Glucocorticoid Receptor Domain Requirements for Chromatin Remodeling and Transcriptional Activation of the Mouse Mammary Tumor Virus Promoter in Different Nucleoprotein Contexts*

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The glucocorticoid receptor (GR) contains several activation domains, ρ1 (AF-1), ρ2, and AF-2, which were initially defined using transiently transfected reporter constructs. Using domain mutations in the context of full-length GR, this study defines those domains required for activation of the mouse mammary tumor virus (MMTV) promoter in two distinct nucleoprotein configurations. A transiently transfected MMTV template with a disorganized, accessible chromatin structure was largely dependent on the AF-2 domain for activation. In contrast, activation of an MMTV template in organized, replicated chromatin requires both domains but has a relatively larger dependence on the ρ1 domain. Domain requirements for GR-induced chromatin remodeling of the latter template were also investigated. Mutation of the AF-2 helix 12 domain partially inhibits the induction of nuclease hypersensitivity, but the inhibition was relieved in the absence of ρ1, suggesting the occurrence of an important interaction between the two domains. Further mutational analysis indicates that GR-induced chromatin remodeling requires the ligand-binding domain in the region of helix 3. Our study shows that the GR activation surfaces required for transcriptional modulation of a target promoter were determined in part by its chromatin structure. Within a particular cellular environment the GR appears to possess a significant degree of versatility in the mechanism by which it activates a target promoter.

Nuclear receptors have provided fruitful models for studying the mechanisms by which transcription factors work (reviewed in Ref. 1). The steroid receptors are a ligand-inducible class of this family and the glucocorticoid receptor (GR)1 cDNA was the first of these to be cloned and sequenced (2). The GR has a ubiquitous expression pattern in mammals and is involved in regulation of a number of biological processes through transcription of various target genes. In the mammary gland, it is essential for the differentiation process that leads to lactation.

The mechanism of GR transactivation has been the focus of a large number of studies, and domains of the GR involved in transcriptional activation have been defined. One of these domains, ρ1 or AF-1, is located in the amino-terminal region of the receptor (3–5). Its core region is unstructured in solution but can form an α-helical structure in a hydrophobic environment (6, 7). Helix-breaking proline substitutions (7) or mutations in hydrophobic amino acid residues decrease its transactivation potential (8, 9). The ρ1 domain is also important in mediating transcriptional repression by the GR (10). It has been shown to interact with a number of factors and complexes including TBP (11), TFIID (11, 12), CBP (9), members of the DRIP/vitamin D receptor-interacting proteins complex (13), the yeast histone acetyltransferase complex SAGA (14), and the ATP-dependent chromatin remodeling complex, SWI/SNF (15).

Two other GR transactivation domains are located in the ligand-binding region. The first, ρ2, is a small region at the amino-terminal end of the ligand-binding domain (LBD) (5). It contains sequences that are conserved among the steroid receptors and has been shown to mediate transactivation when fused to a DNA-binding domain (DBD) (5, 16). It also harbors a nuclear matrix targeting signal and interacts with Hic-5, a protein that associates with the nuclear matrix and also potentiates GR action (17, 18). The other known transactivation domain of the GR, termed AF-2, is a larger region within the COOH terminus of the LBD. In other nuclear receptors it is thought to comprise a surface formed by α-helices 3, 5, 6, and 12 (19, 20). Helix 12, termed the AF-2 AD core, undergoes a conformational change upon binding of ligand that generates a surface for interaction with either corepressors or coactivator proteins, dependent on whether the ligand is an agonist or antagonist (reviewed in Ref. 1). Coactivators include SRC1, TIF2/GRIP1, CBP/p300, and P/CAF, some of which are histone acetyltransferases (reviewed in Ref. 21).

