRE	2.5	3.1	2.3	4.4
pC3ΔAAΔGRE	1.4	4.4	1.4	32.1

Data are from experiments presented in Figs. 1 and 3.

Cell-Type Differences in Proteic Factors Do Not Account for the Different Contribution of the *AA* Element to Glucocorticoid- and Progestin-Induced MMTV Promoter Activation. One possible explanation for these differences in induction of luciferase expression by the two hormones could be differences in general factors, such as NF-I or OTF-1, in the two cell lines. We investigated this by DNase I footprinting using nuclear extracts from 34i or T47D cells. Results presented in Fig. 2 show that the footprints generated by the two nuclear extracts are distinct. The

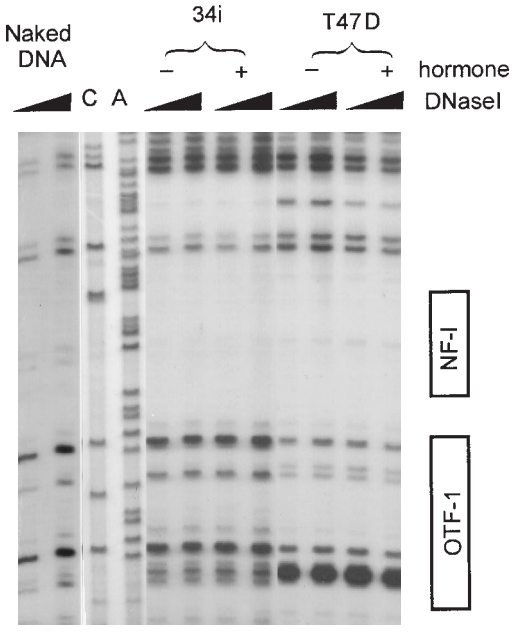

FIG. 2. *In vitro* DNase I footprint analysis of the factors interacting with the proximal MMTV promoter. Footprints of nuclear extracts from 34i and T47D cells, treated or not with, respectively, dexamethasone or R5020, on the MMTV LTR region spanning positions −30 to −105. The samples, including control naked DNA, were digested with two concentrations of DNase I. A and C are marker lanes containing the corresponding MMTV LTR region sequenced by using oligonucleotide SO2 as a primer.

major difference is in the OTF-1 binding region, which is strongly protected with T47D extract and not significantly protected with 34i extract. This cannot result from a PR-facilitated binding of OTF-1 to its target, since the same footprint is obtained with extracts from hormone-treated and untreated cells.

To analyze the relevance of these differences in the activation of MMTV promoter by glucocorticoids and progestins, we decided to study each receptor in the same cell line. We selected NIH 3T3 cells, which contain only low levels of GR (23) and no PR. NIH 3T3 cells were cotransfected with the constructs pC3ΔGREluc, pC3ΔAAΔGREluc, and pC3ΔPSsluc, along with either GR or PR expression vectors (Fig. 3). Deletion of the *AA* element (pC3ΔAAluc) resulted in a decrease of ≈50% in the level of hormone-induced luciferase expression, while the basal luciferase level remained unchanged (Table 1 and Fig. 3). Further deletion of all the MMTV LTR sequences located upstream of the *AA* element (pFC3ΔPAluc) had no additional effect (the higher basal luciferase level shown in Fig. 3 for this construct in cells expressing GR is not significant, since in these transfection experiments the basal luciferase level was the same with pFC31luc). For both receptors, deletion of the two distal GREs (pFC3ΔGREluc) did not affect significantly basal luciferase expression while, as previously observed in 34i and T47D cells, the hormone-induction level was significantly reduced (Table 1 and Fig. 3). The simultaneous deletion of the *AA* element and of the two distal GREs did not change significantly the basal luciferase expression; it resulted in a complete loss of the glucocorticoid induction and, contrasting with it, in a significant restoration of the progestin induction (Table 1). Construct pC3ΔPSsluc, containing a LTR lacking its 5′ end up to position −110, behaves similarly to pC3ΔAAΔGREluc since induction of luciferase expression by glucocorticoids is abolished. However, progestin-induced luciferase expression was lower for pC3ΔPSsluc than for pC3ΔAAΔGREluc, suggesting that elements upstream of the *AA* element may also contribute to progestin-induction of luciferase expression. These results demonstrate that the effect of the simultaneous deletion of the *AA* element and of the two distal GREs is the same (and even stronger) in NIH 3T3 cells as in 34i and T47D cells.

