Antagonism between Nur77 and Glucocorticoid Receptor for Control of Transcription

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Two important functions of glucocorticoids (Gc), namely, suppression of immune system function and feedback repression of the hypothalamo-pituitary-adrenal (HPA) axis, are mediated through repression of gene transcription. Previous studies have indicated that this repression is exerted in part through antagonism between the glucocorticoid receptors (GR) and the AP-1 family of transcription factors. However, this mechanism could not account for repression of the pro-opiomelanocortin (POMC) gene, an important regulator of the HPA axis. Our recent identification of the orphan nuclear receptor Nur77 as a mediator of CRH induction of POMC transcription led us, in the present work, to show that Gc antagonize this positive signal at two levels. First, Gc partly blunt the CRH induction of Nur77 mRNA, and second, they antagonize Nur77-dependent transcription. GR repression is exerted by antagonism of Nur77 action on the NurRE element of the POMC gene. Gc antagonism of NurRE activity was observed in response to physiological stimuli in both endocrine and lymphoid cells. The finding of a Nur77 signaling pathway activated in POMC-producing cells, and their receptor antagonize at two levels, signals mediated through the CRH/Nur77 signaling pathway. Indeed, Gc partly blunt the transcriptional response of the Nur77 gene to CRH, and, in addition, they antagonize in a competitive fashion the transcriptional effect of CRH on POMC expression may be mediated through activation of Nur77. We now report that Gc and their receptor antagonize at two levels, signals mediated through the CRH/Nur77 signaling pathway. Indeed, Gc partly blunt the transcriptional response of the Nur77 gene to CRH, and, in addition, they antagonize in a competitive fashion the transcriptional activation of POMC by Nur77. Mutagenesis of the GR and in vitro binding studies suggest that the mechanism of Nur77-GR antagonism is similar to that of the AP-1-GR interaction. Similar regulatory interactions were also observed in T-cell hybridomas in response to cross-linking of the T-cell receptor (TCR), suggesting that cross talk between Gc repression and the activation of cellular responses by the Nur77 pathway is a regulatory mechanism common to endocrine and lymphoid cells.

Glucocorticoids (Gc) and their receptors have been shown to repress transcription of target genes by interaction of the Gc receptor (GR) with members of the AP-1 family of transcription factors (24, 41, 48). However, this mechanism does not account for repression of all Gc-sensitive genes. This is the case for the Gc-repressed pro-opiomelanocortin (POMC) gene, which encodes the precursor to adrenocorticotropin (ACTH), the major stimulus of adrenal Gc synthesis. Indeed, various mechanisms have been invoked to account for Gc repression of POMC transcription, including the formation of complexes containing three GR molecules on a negative Gc response element (nGRE) present at bp −63 in the POMC promoter (9). Some authors have suggested that a fos-dependent pathway may be involved in part but not exclusively (2), and others have implicated distal promoter sequences which are targets neither for AP-1-related factors nor for GR (38). The finding of a Nur77 signaling pathway activated in POMC-expressing cells in response to the corticotropin-releasing hormone (CRH) (36) led us to consider its relationship to the mechanisms of Gc repression.

The hypothalamic hormone CRH is a major stimulus of pituitary ACTH secretion and of POMC transcription (23). Its actions are antagonized by Gc in a classical negative-feedback loop. Indeed, the hypothalamo-pituitary-adrenal (HPA) axis is activated by CRH, which, through ACTH, leads to increased production of Gc; these steroids give feedback at both hypothalamic and pituitary levels to inhibit hormone secretion and repress gene (CRH and POMC) activity. Thus, the in vivo interactions of positive signals channeled through CRH and the negative feedback of Gc at both the hypothalamic and pituitary levels is thought to set the activity of the HPA axis. We have recently identified a POMC promoter regulatory target for CRH and shown that it is a novel binding site for the orphan nuclear receptor, Nur77 (36). Current evidence suggests that the transcriptional effect of CRH on POMC expression may be mediated through activation of Nur77. We now report that Gc and their receptor antagonize at two levels, signals mediated through the CRH/Nur77 signaling pathway. Indeed, Gc partly blunt the transcriptional response of the Nur77 gene to CRH, and, in addition, they antagonize in a competitive fashion the transcriptional activation of POMC by Nur77. Mutagenesis of the GR and in vitro binding studies suggest that the mechanism of Nur77-GR antagonism is similar to that of the AP-1-GR interaction. Similar regulatory interactions were also observed in T-cell hybridomas in response to cross-linking of the T-cell receptor (TCR), suggesting that cross talk between Gc repression and the activation of cellular responses by the Nur77 pathway is a regulatory mechanism common to endocrine and lymphoid cells.