Consistent with the fact that it interacts with histone acetyltransferases and ATP-dependent remodeling complexes, the GR is known to induce modification of chromatin structure. A number of target genes have glucocorticoid-inducible nucleosome hypersensitive sites (22–24). Perhaps the best studied of these is the mouse mammary tumor virus (MMTV) promoter. The GR has been shown to activate this promoter by two distinct mechanisms, depending on its chromatin structure (25). When the promoter is organized into replicating chromatin, either as an episome or integrated into the genome, the GR induces a chromatin remodeling event in the nucleosomes containing its binding sites (24, 26). This is mechanistically necessary for transcriptional activation because it allows access of previously

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excluded transcription factors NF1 and Oct1 (25, 27, 28). The GR also facilitates the association of the basal transcription machinery with the promoter, which presumably activates transcription (28). Therefore, this form of the MMTV promoter is derepressed and then activated through GR action.

When the MMTV promoter is introduced into cells as a transiently transfected reporter construct, it adopts a disorganized and accessible chromatin structure to which NF1 and Oct1 bind constitutively rather than in a hormone-dependent fashion (25, 28). This template does not undergo remodeling but is activated by GR, probably through increased association of the basal machinery. Once the MMTV template in organized chromatin is remodeled and derepressed, it is unclear whether GR activates it by the same mechanism employed at the transiently transfected MMTV template. One way to approach this question would be to assess the effect of various GR activation domain mutants on the ability of the two templates to be activated. These domains were defined using transiently transfected reporter constructs. It is not known whether these same domains would be necessary for activation of a promoter in a repressed context.

There is conflicting information on the domains required for chromatin remodeling induced by the GR. Several studies indicate that the SWI/SNF family of ATP-dependent remodeling complexes are involved in transactivation by GR in mammalian cells (15, 29, 30). Both in vivo (31) and in vitro (32, 33) studies suggest a role for the SWI/SNF complex in the induction of nucleosome hypersensitivity by GR at the MMTV promoter. GR has also been shown to interact physically with the SWI/SNF complex but none functionally and physically with this complex in an AF-2-deleted context (34). Recent studies of DiRenzo et al. (35) and Muchardt and Yaniv (29) showed that expression of the SWI/SNF ATPase, brahma, greatly potentiated GR-mediated promoter activation in a manner dependent on the GR DBD. Further complexity emerged from the studies of DiRenzo et al. (35) who showed that the estrogen receptor interacts functionally and physically with this complex in an AF-2-dependent fashion. Thus, multiple receptor domains have been implicated in interactions with the SWI/SNF complex but none of these assays assayed chromatin remodeling directly.

In this study we examine the role of each of the activation domains in GR function in vivo. We have taken advantage of a GR mutant, C656G, which binds its ligand with higher affinity than wild type GR (36). We showed previously that this receptor is capable of remodeling chromatin and activating transcription from an MMTV promoter in ordered chromatin (37). In the context of the full-length C656G receptor, mutations were introduced into the previously defined activation domains. These mutations were assayed for their role in transcriptional activation of the transiently transfected and stably replicating MMTV templates as well as in GR-dependent chromatin remodeling at the latter. The results show that the GR uses its domains differently in the transactivation of the MMTV promoter in distinct nucleoprotein environments. In addition, through direct measurement of chromatin remodeling we have determined that the ligand-binding domain plays a critical role in mediating this process. Our findings strongly support the idea that the mechanism by which the GR modulates transcription is not only dependent on the nature of the target promoter but also on its chromatin configuration.

**EXPERIMENTAL PROCEDURES**

**Cloning of GR Mutants**—The C656G expression vector, pClnH6HA-C656G, was made, as previously described (37), by insertion of C656G cDNA (kindly provided by Stoney Simons) into a pCl-nH6HA vector, such that the full-length receptor was fused with a histidine tag and a hemagglutinin A (HA) epitope at its NH2 terminus. The Δ1 mutant was created as follows. A SalI site in the multiple cloning sequence was deleted from the C656G expression vector by digestion with XhoI (in the multiple cloning sequence) and EcoRI (just downstream of the multiple cloning sequence) and subsequent ligation with linker sequences. The resulting vector (C656GΔSalI) was digested with SalI and Bsp120I, which cleave the receptor cDNA at sites within or at the COOH-terminal end of the Δ1 domain, respectively. The cleaved ends were then ligated with the following complementary oligonucleotides: 5'-TCGAC- CAGCGTT-3' and 5'-GGCCAACGCTGAG-3'. The resulting product has amino acids 157–317 in the Δ1 domain deleted (Δ1r1). The AF2dm12 mutant (MT70A/E773A) and Δ1rAF2dm12 mutants were created using a ChameleonTM double-stranded, site-directed mutagenesis kit (Stratagene). The mutagenic primer used to change methionine 770 and glutamic acid 773 of the helix 12 domain of C656G and, when fused to a gene). The mutagenic primer used to change methionine 770 and glutamic acid 773 of the helix 12 domain of C656G and, when fused to a

