A Single Amino Acid Change in the First Zinc Finger of the DNA Binding Domain of the Glucocorticoid Receptor Regulates Differential Promoter Selectivity*

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Mammalian species are well known to differ in their sensitivity to glucocorticoids, but the molecular basis for this difference remains largely uncharacterized. To address this issue, the transcriptional activity of the mouse and human glucocorticoid receptor (GR) was analyzed on two model glucocorticoid-responsive promoters. Mouse GR (mGR) displayed unique promoter discrimination in response to a range of glucocorticoids, with enhanced activity on a simple glucocorticoid response element (GRE)-based promoter and diminished activity on the complex mouse mammary tumor virus promoter compared with human GR (hGR). Promoter discrimination between mGR and hGR was mapped to a single amino acid change at residue 437 (glycine to valine) of mGR and to sequence differences within individual GREs of the different promoters. Mouse GR displayed higher activation on GREs with a guanine rather than a thymine at the −6 position. Binding studies indicated mGR (mGR437V) displayed a weaker affinity for GREs containing a thymine at the −6 position than a mGR mutant containing a glycine at residue 437 (mGR437G). Despite distinct transcriptional activities, both receptors had similar affinities for response elements that contain a guanine at the −6 position. Our findings support a model by which the presence of a valine residue at position 437 of mGR induces a conformational change that leads to alterations in affinity and/or transcriptional activation in a promoter-dependent context.

The biological actions of glucocorticoid hormones are mediated through the intracellular glucocorticoid receptor (GR), which belongs to the nuclear receptor subfamily that includes receptors for mineralocorticoids, estrogen and thyroid hormones, retinoic acid, and vitamin D (1). In the absence of hormone, GR resides in the cytoplasm as a multichain complex with the chaperone proteins Hsp90 and Hsp70, the immunophilin p59, and the phosphoprotein p23 (2). Upon binding hormone, GR dissociates from this complex and undergoes a conformational change that unmasks nuclear localization signals found within the receptor (3). The GR translocates to the nucleus and binds glucocorticoid response elements (GREs) as a dimer in the promoter regions of target genes (4–6). The association of the glucocorticoid receptor dimer with the response element results in an allostatic induced conformational change within the receptor and subsequent recruitment of coactivator complexes critical for chromatin remodeling and transcription (4–7). The functional result of the GR-GRE interaction can be largely cell type-, promoter-, and ligand-specific. In some instances, the GR binds negative GREs in promoters of genes and inhibits gene transcription (8). Alternatively, GR represses gene transcription by physically interacting with other transcription factors such as AP-1 and nuclear factor-κB (8–11).

As with other members of the nuclear receptor family, the GR contains a modular structure consisting of three major domains: an N-terminal domain, a central DNA binding domain (DBD), and a C-terminal ligand binding domain (LBD) (12, 13). The N-terminal domain represents the most variable region among the species of the GR and the nuclear receptor family. The N-terminal domain contains the AF-1 transcriptional activation domain required for transcriptional enhancement and association with basal transcription factors (12–14). The central DBD is composed of two highly conserved zinc finger regions and is most conserved region among nuclear receptors (15). The first zinc finger is primarily thought to be responsible for target site recognition. Three residues in the carboxyl half of the first zinc finger termed the “P box” are thought to be responsible for response element discrimination (16, 17). The second zinc finger stabilizes protein-DNA interactions and contains the “D box” region critical for receptor dimerization (5, 6). The central DBD is also required for the repression of other transcription factors such as nuclear factor-κB and AP-1 (9, 11, 18). The C-terminal LBD serves as the binding site for hormones, chaperone Hsp90, and coactivators (12, 14). In addition, the DBD and LBD are important determinants in receptor mobility (19).