MATERIALS AND METHODS

Transfections. Transfections in AtT-20 cells were performed by lipofection (Lipofectin; Gibco) with 7.5 × 105 exponentially growing AtT-20 cells in 35-mm petri dishes. The cells were grown in Dulbecco modified Eagle medium with 10% fetal calf serum stripped with dextran-coated charcoal (8). The cells and media were harvested 16 h after lipofection. Each sample for lipofection contained a total of 1.5 μg of DNA, including 300 ng of reporter plasmid, 300 ng of Rous sarcoma virus (RSV)-growth hormone (GH) as an internal control, 100 ng of pCMX-Nur77 expression vector, and pSP64 to make up the total amount. Where indicated, CRH and dexamethasone (Dex) were added at 10−7 M. The POMC promoter constructs were described previously (43, 44). In particular, the NurRE promoter constructs were described previously (43, 44).
RESULTS

Localization of Dex-responsive sequences. Promoter sequences required for Gc repression of the POMC promoter were localized by lipofection of POMC promoter-luciferase constructs into POMC-expressing AtT-20 cells. This analysis revealed that a composite regulatory element in the distal region (43, 44) of the promoter, DE2, is required, since its deletion completely blunts responsiveness to the synthetic Gc dexamethasone, Dex (Fig. 1A, compare construct 3 with constructs 1 and 2). The magnitude of the repression (two- to threefold) is similar to that observed in nuclear run-on experiments with in vivo (14) and tissue culture (15) models; thus, repression of transfected reporters is very similar to that measured for the POMC gene in normal pituitary cells. In contrast, deletion of the PE-1 regulatory element (43), which contains an AP-1 site (construct 5), does not prevent Gc repression, although the repression may be less intense. Furthermore, deletion of the proximal region of the promoter that contains a previously described nGRE (9) did not prevent Gc repression either (Fig. 1B, construct 2). However, addition of a DE2 oligonucleotide to a basic reporter construct conferred Gc sensitivity (constructs 3, 4, and 5), and deletion of its upstream region (previously described as DE2A [44]) abolished this response (construct 6). Thus, sequences required for responsiveness to Gc lie upstream of those required for synergistic interaction between the basic helix-loop-helix (bHLH)-restricted

and nGRE linker-scanning replacement mutations were described previously as mutants 3 and 15 in a series of promoter mutations (45). DO 11.10 cells (3.5 × 10^6 in 0.35 ml of DMEM) were electroporated in a Bio-Rad instrument at 250 V and 960 μF, with 10 μg each of reporter plasmid and expression vectors. After electroporation, the cells were plated in DMEM containing 5% fetal calf serum with or without 10^{-7} M Dex; anti-CD3 (clone 145-2C11) was used at 1 μg/ml to coat the dishes (22). A terminal deoxynucleotidyltransferase-mediated (TUNEL) assay was performed as described previously (42). CV-1 cells (5 × 10^5 cells per 35-mm dish) were grown in charcoal-stripped serum and transfected by the calcium phosphate method with 2 μg of reporter plasmid per dish, 1 μg of the internal control plasmid RSV-GH per dish, and the indicated amounts of expression plasmids. Total transfected DNA was kept constant at 5 μg/dish with pSP64. Data are presented as the means ± standard errors of the mean (SEM) for three to five experiments each performed in duplicate.