**Cells, Transfection, and Sorting**—Cell line 147.11 was maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum (HyClone). This cell line was derived from C127 mouse mammary adenocarcinoma cells and contains multiple stably replicating copies of MMTV-chloramphenicol acetyltransferase fusions in the context of bovine papilloma virus sequences, as previously described (37). The 147.11 cells express moderate levels of GR.

Transfections were carried out by electroporation using a BTX Electro Square Porator T820 (Genetronics) as described previously (37). Cells were transfected with 5 µg of pCMVIL2R (interleukin 2 receptor Tac subunit expression vector) for cell sorting and varying amounts of receptor expression vector DNA (3–12 µg). Cells were also transfected with either 10 µg of pLTRLuc (full-length MMTV long terminal repeat driving luciferase) or 10 µg of pMTVbgl2 (full-length MMTV long terminal repeat driving expression of the rabbit β-globin gene) for transient template assays. After electroporation, cells were plated in phenol red-free Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% charcoal/dextran-treated fetal bovine serum (HyClone). The following day cells were treated with dexamethasone as indicated in the figure legends and sorted with goat anti-mouse IgG (H+L) conjugated with a biotinylated monoclonal antibody (Upstate Biotechnologies, Inc.), as previously described (37). Sorted cells were either used immediately or frozen for later analysis.

**RNA and Luciferase Analysis**—RNA was isolated as described previously (38). RNA induction was measured by S1 nuclease protection assay, as previously described (37). Briefly, isolated RNA (10 µg) was hybridized with probes specific for MMTV and β-actin sequences (overnight and then digested with S1 nuclease (25 units/10 µg of RNA). Digestion products were separated on 8% denaturing gels that were dried and exposed to phosphorimaging screens. Quantitation was carried out using ImageQuant software (Amersham Biosciences).

Cell extracts for luciferase assays were made from transfected, non sorted cells as previously described (39). Luciferase activities were measured using a Microlumat LB 96P luminometer (EG&B Berthold). For each sample luciferase activity was normalized to the amount of
cellular protein used in the assay. Dose-response curves and EC_{50} values were generated using GraphPad Prism software.

Western Blotting—Whole cell extracts were generated as follows. Sorted cells were resuspended in HEGDM (10 mM HEPES, pH 8.0, 1 mM EDTA, 10% glycerol, 2 mM dithiothreitol, and 10 mM sodium molybdate) containing 0.1% Nonidet P-40 and a protease inhibitor mixture (Calbiochem), after which NaCl was added to a final concentration of 250 mM. After incubation on ice for 20 min, cell lysates were centrifuged at 30,000 × g. The supernatants were stored at −80 °C. Generation of cytosolic extracts was similar except that NaCl addition was omitted and incubation on ice was reduced to 5 min before centrifugation at 12,000 rpm in a microcentrifuge.

Proteins (20–40 µg) were separated by SDS-PAGE (3% stacking gel, 8% separating gel), and transferred to Hybond ECL nitrocellulose (Amersham Biosciences) for 1.5 h in 25 mM Tris, 192 mM glycine buffer with 20% methanol at 400 mA. Immunoblotting was carried out with polyclonal anti-HA antibody (HA.11, Covance) diluted 1:1000. Detection was carried out using the Pierce SuperSignal chemiluminescence substrate followed by scanning with a Fluorchem 8000 chemiluminescence imager (Alpha Innotech Corp.).