The GR is an evolutionary conserved transcription factor found in such species as human, rat, mouse, frog, rainbow trout, flounder, and others. The highest sequence homology exists among mammalian species, particularly in the highly conserved DBD and LBD. Nonmammalian species display less homology overall, both in conserved (DBD, LBD) and variable domains (N terminus) of the GR. Despite relatively high sequence homology, striking differences in glucocorticoid sensitivity have been observed among mammalian species. In some
instances, species-specific differences in glucocorticoid action result from amino acid changes within functional domains of the GR protein. For example, the guinea pig displays resistance to cortisol because of the presence of several amino acid residues in the LBD which differs from the human GR (hGR) (20). Similarly, sequence differences in the LBD are responsible for humans and other cortisol-secreting species (rabbit, pig) playing a higher affinity for cortisol than rodents (rat, mouse), which bind corticosterone with a higher affinity (21). In addition, phosphorylation patterns vary among species, providing a mechanism for species-specific differences in phosphorylation-influenced functions of GR which include protein stability, transcriptional activation, and subcellular localization (22–24). For example, glycogen synthase kinase-3 inhibits the transcriptional activity of rat GR through phosphorylation but has no effect on hGR (24). Alternatively, species-specific differences in GR signaling may result from the elevated expression of proteins that alter GR function. For example, the elevated expression of the FK506-immunophilin FKBP51 causes glucocorticoid resistance in several New World primates by decreasing binding of glucocorticoids to GR (25). In addition, the dominant-negative form of the GR, hGRβ, is expressed in humans but not other species (26).

In the present study, we investigated the molecular basis for species-specific differences in the transcriptional activation of simple and complex promoters by mouse GR (mGR) and hGR in response to glucocorticoid agonists and antagonists. Our studies reveal that mGR displays distinct promoter-specific transcriptional activities compared with hGR. A comprehensive analysis of GR domains and hormone-responsive promoters indicated that promoter discrimination mapped to a single amino acid at residue 437 of the DBD of mGR and to the identity of the −6 position of the GRE.

**EXPERIMENTAL PROCEDURES**

**Materials**—Specific antibodies against GR protein (57) were identical to those described previously (27). Antibodies against the VP16 activation domain were purchased from Clontech Laboratories (Palo Alto, CA). Goat anti-rabbit IgG conjugated to horseradish peroxidase (GAR-HRP) was purchased from Jackson Immunoresearch (West Grove, PA). Dexamethasone, cortisol, corticosterone, triamcinolone, and RU486 were purchased from Steraloids (Wilton, NH).

**Construction of Receptor Mutants**—Glucocorticoid receptor DBD mutants were generated with the QuickChange mutagenesis kit as specified by the manufacturer (Stratagene, La Jolla, CA). The primer sequences used to generate the following mutants were: mGR437G, 5'-ccgaagctttgtacaggatgttctagctacctcgagatata; mGR437A, 5'-ccgaagctttgtacaggatgttctagctacctcgagatata; mGR437D, 5'-ccgaagctttgtacaggatgttctagctacctcgagatata; and mGR437L, 5'-ccgaagctttgtacaggatgttctagctacctcgagatata. The product, digested with KpnI and EcoRI enzymes and ligated into digested pDNA3.1 (Invitrogen). The VP16 activation domain was amplified from pAkt (Clontech) with primers 5'-tatagtagctactatgtcgtgagaagcc-3' and 5'-tatagtagctactatgtcgtgagaagcc-3' The product was digested with KpnI and EcoRI and ligated into pCDNA3.1 (Invitrogen). The VP16 activation domain was amplified from pAkt (Clontech) with primers 5'-tatagtagctactatgtcgtgagaagcc-3' and 5'-tatagtagctactatgtcgtgagaagcc-3'. The product was digested with KpnI and EcoRI and ligated into pCDNA3.1 (Invitrogen). The VP16 activation domain was amplified from pAkt (Clontech) with primers 5'-tatagtagctactatgtcgtgagaagcc-3' and 5'-tatagtagctactatgtcgtgagaagcc-3'. The product was digested with KpnI and EcoRI and ligated into pCDNA3.1 (Invitrogen). The VP16 activation domain was amplified from pAkt (Clontech) with primers 5'-tatagtagctactatgtcgtgagaagcc-3' and 5'-tatagtagctactatgtcgtgagaagcc-3'

**Construction of Reporter Gene Constructs**—The TAT2-LUC reporter gene was generated by excising a BamHI-HpaI fragment from GRE2-CAT (28) which contained the TAT GREs and TATA box sequence. The fragment was subcloned into BamHI-HpaI sites of pX2 plasmid (29). The TAT-LUC plasmid served as a positive control. The TAT-LUC plasmid was then digested with EcoRI and XhoI, and the insert was then inserted downstream of the DBD to generate the pCD437TAT2-LUC fusion expression vector. The expression vector pCD437TAT2-LUC was generated by mutagenesis using mGR437 primers as described above.