GR and PR N-Terminal Domains Are Responsible for the Different Behavior of the Two Receptors Regarding the *AA* Element. To determine which domain of the receptors is involved in this cooperation process with the *AA* element, we investigated the ability of truncated PR lacking its N-terminal domain or its C-terminal domain to induce luciferase from constructs pFC31luc, pC3ΔAAluc, pC3ΔGREluc, and pC3ΔAAΔGREluc. The level of luciferase expression obtained with the N-terminal truncated PR was low when compared to the full-length receptor, making results impossible to interpret (not shown). In contrast truncation of the C-terminal domain of PR, which generates a receptor form that is constitutively active, resulted, when using pFC31luc, in a luciferase expression that was ≈8–16% of that obtained with the wild-type receptor (not shown). Fig. 4*A* shows the results of an experiment in which NIH 3T3 cells were cotransfected with the vector encoding either PR or the C-terminal truncated PR (hPR5) together with either pFC31luc, pC3ΔGREluc, or pC3ΔAAΔGREluc. The effect of the deletions within the MMTV LTR on luciferase expression in hormone-treated cells expressing PR or in cells expressing hPR5 was very similar. The deletion of the two distal GREs led to a decreased luciferase expression and the deletion of the *AA* element along with the distal GREs resulted in a substantial restoration of luciferase expression when compared with the wild-type MMTV. This demonstrates that PR deleted of its C-terminal domain behaves as the wild-type receptor, regarding the *AA* element. These results however did not allow us to discriminate between effects due to the N-terminal or the DNA binding domain of PR.

To investigate further the role of the N-terminal domains of GR and PR, and since the deletion of the N-terminal domain decreased considerably the ability of GR and PR to activate MMTV promoter, we constructed GR/PR chimeras, in which the N-terminal domains of the receptors were exchanged. Fig. 4*B* shows the structure of the two chimeras at the junction point. NIH 3T3 cells were cotransfected with either pFC31Puc, pC3ΔGREluc, or pC3ΔAAΔGREluc together with vectors expressing either PR or each of the chimeras. The results are presented in Fig. 4*C*. In the presence of the appropriate hormone, both chimeras transactivated efficiently the wild-type MMTV promoter. GRN/PRC is as potent as PR, and PRN/GRC retains an activity that is one-third that of PR. For both chimeras, as observed for the wild-type receptors, the induction of luciferase expression was low when the two distal GREs had been deleted (pC3ΔGREluc). When the distal GREs and the *AA* element were simultaneously deleted (pC3ΔAAΔGREluc), in the presence of the chimera containing the N-terminal domain of PR, the luciferase expression obtained in the presence of hormone was twice as high as that obtained when the *AA* element was not deleted. In contrast, in the presence of the chimera containing the N-terminal domain of GR, the luciferase expression obtained upon hormonal treatment was slightly lower than that obtained when the *AA*