Nuclei Digestion and DNA Analysis—Nuclei were isolated from magnetically sorted cells as previously described (37). Aliquots of nuclei containing 75–100 µg of DNA were resuspended in 100 µl of nuclei digestion buffer (2.5% glycerol, 1 mM MgCl₂, 50 mM NaCl, 50 mM Tris, pH 8.0, and 1 mM β-mercaptoethanol). Nuclei were digested with SacI (10 units/µg of DNA) for 15 min at 30 °C or with λ-exonuclease (100 units/ml) and HaeIII (1000 units/ml) at 37 °C for 15 min. Digestion was terminated with 5 volumes of nuclei stop buffer (10 mM Tris, pH 7.5, 10 mM EDTA, 0.5% SDS) containing 100 µg/ml proteinase K, and samples were incubated at 37 °C overnight. DNA was purified by phenol/chloroform/isoamyl alcohol extraction, precipitated, and resuspended in 10 mM Tris, pH 8.0, 1 mM EDTA. DNA from nuclei cleaved with ScaI was digested to completion with DpnI. Digestion products were detected by linear amplification using Tqs polymerase and a radiolabeled oligonucleotide (MMTV sequence from –27 to +1 bp) followed by separation on 8% denaturing gels. Dried gels were exposed to Phosphorimager screens. Quantitation of the radiolabeled digestion products was carried out using ImageQuant software (Amersham Biosciences).

RESULTS

Effects of Mutations in the 1 domain and AF-2 Helix 12 Domains on Transactivation—Defining domains of the GR required for activation or repression of endogenous mammalian target promoters has been hampered by the fact that most cultured cell lines that express a known endogenous target gene also express endogenous GR. This makes it difficult to separate effects of transfected mutant receptors from those of the wild type resident receptor. We have circumvented this problem by using the rat GR mutant, C656G, which has higher affinity for ligand than the wild type GR. The receptor has been shown previously to fully activate the MMTV promoter at concentrations of ligand that are too low to induce the endogenous GR in our murine mammary adenocarcinoma-derived cell lines (37). Thus, the activity of C656G and any activation domain mutants derived from it can be determined in the presence of endogenous GR. Although the C656G contains a point mutation in the LBD, we have shown that it does not affect its ability to induce remodeling or activation of the MMTV promoter in organized chromatin (37).

As shown in Fig. 1A, we introduced mutations into the C656G receptor to disable either the 1 domain or AF-2 helix 12 domains, or both. The 1 domain was originally characterized as a rather large region of the NRE-terminal area of the GR (5). Later, a smaller core region was defined that was sufficient, when fused to a DBD, to activate transcription (40). However, we deleted most of the 1 domain to avoid missing any relevant sequences that might be necessary for activation in chromatin. Mutation of the AF-2 domain was more problematic because of the possibility of deleterious effects on ligand binding. We introduced two mutations, M770A and E773A, into the helix 12 region, which had been reported to have varying effects on ligand binding (41–43).
The transient and stable MMTV templates in the same cells, we
ured for C656G (Table I).
ations alone. The same pattern of effects for both templates was
showed that activation of the transient template was driven
largely by the AF-2 helix 12 domain with a small contribution
of glucocorticoid-regulated reporters in F9 embryonal carcinoma cells (10). In addition, the same set of
mutations prevents the interaction of GR with members of the
DRI complex (13). We constructed a receptor containing these
mutations (Δr1/AF2dm12 receptor) restored the EC50 to that measured for C656G (Table I).
Table I

| Receptor                | EC50  |
|-------------------------|-------|
| C656G                   | 3.4 × 10⁻¹⁰ |
| Δr1/AF2dm12             | 3.4 × 10⁻¹⁰ |
| K597A                   | 4.8 × 10⁻¹⁰ |
| SS73A                   | 4.2 × 10⁻¹⁰ |
| AF2dm12/SS73A           | 1.2 × 10⁻⁹  |
| AF2dm12/K597A           | 2.1 × 10⁻⁹  |
| Δr1/AF2dm12/K597A       | 1.4 × 10⁻⁹  |
| Δr1/AF2dm12             | 6.0 × 10⁻¹⁰ |