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RESULTS

Comparison of Promoter Activation by mGR and hGR—The transcriptional activities of the mGR and hGR were evaluated on two model glucocorticoid responsive promoters: 1) a simple GRE promoter, and 2) the complex MMTV promoter (Fig. 1A). The simple reporter gene, TAT2-LUC, contains two copies of the GRE from the rat TAT gene upstream of the TATA box sequence and the LUC reporter gene (Fig. 1A). The complex MMTV-LUC reporter gene is comprised of two dissimilar GREs, two half-site GREs (TGTTC), and sites for the transcription factors sites NF-1 and Oct-1 (Fig. 1A). The complex MMTV-LUC reporter gene is inserted upsteam of the simple TAT2-LUC promoter (Fig. 1B). In contrast, hGR activated transcription better in the context of the complex MMTV promoter than the simple TAT2-LUC reporter gene. Mouse GR displayed enhanced activity on the simple and complex glucocorticoid responsive reporter genes in COS-1 cells in response to dexamethasone and synthetic glucocorticoids (Fig. 1B). Mouse GR displayed enhanced activity on the simple TAT2-LUC reporter gene over the complex MMTV promoter (Fig. 1B). In contrast, hGR activated transcription better in the context of the complex MMTV promoter than the simple TAT2-LUC reporter gene. Mouse GR displayed -5-fold higher activation over hGR on TAT2-LUC and 1.6-fold less activity on the MMTV promoter in response to dexamethasone. Importantly, the promoter-specific activities were independent of the agonist tested and were observed with corticosterone, cortisol, and triamcinolone, ligands that display differing affinities for GR (27, 39, 41-43). The agonists represent a structurally similar class of compounds that are thought to induce similar conformational changes within the GR. The expression levels of each protein in the reporter gene assays were nearly identical as determined by Western blot analysis (Fig. 1C). Collectively, these data revealed that mGR displayed distinct promoter-specific transcriptional activities compared with hGR in response to glucocorticoid agonists.

A Single Amino Acid Change in the DBD of mGR Exerts Promoter Discrimination—We next focused on identifying the functional domains that contributed to the species-specific promoter discrimination by mGR. In preliminary studies, we examined the role of the N terminus of GR and found that the domain was not responsible for the distinct promoter activities between mGR and hGR in response to glucocorticoids (data not shown). This led us to investigate the consequence of a single amino acid change at the tip of the first zinc finger of the DBD of the mGR (Fig. 2). The mGR utilized in this study, originally reported by Danielsen et al. (44), contains a valine at position 437 instead of a glycine found in hGR. The amino acid change results from codon change of GGA to GTA (44). The cDNA sequence (GenBank™ accession number X04435) containing this nucleotide change acts as the reference standard for the mGR by genomic data bases such as NCBI SwissProt, and MGI. Residue 437 of mGR also appears to be polymorphic among mouse cell lines (45, 46). Similar polymorphisms have been found in the vitamin D receptor (G30D) and the androgen receptor (G551V) and have been associated with hypocalcemic rickets and partial androgen insensitivity syndrome, respectively (47-49). The mutations in androgen receptor and vitamin D receptor appear to disrupt DNA binding and transcriptional activation properties of the receptor.

Amino acid 437 of mGR is located outside the P box (Fig. 2),
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Fig. 2. Schematic representation of the zinc finger DBD. The amino acid sequence of the first and second zinc finger domains of mGR is indicated by the standard one-letter amino acid abbreviations and numbered accordingly. A codon change of GGA to GTA in mGR results in the change of a glycine to a valine at position 437. A □ symbol indicates a phosphate contact at a nonspecific site; ■, phosphate contact at a specific site; □, base contact at a nonspecific site; □, base contact at a specific site; ○, residues of the P box; ○, D box region. The P box and D box regions are required for target site recognition and dimerization, respectively.