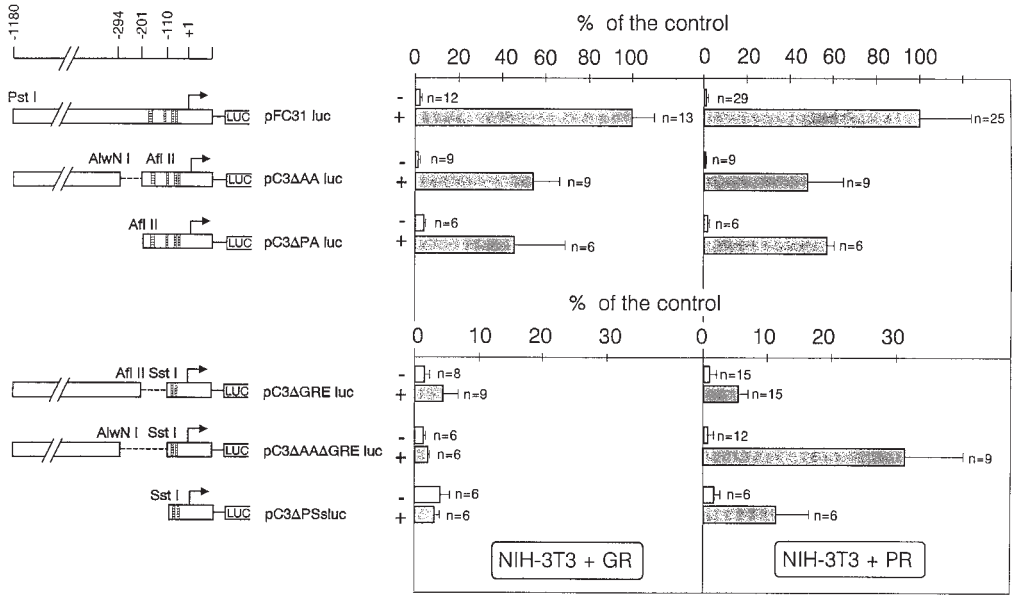

FIG. 3. Contribution of the *AA* element to glucocorticoid- and progestin-induced MMTV activation in different cell types. NIH 3T3 cells were cotransfected with the constructs shown, together with a vector expressing GR or PR. Cells were treated or not with the appropriate hormone, and luciferase was assayed. Open bars, untreated cells; grey bars, cells treated with hormone. Results are expressed as in Fig. 1.