To compare the activity of the various mutant receptors on the transient and stable MMTV templates in the same cells, we
cotransfected cell line 1471.1 with expression vectors for the
GR mutants, the Tac subunit of the interleukin 2 receptor, as
well as an MMTV-β-globin reporter, which served as the trans
tient template. The interleukin 2 receptor subunit serves as a
tag for magnetic affinity cell sorting (44). This was carried out
after transfection and dexamethasone treatment to isolate a
population of cells highly enriched for the presence of both
MMTV templates and the expression of the GR mutants. RNA
was collected from the transfected cell populations and sub-
jected to S1 nuclease analysis with an MMTV probe designed to
detect transcripts from both the transient and stable MMTV
templates as well as a β-actin probe. A representative S1 anal-
ysis is shown in Fig. 2A. Whole cell extracts were prepared
from the transfected cell populations and subjected to Western
analysis to ensure that roughly equivalent amounts of the
various receptor mutants were expressed (Fig. 2B).

A summary and statistical analysis of the S1 data are shown
for the transient and stable templates in Fig. 2C. Relative to
C656G, activation of the transient MMTV template (left panel)
by AF2dm12 was about 60% lower, whereas activation by Δr1
was only about 20% lower. The double mutant, Δr1/AF2dm12,
was a less efficient activator than AF2dm12. In contrast, both
domains assayed separately had significant effects on dexam-
ethasone activation of the stable MMTV template (right panel),
Δr1 resulting in a 60% loss of activation and AF2dm12 resulting
in a 40% loss. The double mutant was only 25% as effective
as C656G and had less activity than either of the two muta-
tions alone. The same pattern of effects for both templates was
also observed in a different cell line having integrated copies of
an MMTV-Ras transcription unit (data not shown). The results
show that activation of the transient template was driven
largely by the AF-2 helix 12 domain with a small contribution
from the r1 domain. However, in the same cells activation of
the stable MMTV template requires both domains with the
larger contribution coming from r1. Mutation of both domains
does not have a synergistic effect on loss of activation at either
template but does not eliminate activation altogether.3

Hydrophobic residues in the r1 core region (amino acids
208–264 in rat GR) have been shown to be important for its
activation function. In particular, a combination of three mu-
tations was found to severely impair r1-dependent activation of
transiently transfected glucocorticoid-regulated reporters in F9
embryonal carcinoma cells (10). In addition, the same set of
mutations prevents the interaction of GR with members of the
DRI complex (13). We constructed a receptor containing these
mutations (Δr1/AF2dm12 receptor) and tested its ability to transactivate the
two MMTV templates. A representative Western blot and S1 RNA analysis are shown in Fig. 3, panels B and C, respectively.

At the transient template (Fig. 3D, left panel) the r1EFW
mutant was only 65–70% as effective in activation as C656G.
Its efficiency was somewhat lower than that of Δr1, which
italizes a large deletion, indicating that these amino acids were
probably responsible for the small contribution of the r1 domain
to activation at the transient template. However, this combina-
tion of amino acid substitutions may cause r1 to be somewhat
inhibitory to receptor function in the context of the transient
template relative to removal of the entire domain, as in Δr1. At
the stable MMTV template the r1EFW mutant was 80% as
effective as C656G (Fig. 3D, right panel). This provides a sharp
cost to the Δr1 mutant, which was only 40% as effective.

Because the Δr1/AF2dm12 receptor has a significant amount
of residual activity at the two MMTV templates, we considered
the possibility that other regions of the AF-2 domain partially
compensate for the two mutated residues in the AD core.
Met 770 and Glu 773 lie within the helix 12 region. However, the
AF-2 surface to which various coactivators bind in other nuclear receptors also includes residues in helices 3, 5, and 6 (19, 20). Mutation of a lysine residue at the COOH-terminal edge of helix 3 has been shown to impair transactivation in the estrogen receptor (45), thyroid receptor (19), and vitamin D receptor (46) without a severe effect on ligand binding. This mutation in the context of the C656G receptor (45), thyroid receptor (19), and vitamin D receptor (46) shown.