A region thought to act as the primary determinant of sequence recognition among glucocorticoid and estrogen receptors (16, 17). Response element specificity depends largely on the identity of the amino acids within the P box. Residues outside the P box region have been previously reported to make contacts with phosphate backbone of DNA and are thought to help provide structural information (5, 6, 15). The functional importance the conserved glycine at positions corresponding to residue 437 of structural information (5, 6, 15). The functional importance the conserved glycine at positions corresponding to residue 437 of structural information (5, 6, 15). The functional importance the conserved glycine at positions corresponding to residue 437 of structural information (5, 6, 15). The functional importance the conserved glycine at positions corresponding to residue 437 of structural information (5, 6, 15).

mGR 437 mutants were assayed for their ability to activate the simple TAT2 and complex MMTV reporter genes in response to a concentration range of dexamethasone (Fig. 4B). On the TAT2 promoter, potency of activation occurred in the order of mGR437L, mGR437A, mGR437V, mGR437H, mGR437D and mGR437G. All mutants displayed higher activation than mGR437G on the TAT2 promoter. Mutants containing residues at position 437 with smaller side chains (Leu, Ala, Val) showed the strongest activation. The activation profile on the complex MMTV promoter differed drastically with no direct correlation to the identity of the amino acid at position 437. The mGR437L mutant displayed the highest activation on the simple promoter but the lowest on the complex MMTV promoter. In contrast, the mGR437A mutant maintained strong activation on both promoters. Aspartic acid was the least favorable substitution, with mGR437D exhibiting diminished activity on both promoters. In addition, the dose-response curve was shifted to the right for mGR437D on the TAT2 reporter gene. The diminished activity of mGR437D may relate to its stability as a major degradation product of the protein as detected by Western blot analysis (Fig. 4B). The expression levels of the other mutants were comparable. Together, these findings suggest that the identity of the amino acid at position 437 influences the transcriptional properties of mGR on the simple and complex promoters, demonstrating its role in promoter discrimination.

Molecular Basis of Promoter Specificity—Studies thus far have identified residue 437 as a primary determinant within the mGR responsible for promoter discrimination. We next analyzed the composition of the simple and complex promoters to determine the role of the DNA elements in the differential transcriptional activity of mGR437V. The simple TAT2 promoter differs from the MMTV promoter in several aspects: the number of GREs, the absence of NF-1 and OCT-1 sites of the MMTV promoter, and the individual response element sequence (Figs. 1A, and 5B). We first investigated whether the number of GREs could be a determinant of promoter-specific activation because the number of GREs in the context of a simple promoter has been reported to lead to cooperativity of GR activity (51). The TAT2 promoter contains two full-length GREs, whereas the MMTV promoters contain two full-length and two half-site GREs (Figs. 1A and 5B). The half-site GREs correspond to the conserved 3′-site (TGTTC), and each has been shown to be functionally occupied by one subunit of the GR (52). To evaluate whether the number of GREs within the simple promoter influenced the activity of mGR437V, the mutant was analyzed on a simple promoter comprised of one, two, or three copies of the TAT GRE. In response to dexamethasone,
mGR437V displayed enhanced activity over mGR437G regardless of the number of copies of GRE (Fig. 5A). We next investigated the consequence of the NF-1 and Oct-1 sites present in the MMTV promoter but absent in the simple TAT2 promoter. A chimeric reporter gene was generated which contained the tandem GRE elements of the TAT2 promoter fused with the NF-1 and Oct-1 sites of the MMTV promoter (Fig. 5B). The presence of the NF-1 and Oct-1 sites in the context of TAT