Northern blots. Total RNA (20 μg) extracted from AtT-20 cells was used in Northern blot experiments performed as described previously (30).

Gel retardation. NurRE, NBRE, and TRE oligonucleotides were 3'-end labeled with Klenow polymerase and purified on polyacrylamide gels. The binding conditions were as previously described (9, 10, 36, 48). In vitro-translated Nur77, GR, and c-jun were produced with reticulocyte lysate kits purchased from Promega (TNT SP6/T7). The efficiency of protein synthesis was monitored by [35S]methionine labeling of the reaction products and analysis by gel electrophoresis; similar amounts (about 10 ng) of in vitro-synthesized Nur77, GR, and c-jun were used.
factor NeuroD1, which binds the DE2C subelement, and Ptx1, a bicoi’d-related homeobox factor that binds the downstream CE3 element of the promoter (30, 37, 44). The strict dependence on those upstream DE2 sequences was shown by using promoter constructs constituted only of oligonucleotides (Fig. 1C). These contained the targets for Ptx1 and the bHLH NeuroD1-BETA2 heterodimers (30, 37). This construct (Fig. 1C, construct 2) exhibited cell specificity (30) and responsiveness to CRH and Nur77 (36) and was sensitive to Gc (Fig. 1C).

The NurRE confers responsiveness to Dex. To test this hypothesis, we determined whether the activation of POMC transcription by CRH (Fig. 2A) and Nur77 (Fig. 2B) was antagonized by Dex. Indeed, in addition to repressing basal POMC promoter activity, Dex reversed the activation by CRH and Nur77 overexpression (Fig. 2, construct 1). Whereas mutagenesis of the nGRE (construct 2) had no effect on either response, mutagenesis of the NurRE (by transversion of 10 bp; construct 3) decreased promoter activity to that of the Dex-repressed intact promoter and completely blunted Dex repression under basal and CRH or Nur77-stimulated conditions. Under our experimental conditions, the bp –323 promoter (construct 4) that contains both nGRE (which itself contains a NBRE target for Nur77) and an AP-1 site did not respond to CRH, Nur77, or Dex; similarly, the minimal promoter (construct 5) that still contains the AP-1 site in its exonic portion did not respond. The NurRE thus seems to be the point of convergence for both positive (CRH and Nur77) and negative (Gc) signals controlling POMC transcription.

The convergence of CRH, Nur77, and Gc signals at the NurRE was clearly shown by using a reporter that contained only that regulatory element in three copies (Fig. 3). Indeed, the high activity elicited by CRH stimulation with the NurRE-luc reporter was completely reversed by Dex (Fig. 3A), and even the supraphysiological induction produced by overexpression of Nur77 was partly reversed by Dex (Fig. 3B). In similar experiments, overexpression of GR led to a greater Dex-dependent reversal of Nur77 activation (data not shown). Antagonism between Nur77 and Dex was also observed with a NBRE-luc reporter (36), although the effects were less pronounced (Fig. 3C).

Dex antagonism is partly exerted on Nur77 expression. Although the Gc reversal of Nur77-dependent reporter activity suggested an interaction at the transcriptional level, we tested whether these signals may not also converge at the Nur77 gene itself, since it is very strongly induced in response to CRH (36). Northern blot analysis of AtT-20 cells treated with CRH and/or Dex indicated that Dex reduced the CRH induction of Nur77 mRNA but only by about 50% (Fig. 4). Thus, it appears that Gc blunt the Nur77 signaling pathway at two levels, namely, induction of Nur77 transcription (Fig. 4) and the transcriptional effect of Nur77 itself on its target (Fig. 3).