Effects of an LBD helix 3 mutation on MMTV activation. A, expression vectors for the K597A and Δr1/AF2dm12/K597A receptors were transfected into cells with the MMTV reporter construct, pLTRluc. Dexamethasone dose-response assays were carried out as described under "Experimental Procedures." Receptor activity was expressed as fold induction (relative to activity at 1 pm dexamethasone). Results from at least three independent experiments are shown. B, a representative S1 nuclease experiment. Dexamethasone (Dex) treatments were 3.5 h in length. C, data from three independent experiments were analyzed as described in the legend to Fig. 2. The dashed line indicates the average induction observed with the AF2dm12 receptor for each MMTV template as shown in Fig. 2C.

AF2dm12 mutants vary depending on the presence of the K597A receptor has near maximal activity (90%) at 1 nM dexamethasone, and its EC50 value was close to that of C656G. The EC50 values of the two receptors carrying both the K597A and Δr1/AF2dm12/K597A receptors in Ta-TF. Thus helices 3 or 12 were important for activation of the MMTV promoter in either structural context. The Lys597 mutation in the context of Δr1/AF2dm12 (Δr1/AF2dm12/K597A) resulted in a further decrease in activation efficiency relative to the K597A and the Δr1/AF2dm12 receptors at both templates, presumably reflecting the contributions of the r1 and helix 12 domains to the overall activity of the GR. Notably, Δr1/AF2dm12/K597A has residual activity at both templates.

The remaining activity of GR with disabled r1 and AF-2 domains may reflect a contribution from the third known activation domain in the GR, r2. Because r2 lies within the ligand-binding domain, it was difficult to mutate without disrupting ligand binding. However, Milhon et al. (16) had characterized a mutation in the mouse GR r2 region that bound ligand with normal affinity but impaired transactivation. This mutation was introduced into the C656G, AF2dm12, or Δr1/AF2dm12 receptors (see Fig. 1A). As seen in Table I, dose-response analysis showed that the S573A receptor had an EC50 value close to that of C656G. However, combination of the S573A and AF2dm12 mutations had a deleterious effect on the EC50 independent of the presence of the r1 domain (see EC50 values for the AF2dm12/S573A and Δr1/AF2dm12/S573A receptors in Table I). Therefore, the S573A receptor was transfected into cells and tested for expression levels (Fig. 5A) as well as transactivation potential (Fig. 5B). The summary of results, as shown in
Fig. 5. Effects of a 12 domain mutation on MMTV activation. A, receptor levels in transfected cells. B, a representative S1 analysis. C, data from at least three independent experiments were analyzed as described in the legend to Fig. 2. The dashed line indicates the average induction observed with the AF2dm12 receptor for each MMTV template as shown in Fig. 2C. Dex, dexamethasone.

Fig. 5, C and D, demonstrates that the S573A mutation by itself does not impair transactivation at either template.

Effects of Activation Domain Mutations on Chromatin Remodeling at the Stable MMTV Template—To determine which GR domains were essential for the structural transition in chromatin at the stable MMTV template, we tested the ability of various mutants to induce nuclease hypersensitivity and binding of NF1 in the proximal promoter region. Fig. 6 shows the results of restriction enzyme access experiments using SacI, which cleaves the promoter within the induced region of hypersensitivity (26). A representative set of data is shown in Fig. 6A. In each experiment, the dexamethasone-induced change in fractional cleavage was calculated for each receptor. The average changes in fractional cleavage, expressed as a percentage, are shown in Fig. 6B. As shown previously, C656G induces hypersensitivity at 1 nM dexamethasone to the same extent as the endogenous receptor (indicated as mock) at 100 nM dexamethasone (37). Deletion of the 1 domain has only a slight effect on the induction of SacI cleavage, indicating that it functions downstream of chromatin remodeling and derepression in the mechanism of activation. The receptor mutated in helix 12 (AF2dm12) was significantly impaired for induction of hypersensitivity, being less than half as effective as C656G. This degree of impairment correlates well with its relative ability to activate transcription from this template (see Fig. 2D), which underscores the fundamental role depression plays in the mechanism of GR action in the chromatin context. Surprisingly, the double mutant, Δ1/AF2dm12, has normal remodeling activity. These results indicate that the helix 12 region of AF-2 contributes to the chromatin remodeling activity of GR only in the presence of an intact 1 domain.