**Fig. 3. Functional analysis of the valine to glycine change at residue 437 of mGR.** A, activation of the simple TAT2 and complex MMTV reporter genes. COS-1 and E8.2 cells transfected with either pCMVmGR437V or pCMV437G and the indicated reporter gene were incubated with 10 nM dexamethasone (Dex) for 16 h, harvested, and luciferase activity measured. The Western blot to the right shows the expression levels of the mGR437 mutant proteins in the reporter gene assay. B, dose-response curves for mGR437 mutants. COS-1 cells transiently transfected with the indicated mGR437 mutant and reporter gene were treated with increasing concentrations of dexamethasone (0–1,000 nM) for 16 h and luciferase activities determined. C, reporter gene activation in response to increasing concentrations of mGR437 mutants. COS-1 cells were transfected with the TAT2 or MMTV-LUC reporter gene and increasing concentrations of the indicated mutant (5–800 ng). Cells were incubated with 10 nM dexamethasone for 16 h and harvested for luciferase activity and Western blot analysis. Each data point represents the mean ± S.E. of triplicate samples from a representative experiment. Differences in relative activities were reproducible in three independent experiments. The Western blot indicates the expression levels of the proteins in the reporter gene assay.
GREs had no effect on the transcriptional activities of the mGR437 mutants (Fig. 5C). The mGR437V mutant displayed enhanced activity over mGR437G on simple GRE and chimeric TAT2-MMTV promoters.

The findings suggest that a second determinant of promoter-specific activity of mGR437V resides in the response element sequences, which differ among the promoters. To test this hypothesis, reporter genes were constructed which contained a single copy of the rat TAT, human MTIIA, rat TO, MMTVI or MMTVII GREs fused upstream of a minimal TATA promoter and luciferase reporter gene. The MMTV response elements represent the first (MMTVI) and second (MMTVII) GREs from the complex MMTV promoter. The sequences of the various GREs differ mainly in the 5'-variable half-site and spacer regions (Fig. 6A). The mGR437 mutants were analyzed for their ability to activate the individual reporter genes in response to a concentration range of dexamethasone. The mGR437V mutant displayed higher transcriptional activity on the TAT, MMTVII, and MTIIA response elements and lower activity on the MMTVI and TO GREs compared with mGR437G (Fig. 6B).

The transcriptional activity of the mutants correlated directly with the identity of the nucleotide present at the −6 position of the response element. Higher activity was observed with mGR437V on individual response elements (TAT, MTIIA, MMTVII) that contain a guanine at the −6 position of the GRE and lower activity on GREs (MMTVI, TO) that contained a thymine at this position. A guanine at the −6 position is the preferred nucleotide in the consensus response element of nuclear receptors (Fig. 7A). The GR has been reported to display a higher affinity for a GRE with a guanine at the −6 base than any other base (53, 54). To determine whether the identity of the −6 base was actually responsible for influencing the activity of mGR437, the 5'-half-site of MMTVI GRE was mutated to that of the TAT GRE. The TAT and MMTVI GREs differ at the −7 and −6 positions and the spacer region (Fig. 7B). The −7G and −6T of the MMTVI GRE were mutated alone and in combination to the −7T and −7G of the TAT response element. Mutation of the guanine at the −7 position of MMTVI GRE to a thymine caused a minimal increase in mGR437V and a decrease in mGR437G transcriptional activities (MMTVI −7G versus MMTVI −7T, Fig. 7B). The minimal decrease in transcriptional activity of mGR437G is consistent with previous DNA binding specificity studies in which the GR displayed a lower affinity for GREs with a −7 thymine instead of a guanine (53, 54). In contrast, mutation of the −6 thymine to guanine in MMTVI strongly increased transactivation by mGR437V and mGR437G by 8- and 1.5-fold, respectively (MMTVI versus MMTVI T−G, Fig. 7B). At least for mGR437G, the enhanced activity is consistent with a reported increased affinity of wildtype GR for GREs containing a guanine at the −6 position.
More importantly, the substitution of a guanine at the −6 position restored the enhanced activity of mGR437V over mGR437G, confirming the role of this residue in the observed promoter discrimination. Mutation of both −7 and −6 positions in the MMTV GRE essentially converted the response element into the TAT GRE (TAT versus MMTV I G−7T, T−6G). Transcriptional activation by the mGR mutants was similar on both response elements. Collectively, these findings indicate that a major determinant of the response element discrimination of mGR resides with the identity of −6 position of the GRE. The −7 position of the GRE can further regulate the potential of transcriptional activation depending on the identity of −6 base.