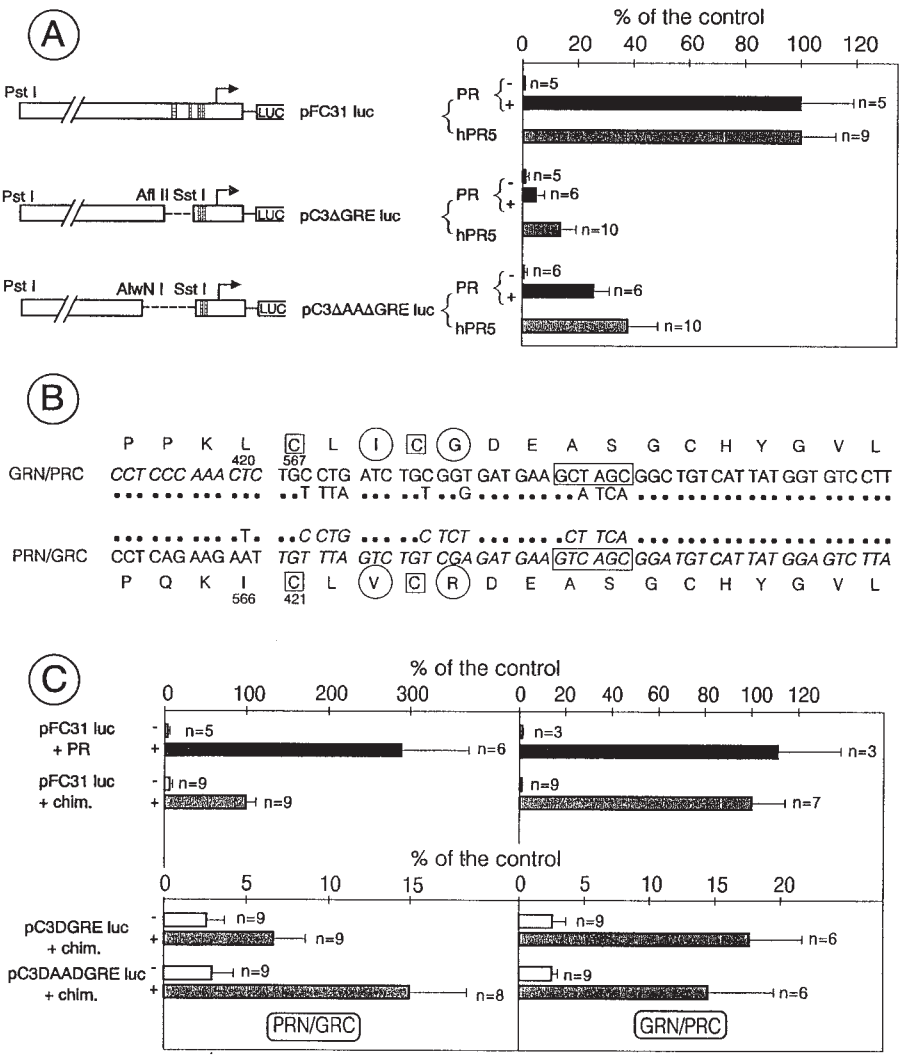

FIG. 4. Analysis of the GR and PR receptor domains that display functional interaction with the *AA* element. (*A*) NIH 3T3 cells were transfected with the constructs shown, together with a vector expressing either PR or hPR5. PR (solid bars), cells treated with R5020; PR (open bars), cells untreated with the hormone; hPR5 (grey bars), hormone-treated and untreated cells. Results are expressed as the percentage of the values obtained with pFC31luc in cells expressing PR and treated with hormone, or in cells expressing hPR5 treated or not with hormone. (*B*) Sequences of the regions of PR and GR that were engineered to generate the two chimeras PRN/GRC and GRN/PRC. Underneath (GRN/PRC) and above (PRN/GRC) are indicated the nucleotide sequence for the wild-type receptor. The *Nhe* I site is boxed. Above (GRN/PRC) and underneath (PRN/GRC) are shown the amino acid sequences in single letter code. The numbers refer to wild-type receptors. Cysteines are boxed, and the amino acids of the DNA-binding domains of the two chimeras that are different are circled. (*C*) NIH 3T3 cells were cotransfected with the constructs indicated together with PR (*C Upper*), the PRN/GRC chimera (*C Left*), or the GRN/PRC chimera (*C Right*). Bars: solid, PR, cells treated with R5020; grey, chimeras, cells treated with the appropriate hormone; open, PR or chimeras, cells untreated with the hormone. Results are expressed as the percentage of the value obtained for construct PFC31luc in the presence of either PRN/GRC or GRN/PRC in cells treated with the appropriate hormone.

element was still present. Although the effects are more moderate than in the presence of wild-type receptors, the chimeras behave in the same way as the receptor corresponding to their N-terminal domain.

DISCUSSION

It is well established that mutations in the GREs affect differently the response of MMTV to glucocorticoids and progestins (6, 17). In addition, the involvement of at least one of the general transcription factors, namely NF-I, appears to be different upon activation of the MMTV promoter by the two hormones (6, 17). We have previously identified a regulatory element located within MMTV LTR, immediately upstream of the HREs, termed *AA* element (23). This element cooperates with the HREs for glucocorticoid-induced MMTV promoter activation. In the present study we focused on the role of GR and PR in their functional interaction with the *AA* element. A detailed characterization of the *AA* element will be reported elsewhere (unpublished data). Here we show that, as previously described for glucocorticoids, in a wild-type MMTV LTR context, the deletion of this element decreased ≈50% the progestin-induced MMTV promoter activation. In contrast, when PR and GR were forced to bind the same HREs, deletion of the *AA* element resulted in a complete loss of glucocorticoid induction, while progestin induction (dramatically decreased by the deletion of the two distal GREs) was substantially restored. This demonstrates that the apparently identical effect of the deletion of the *AA* element, in the context of all four GREs, on glucocorticoid- and progestin-induced activation may reflect a complex set of events, some of which may be steroid hormone specific. The diagram in Fig. 5 summarizes one possible model drawn from our observations.

Several groups have investigated the mechanisms by which specific hormonal regulation of gene expression is attained *in vivo* for steroid hormone receptors that recognize the same DNA elements *in vitro*. It has been shown, for a composite element containing a GRE and an AP1 binding site, that GR repressed AP1-induced transcription while MR did not. The authors conclude that a region located within the N-terminal domain of GR is responsible for this specificity and that the distinct physiological effects mediated by MR and GR may be determined by different interactions of nonreceptor factors with specific receptor domains at composite response elements (29). However, in these experiments it is impossible to discriminate between the existence of a so-called "composite specificity" receptor domain and steric hindrances resulting from the much larger N-terminal domain of MR. Another group addressed the question of androgen-specific gene activation via the *slp* (mouse sex-limited protein) enhancer which contains several GREs. From a detailed analysis of the enhancer, they conclude that a subtle interplay of the precise sequence of the response element and the particular array of nonreceptor binding sites may allow gene expression to vary *in vivo* with hormone, concentration, and cell type (30, 31). The mutated MMTV promoter that we have engineered is activated in a hormone-specific fashion, and it differs from the *spl* enhancer in that this specificity is cell type-independent. This construct retains the ability to bind GR, but deletion of the *AA* element