The binding of NF1 to the stable MMTV template was tightly correlated to the induction of nuclease hypersensitivity by GR. We tested the ability of the various GR mutants to induce NF1 binding as measured by exonuclease block assay. As shown in Fig. 7, the C656G receptor was able to induce NF1 binding when activated by 1 nM dexamethasone to approximately the same level as that induced by the endogenous GR at 100 nM. The Δ1 mutant induces NF1 binding efficiently, but the AF2dm12 mutant does not. The double mutant, Δ1/AF2dm12, was also able to induce NF1 binding to the MMTV promoter. These results correlate well with the SacI access data.

To better define the region of the GR important for induction of chromatin remodeling, we assayed the K597A mutation alone or in the context of the Δ1/AF2dm12 receptor, which induces wild type levels of nuclease access. As shown in Fig. 8, both the K597A and Δ1/AF2dm12/K597A receptors were significantly impaired in their ability to remodel MMTV chromatin. The Δ1/AF2dm12/S573A receptor was also assayed and found to be ~50% as effective as C656G in inducing SacI access. This was likely a reflection of its impaired dose response rather than a contribution to remodeling because its EC50 falls at 1 nM dexamethasone (Table I). Thus, our results indicate
that the helix 3 region of the GR LBD was also involved in chromatin remodeling.

**DISCUSSION**

Our study into the mechanism by which the GR activates the same promoter in different nucleoprotein contexts shows that the GR was a highly versatile protein that utilizes distinct domains and activation mechanisms to regulate transcription. A previous study reported that distinct domains are required for GR activation in different cellular contexts (47); whereas the GR was highly active in CV1 cells, it had much less transactivation capacity in HeLa cells where the LBD was much more active. They speculated that these differing domain dependences reflected varying cellular concentrations of cofactors. However, our study shows that this was true even within a particular cellular environment and was dependent on nucleoprotein context of a specific target promoter. In addition, we have defined a region of the GR important for productive interaction with machinery that catalyzes chromatin remodeling in mammalian cells and have provided evidence for a functionally important cross-talk between the AF-2 and r1 domains. The results of our study have implications for understanding the tissue-specific regulation of target genes by steroid receptors, which may be determined by the availability of various cofactors as well as the chromatin structure of the target promoter.

The GR domains required for various steps in activation of the two structurally distinct MMTV templates are summarized in Fig. 9. Activation of a transiently transfected MMTV promoter construct by the GR was largely dependent on the GR LBD in our cell lines. Mutation of amino acids in helices 3 or 12 significantly reduced activation. However, a mutation in the $\Delta r1$ region had no effect, which differs from the results of Milhon et al. (16). The disparity may lie in the species of receptor used (rat versus mouse) or the cell type assayed (mammary versus kidney origin). In contrast to the LBD mutations, deletion or mutation of $\Delta r1$ impaired activation of the transient template by only 20–30%. The importance of the $\Delta r1$ region for activation of transiently transfected promoters has been found to vary depending on the cell line used (5, 10, 47, 48).

Activation of the stably replicating MMTV promoter required both the $\Delta r1$ domain and amino acid residues in helices 3 and 12 of the AF-2 domain. Interestingly, the hydrophobic residues in $\Delta r1$ required for full activation of the transient template made only a small contribution to activation of the stable MMTV template. Thus the region of $\Delta r1$ necessary for activation in the context of ordered chromatin does not completely coincide with that required for activation of transient templates and interaction with the DRIP complex (13). This result strongly suggests that $\Delta r1$ interacts with different sets or domains of transcriptional cofactors at the two MMTV templates.

The $\Delta r1$ domain was not required for chromatin remodeling, which indicates that its main contribution to transactivation at the stable template was downstream of template derepression and NF1 binding, and may be mediated through interactions with the basal transcription machinery. Consistent with this idea is the fact that the $\Delta r1$ domain has been shown to interact with TBP and the TFIID complex (11, 12) as well as CBP (9). The AF-2 helix 12 domain also functions downstream of template derepression. This was evidenced by the fact that the $\Delta r1/AF2dm12$ receptor is a less efficient activator than the $\Delta r1$ receptor even though it can fully remodel chromatin at the stable MMTV template. The helix 12 domain may thus participate in a common activation step at both MMTV templates.