**Basis of Enhanced Transcriptional Activity on Promoters**—Our studies have identified the key positions in mGR and response elements responsible for mGR promoter discrimination. We next focused on determining whether the differential promoter activities of mGR437V resulted from alterations in DNA binding affinity and/or transcriptional activity. DNA binding was first measured with isolated DBDs of the receptor mutants by a reporter gene approach. The DBDs of mGR437V and mGR437G were fused to the VP16 activation domain (Fig. 8A). This approach allows the determination of DNA binding properties without ligand or influences by the N- and C-terminal domains of GR. DNA binding was measured by determining activation on the simple TAT2 and complex MMTV promoters and reporter genes containing a single copy of the TAT or MMTV GREs (Fig. 8B). The mGR437V and mGR437G mutants showed similar DNA binding on reporter genes containing the TAT GRE (TAT1, TAT2). In contrast, the DNA binding of mGR437V was reduced dramatically on MMTV and full-length MMTV promoters compared with mGR437G. The overall DNA binding on the MMTV response element was also lower for both mutants compared with the TAT GRE, consistent with the reported decreased affinity of GR for GREs containing a thymine at the −6 position (53, 54). The DNA binding affinities were also confirmed by determining the relative affinity of the full-length mGR437 proteins for the TAT and MMTV GREs using competitor DNA binding ELISAs. Consistent with the DBD studies, the full-length mGR437V and mGR437G proteins displayed similar relative affinities for the TAT GRE (Fig. 8C). Similarly, a lower affinity was observed for the MMTV GRE for mGR437V with respect to mGR437G. Collectively, these data suggest that the diminished transcriptional activities of mGR437V on MMTV GRE and the complex MMTV promoter may in part come from a decrease in DNA binding affinity. The decreased DNA affinity of mGR437V for MMTV GRE correlates with the presence of a thymine at the −6 position of the response element. However, the basis for the decreased DNA binding affinity for the full-length MMTV promoter is complicated by the additional presence of the MMTVII and half-site GREs. The contribution and cooperativity of the individual GREs of the MMTV promoter to GR-DNA binding and transactivation remain to be fully characterized. In contrast, mGR437 displayed enhanced activity on the TAT GRE-based promoters despite having DNA binding similar to that of mGR437G. These findings suggest that the glycine to valine change at position 437 can enhance the transcriptional activation function of GR without affecting DNA affinity.

**DISCUSSION**

Promoter specificity among nuclear receptors has largely been attributed to the identity of P box residues located at the base of the first zinc finger (16, 17). In this paper, we illustrate that residues outside the P box are critically important for maintaining the functional integrity of promoter discrimination by GR. By investigating species-specific differences in promoter activation, we identified a single amino acid change (G437V) at the tip of the first zinger finger domain of mGR which has a profound impact on target site recognition, transcriptional activation, and receptor mobility. A glycine residue is critically important at this position as demonstrated by its conservation among the nuclear receptor family and conse-
sequence of its mutation. For example, patients with the corresponding mutation at this position (G551V) in the human androgen receptor gene exhibit the partial androgen insensitivity syndrome (47, 48). Similarly, the mutation (G30D) in the human vitamin D receptor gene has been associated with hypocalcemic rickets (49). In both androgen receptor and vitamin D receptor studies, the corresponding Gly to Val mutation appears to influence DNA binding and transactivation negatively.

**Fig. 6.** Transcriptional activities of mGR437 mutants on different GRE-containing promoters. A, sequence of the GREs from the rat TAT (31), human MTTII (32), rat TO (33), and MMTVI and MMTVII (30). The nucleotide and half-site locations of the individual response elements are indicated. The nucleotide at position -6 of the GRE is highlighted in gray. B, reporter genes were generated which contained a single copy of the individual GREs fused upstream of a minimal TATA promoter and luciferase reporter gene. COS-1 cells were transiently transfected with pCMV44-mGR437V or pCMV44-mGR437G and the indicated reporter gene were incubated with 10 nM dexamethasone (Dex) for 16 h and harvested for luciferase activity determination. Each bar represents the mean ± S.E. of triplicate samples from a representative experiment. Differences in relative activities were reproducible in three independent experiments.