Reciprocal titration of Nur77 and GR action. The transcriptional interaction between Nur77 and GR could take place at different levels during activation of transcription. We have previously shown that GR does not bind the NurRE (11), suggesting that protein-protein interactions are involved in the antagonism between GR and Nur77. To test whether the antagonism between GR and Nur77 is reciprocal, we expressed in CV-1 cells increasing amounts of GR in the presence of Nur77 and the NurRE-luc reporter, and vice versa with a GRE-containing reporter (Fig. 5). Both GR and Nur77 titrated each other’s activity on its cognate reporter. Thus, increasing concentrations of GR blunted the Nur77 activation at the NurRE only in the presence of Dex (Fig. 5A), and Dex had no effect in the absence of GR (ratio, 1:0). Conversely, the Dex-dependent transactivation by GR was reversed in a dose-dependent manner by Nur77 (Fig. 5B). Dex did not activate this reporter in the absence of GR expression (data not shown). These data suggest that the two factors antagonize each other by direct interaction or by interaction with a common target.

To assess a putative interaction between Nur77 and GR, we tested the effect of one factor on the DNA binding activity of
the other (Fig. 6). The antagonism between GR and AP-1 was reflected in vitro by an impairment of the DNA binding of each factor to its cognate element (26, 41, 48). The in vivo significance of this interaction has been questioned (29), but the current models of integration of transcription factor signals by CBP/p300 are consistent with interactions between the factors as well as with CBP/p300 (25). Similarly, we found that the binding of in vitro-translated Nur77 to a NurRE probe was impaired in the presence of GR (Fig. 6, lanes 4 to 7) but not with similar amounts of in vitro-translated c-jun (lanes 8 to 10). Conversely, addition of Nur77 in GR binding assays to a GRE probe led to a significant decrease of GR binding (lanes 14 to 17). Interestingly, similar amounts of c-jun did not impair GR binding (lanes 18 to 20). Larger amounts of c-jun were required (data not shown), in agreement with previous work (48) and with data indicating that c-jun is less potent than c-fos for inhibition of in vitro GR binding (26). The c-jun preparation used in these experiments was active in TRE binding (lane 24).

Thus, the GR-Nur77 interaction appears to occur at protein concentrations that are similar to or lower than those involved in GR–AP-1 interactions.

GR domains required for repression of Nur77 action. The domains of GR required for repression of Nur77 action at NurRE were localized by using a panel of GR mutants that were previously assessed for their effect on Gc-dependent activation of transcription and for repression of AP-1-induced transcription (19, 24, 41, 48). Each GR mutant was tested for its capacity to activate transcription from a GRE-containing reporter in the presence or absence of Dex, as well as for its capacity to repress Nur77-induced transcription of the NurRE reporter (Fig. 7). Deletions of the N-terminal transactivation domain of GR did not interfere with Dex-dependent repression, whereas most mutations in the DNA binding domain (DBD) that prevented DNA binding as well as Gc-dependent induction of transcription also prevented repression of Nur77-dependent activity. Interestingly, the LS7 mutant that was previously shown (16, 19, 48) to be deficient in activation function but still active in repression of AP-1-dependent transcription (19, 24, 41, 48). Each GR mutant was tested for its capacity to activate transcription from a GRE-containing reporter in the presence or absence of Dex, as well as for its capacity to repress Nur77-induced transcription of the NurRE reporter (Fig. 7). Deletions of the N-terminal transactivation domain of GR did not interfere with Dex-dependent repression, whereas most mutations in the DNA binding domain (DBD) that prevented DNA binding as well as Gc-dependent induction of transcription also prevented repression of Nur77-dependent activity. Interestingly, the LS7 mutant that was previously shown (16, 19, 48) to be deficient in activation function but still active in repression of AP-1-dependent transcription is also active in repression of Nur77-dependent activity. Deletion of the ligand binding domain produced a receptor (mutant VAN525) that represses Nur77-dependent reporter activity in a hormone-independent fashion; the transactivation activity of
this mutant GR is also hormone independent. Since neither N- nor C-terminal domains of GR appear to be required for the repression function and since many DBD mutations block repression activity, it appears that this function can be mostly ascribed to the DBD. However, the transactivation and repressor activities of DBD appear separate, as evidenced by the LS7 mutant. These findings are strictly similar to those made for GR domains required for repression of AP-1 activity (17, 41, 48), and they suggest that similar GR domains are involved in cross talk with Nur77 and AP-1.