Our study clearly shows that the GR LBD plays an important role in the induction of chromatin remodeling at the stable MMTV template. This is consistent with our previous work.
showing that a different LBD mutant of GR failed to induce remodeling (49). Mutation of helix 12 residues causes a significant impairment of the ability of the GR to induce chromatin remodeling and subsequent NF1 binding, but only in the presence of the r1 domain. This observation suggests that helix 12 is not directly necessary for productive interaction with remodeling machinery but may facilitate this process through cross-talk with r1. A mutation in LBD helix 3 either alone or in the context of the Δr1/AF2dm12 receptor significantly impaired chromatin remodeling, suggesting that this region of the LBD plays a role distinct from that of helix 12. However, remodeling was never completely abolished. The helix 3 region may provide part of an interaction surface for the remodeling complex and/or facilitate its interaction with another domain. Previous studies indicated that the DBD of the GR may be important for interaction and function of SWI/SNF complexes (29, 34). In addition, a recent report showed that the SWI/SNF complex makes physical and functional interactions with transcription factors, which like the GR, contain zinc finger DBDs (50). The proximity of helix 3 to the GR DBD leaves open the possibility that both of these domains may serve to recruit or interact with the remodeling machinery.

A recent report showed that r1 interacted physically and functionally with purified yeast SWI/SNF complex (15). Our results showing that r1 was dispensable for chromatin remodeling at the MMTV promoter in organized chromatin may differ based on subunit differences between yeast and mammalian SWI/SNF complexes and their interactions with other basic transcription complexes (51). The promoter context may also influence the function of various GR domains. In addition, it is possible that a complex other than SWI/SNF can remodel MMTV chromatin in mammalian cells (52). Our results are more in line with those of DiRenzo et al. (35), who showed a physical and functional interaction of human SWI/SNF complex with the LBD of estrogen receptor. However, their trans-activation assays were carried out on transiently transfected reporters that may not undergo the type of remodeling observed at the MMTV promoter in organized chromatin. Our study is the only report to date that directly assays effects of various receptor domains on the induction of nucleosome hyper-sensitivity at a target promoter in mammalian cells.

Our results have also provided evidence for a functionally important cross-talk between the r1 and AF-2 domains. First, the presence of the r1 domain has a deleterious effect on dose response of the GR containing mutations in helices 3 and/or 12 (AF2dm12 and AF2dm12/K597A) because when it is removed (Δr1/AF2dm12 and Δr1/AF2dm12/K597A) the EC50 improves to a value close to that of the C656G receptor (see Table I). This same effect was observed in chromatin remodeling assays as described above (AF2dm12 versus Δr1/AF2dm12, Fig. 6). Although there is no evidence for a direct interaction between r1 and the GR LBD, some steroid receptor coactivators are capable of interacting with both domains (13, 53, 54). It is possible that coincident interaction of the two activation domains with each other and/or bridging proteins may cause a conformational change in the receptor that facilitates stable binding of ligand and allows another region of the GR to make contact with the remodeling machinery.

Why are different GR domains required for activation of the same promoter in distinct nucleoprotein contexts? It has been established that the nucleoprotein structure of the MMTV promoter influences its mechanism of activation by the GR (25). Incorporation of the promoter into organized chromatin necessitates remodeling and derepression prior to activation of transcription. A distinct set of factors is likely to be recruited to the MMTV promoter in organized chromatin to participate in remodeling and the subsequent transcriptional activation and elongation in the context of positioned nucleosomes and linker histones (55). This greater complexity may be reflected by the fact that both transcriptional domains of the GR (r1 and the LBD) are required for activation of the stable template, whereas activation of the transient template is largely dependent only on the LBD. Another interesting possibility is that the interaction of GR with nucleosomes may serve as an allosteric effector, much like its interaction with various GREs (56, 57). At a nucleosome the GR may present various domains necessary for activation in that context.

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