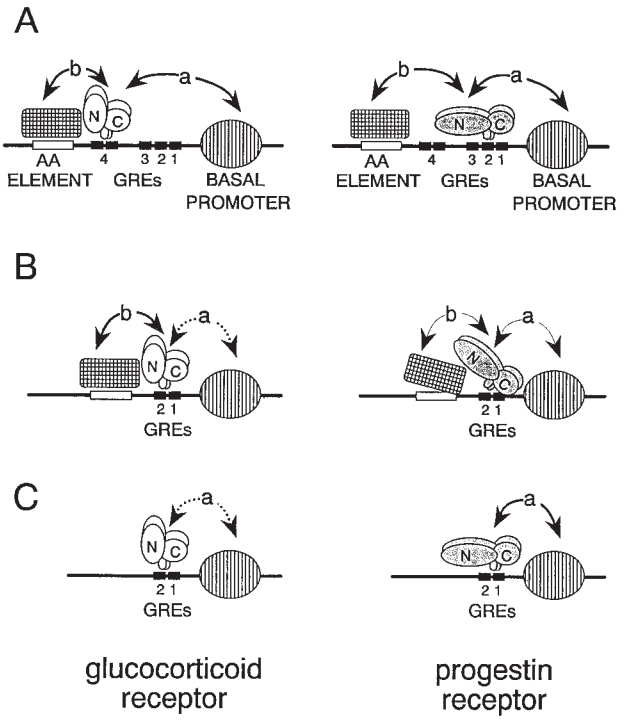

FIG. 5. Model for the different involvement of GR and PR in MMTV promoter activation. (*A*) Intact MMTV LTR. (*Left*) GR interacts mainly with GRE4. (*Right*) PR interacts with GREs 1 and 2. For both receptors, the maximal MMTV promoter activation is achieved through functional interactions of the receptors with factors bound to the basal promoter (path a) and to the *AA* element (path b). N, receptor N-terminal domain, C, receptor C-terminal domain. (*B*) MMTV LTR deleted of GREs 3 and 4. GR and PR are forced to bind the same GREs (GREs 1 and 2). This results for both receptors in a significant decrease in promoter inducibility. However, this decrease may result from different causes for the two receptors. On such a mutated MMTV LTR, GR must interact with GREs 1 and 2 rather than with the preferred GRE4. These sites are close to the NF-I target site, and as has been described *in vitro* (20), GR and NF-I may compete for a binding to the same region. Since NF-I is necessary for MMTV promoter activation by glucocorticoids, this may result in a decrease in promoter inducibility (path a). The interaction between GR and factors bound to the *AA* element should remain identical to that observed on the wild-type promoter (path b), resulting in the 3- to 5-fold induction of the promoter upon glucocorticoid addition. In contrast PR interacts with the same GREs as in wild-type MMTV LTR. However, because of the deletion of the distal GREs, GREs1 and 2 are now very close to the *AA* element. Since the N-terminal domain of PR is much larger than the GR one, this may generate steric hindrances. In such a situation, the functional interactions of PR with proteins bound both to the basal promoter and the *AA* element would be altered (paths a and b), resulting in a dramatic decrease in the level of progestin induction of the MMTV promoter. (*C*) MMTV LTR with the simultaneous deletion of the *AA* element and GREs 3 and 4. GR can only bind to GREs 1 and 2 and, as discussed in *B*, it could not interact with factors bound to the basal promoter (path a). This mutated MMTV promoter is no longer inducible by glucocorticoids. In contrast, PR is now able to bind its natural GRE target and cooperates with factors bound to the basal promoter (path a), allowing the dramatic recovery in progestin-induced activity when compared with the MMTV LTR mutant retaining the *AA* element.

results in a loss of induction of the MMTV promoter by glucocorticoids. Our results support a model in which the interactions between factor(s) binding the *AA* element and GR or PR are different. The factor(s) bound to the *AA* element would then contribute to the specificity of the hormonal response. We have shown that the N-terminal domain of the receptors plays a critical role in specifying this hormonal specificity of induction. However, it remains to be determined whether the specific activation of MMTV promoter by either GR or PR results from specific interactions or from steric hindrance. In addition, other domains of the receptors may also be involved in that process. The comparison of the two chimeras GRN/PRC and PRN/GRC suggests that the hormone-binding domain of PR is more efficient than that of GR in transactivating the MMTV promoter. In that regard it may also play a role in the hormonal specificity of MMTV promoter activation.

The mutated MMTV promoter described here constitutes a unique model to study the specificity of steroid hormone response because it retains inducibility by progestins but not by glucocorticoids. The lack of cell specificity that we report here also makes it a potent tool for pharmacological studies.

We thank R. Evans for plasmid pRSVhGRα, E. Milgrom and H. Loosfeldt for plasmid pSG5PR, and P. Chambon and H. Gronemeyer for constructs hPR3 and hPR5. We are grateful to S. C. Batson and C. Chailleux for critically reading the manuscript. This work was partially supported by the Association pour la Recherche sur le Cancer, Grant 6694, the Conseil Regional Midi-Pupénécs and the Fondation pour la Recherche Médicale. S.L.R. was supported by a fellowship from the Association pour la Recherche sur le Cancer.