**Fig. 7.** Analysis of the 5′-half-site GRE sequence effect on transcriptional activity of mGR437 mutants. A, comparison of consensus response element sequences among nuclear receptors. The guanine conserved at the -6 position among nuclear receptors is highlighted with gray. ARE, androgen response element; MRE, mineralocorticoid receptor; PRE, progesterone response element; ERE, estrogen response element; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; TRE, TAT response element. B, effect of mutation of the 5′-half-site of MMTVI GRE sequence on transcriptional activities of mGR437 mutants. The -7G and -6T of the MMTVI GRE were mutated alone and in combination to the -7T and -6G of the TAT response element. Sequences of the consensus, TAT, MMTVI, and mutated MMTVI response elements are indicated. COS-1 cells transfected with pCMV44-mGR437V or pCMV44-mGR437G and the indicated reporter gene were incubated with 10 nM dexamethasone (Dex) for 16 h and harvested for luciferase activity determination. Each bar represents the mean ± S.E. of triplicate samples from a representative experiment. Differences in relative activities were reproducible in three independent experiments.
although transcriptional analysis was only limited to a few promoters. Our studies indicate that the mutation in the GR also has a profound effect on DNA binding and transcriptional activation in a promoter-specific fashion and the mobility of GR within the nucleus. The functional outcome of the GR mutation depends largely on two determinants: the identity of the amino acid at position 437 of GR, and the nucleotide at the −6 position of the GRE. Mouse GR with a valine at position 437 (mGR437V) displayed reduced DNA affinity and transcriptional activity on GREs that contain a thymine at the −6 position compared with wild-type GR (mGR437G). In contrast, mGR437G mutants. Treatment of mGR mutants for the TAT and MMTVI response elements. Competitive DNA binding ELISA was used to measure DNA binding and calculate relative affinities of full-length mGR mutants for the TAT and MMTVI response elements essentially as described under “Experimental Procedures.” EC50 values were calculated based on the one-site competition curves using GraphPad Prism software.

The findings in this paper support the role of response element sequence as a determinant of both receptor affinity and in the allosteric regulation of GR transactivation. We propose that promoter discrimination involves two interdependent mechanisms of action. First, the DNA binding affinity of GR is influenced by the response element sequence and the conformation of the receptor. Distinct changes in receptor conformation induced by mutation or different ligands influence the ability of the receptor to recognize different response element sequences. Second, the response element sequence allosterically regulates GR conformation to affect transactivation. The outcome of the DNA-induced conformation change is determined primarily by the relationship between the GRE sequence and receptor conformation prior to DNA binding. Understanding how the response element sequence induces changes in receptor conformation is limited primarily by the availability of solved crystal structures of GR bound to different response element sequences. Multiple structures are required for proper analysis of hydrogen bonding patterns between GR and the GRE that differ depending on the nucleotide composition of the response element. Currently, the only available crystal structure is the rat GR DBD-GRE complex solved by Luisi et al. (6). The 5′-half-site including the −6 position of the GRE within the complex differs from the response elements utilized in the present
study. Furthermore, the mechanism by which the DBD of GR could transduce structural changes to other functional domains remains unresolved because of the absence of a crystal structure of the full-length GR complexed to DNA. However, it has been proposed that site-specific GRE binding influences the folding of the AF-1 transactivation domain and alters the ability to bind cofactors crucial for the transactivation process (40). Merit for this hypothesis is supported by recent studies demonstrating estrogen receptor structure, function, and coactivator recruitment can be modulated by response element sequence (59, 60). Collectively, our studies demonstrate that residues outside the P box are critically important for maintaining the functional integrity of promoter discrimination by GR and in communicating with the response element sequences for the regulation of transcriptional activation.

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J. Biol. Chem. 2004, 279:39279-39288.
doi: 10.1074/jbc.M405489200 originally published online June 25, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M405489200

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