1. Payvar, F., DeFranco, D., Firestone, G. L., Edgar, B., Wrange, O., Okret, S., Gustafsson, J. A. & Yamamoto, K. R. (1983) *Cell* **35,** 381–392.
2. Scheideret, C., Geisse, S., Westphal, H. M. & Beato, M. (1983) *Nature (London)* **304,** 749–752.
3. Hynes, N. H., van Ooyen, A. J. J., Kennedy, N., Herrlich, P., Ponta, H. & Groner, B. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3637–3641.
4. Cato, A. C. B., Miksicek, R., Schutz, G., Arenmann, J. & Beato, M. (1986) *EMBO J.* **5,** 2237–2240.
5. Darbre, P., Page, M. & King, R. J. B. (1986) *Mol. Cell. Biol.* **6,** 2847–2854.
6. Gowland, P. R. & Buetti, E. (1989) *Mol. Cell. Biol.* **9,** 3999–4008.
7. Arriza, J. L., Weinberger, C., Cerelli, G., Glaser, T. M., Hendelin, B. L., Housman, D. E. & Evans, R. M. (1977) *Science* **237,** 268–275.
8. Cato, A. C. B. & Weinmann, J. (1988) *J. Cell Biol.* **106,** 2119–2125.
9. Chandler, V. L., Maler, B. A. & Yamamoto, K. R. (1983) *Cell* **33,** 489–499.
10. Strahle, U., Boshart, M., Klock, G., Stewart, F. & Schutz, G. (1989) *Nature (London)* **339,** 629–632.
11. Baulieu, E. E., Lasnitzki, I. & Robel, P. (1968) *Nature (London)* **219,** 1155–1156.
12. Funder, J. W., Pearce, P. T., Smith, R. & Smith, I. (1988) *Science* **242,** 583–585.
13. Huang, A. L., Ostrowski, M. C., Berard, D. & Hager, G. L. (1981) *Cell* **27,** 245–255.
14. Lee, F., Mulligan, R., Berg, P. & Ringold, G. (1981) *Nature (London)* **294,** 228–232.
15. Chalepakis, G., Arnemann, J., Slater, E., Bruller, H. J., Gross, B. & Beato, M. (1988) *Cell* **53,** 371–382.
16. von der Ahe, D., Janish, S., Scheideret, C., Renkavitz, R., Schutz, G. & Beato, M. (1985) *Nature (London)* **313,** 706–709.
17. Cato, A. C. B., Skroch, P., Weinmann, J., Butkeraitis, P. & Ponta, H. (1988) *EMBO J.* **7,** 1403–1410.
18. Gunsburg, W. H. & Salmons, B. (1992) *Biochem. J.* **283,** 625–632.
19. Cordingley, M. G., Riegel, A. T. & Hager, G. L. (1987) *Cell* **48,** 261–270.
20. Bruggemeier, U., Rogge, L., Winnacker, E. L. & Beato, M. (1990) *EMBO J.* **9,** 2233–2239.
21. Kalff, M., Gross, B. & Beato, M. (1990) *Nature (London)* **344,** 360–362.
22. Bruggemeier, U., Kalff, M., Franke, S., Scheideret, C. & Beato, M. (1991) *Cell* **64,** 565–572.
23. Gouilleux, F., Sola, B., Couette, B. & Richard-Foy, H. (1991) *Nucleic Acids Res.* **19,** 1563–1569.
24. Graham, F. L. & van der Eb, A. J. (1973) *Virology* **52,** 456–467.
25. Nguyen, V. T., Morange, M. & Bensaude, O. (1988) *Anal. Biochem.* **171,** 404–408.
26. Giguere, V., Hollenberg, S. M., Rosenfeld, M. G. & Evans, R. M. (1986) *Cell* **46,** 645–652.
27. Meyer, M. E., Pornon, A., Ji, J., Bocquel, M. T., Chambon, P. & Gronemeyer, H. (1990) *EMBO J.* **12,** 3923–3932.
28. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11,** 1475–1489.
29. Pearce, D. & Yamamoto, K. R. (1993) *Science* **259,** 1161–1165.
30. Adler, A. J., Danielsen, M. & Robins, D. M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 11660–11663.
31. Adler, A. J., Scheller, A. & Robins, D. M. (1993) *Mol. Cell. Biol.* **13,** 6326–6335.