**Antagonism between TCR signaling and Gc at the NurRE.**

Nur77 appears to be an essential mediator of T-cell apoptosis (4, 5, 31, 47). Since the apoptotic response of T cells to TCR activation is antagonized by Gc (22, 28), we tested whether Dex antagonizes NurRE-luc reporter activity induced by TCR activation (36) produced by cross-linking with an anti-CD3 antibody (Fig. 8). The DO 11.10 T hybridoma cells used in these experiments are induced into apoptosis by anti-CD3 treatment and by Gc through independent pathways; however, the two pathways antagonize each other (22, 27). Preliminary experiments involving the TUNEL assay (42) to identify apoptotic cells were conducted to determine the optimal concentrations of anti-CD3 and Dex for assessment of NurRE reporter (Fig. 8A). In contrast to AtT-20 cells, where basal NurRE-luc reporter activity is very low and insensitive to Dex treatment (Fig. 3), Dex decreased basal reporter activity in DO 11.10 cells by almost as much as it repressed the anti-CD3-induced activity (Fig. 8B). This is suggestive of a higher endogenous Nur activity in the hybridoma cell line and may account for the lower sensitivity to repression by Gc after anti-CD3 treatment. Part of this basal activity might have been induced by the electroporation itself. Be that as it may, Gc signaling and Nur77 signaling antagonize each other in T cells as in endocrine cells.

**FIG. 5.** Nur77 and GR antagonize each other’s action on cognate target reporters. The ratio of Nur77 and GR expression vectors was varied as indicated in cotransfection experiments in CV-1 cells to assess the effect of excess GR on Nur77-dependent activation of the NurRE-luc reporter and to assess the effect of excess Nur77 on GR and 10^{-7} M Dex-dependent activation of a GRE-containing reporter (9). (A) The Nur77 expression vector was used at 50 ng/dish, whereas the amount of GR expression vector was varied from 100 to 400 ng/dish. (B) The expression vectors were GR at 50 ng/dish and Nur77 at concentrations from 50 to 400 ng/dish.

**FIG. 6.** Nur77 and GR impair each other’s ability to bind their cognate DNA sequence. Gel retardation experiments (9, 36, 48) were used to study the effect of in vitro-translated proteins on DNA binding. The binding of Nur77 to the NurRE probe (lanes 4 to 10) produced mostly complexes containing homodimers of Nur77 (36); the effect of similar amounts of GR (lanes 5 to 7) and c-jun (lanes 8 to 10) on the formation of these Nur77 complexes was assessed. Similar experiments were performed with GR, using a GRE probe (lanes 14 to 20). The c-jun preparation used in these experiments was active in TRE probe binding (lane 24), and this probe was not bound by Nur77 (lane 22) and GR (lane 23). Similarly, c-jun (lane 2) and GR (lane 3) did not bind NurRE, and c-jun (lane 12) and Nur77 (lane 13) did not bind GRE.
DISCUSSION

Whereas previous work has suggested that the GR represses transcription of target genes either through direct GR interaction with DNA or through protein-protein interaction with other classes of transcription factors (7), our present data suggest that GR-mediated repression may also result from antagonism with other members of the nuclear receptor family. Indeed, Nur77 is a member of a small subfamily of orphan nuclear receptors that appear to play important signaling functions in the HPA axis (6, 20, 32, 35, 36) and in T cells (4, 13, 31, 47). At this time, it is not clear whether Nur77 requires ligand activation, since no ligand is known for this factor and since it is obviously active in transcription activation experiments without the addition of any exogenous ligand. The present work supports at least two mechanisms for Gc/GR repression of signals mediated through Nur77. Indeed, we have shown that Gc blunt the responsiveness of the Nur77 gene to a physiological signal, CRH (Fig. 4), and we have shown that GR antagonizes Nur77-dependent activation of transcription in three different systems (Fig. 3, 5, and 8). The mechanism of this antagonism appears to be similar to that for cross talk between GR and AP-1.

Repression of POMC transcription by GR. Repression of POMC expression by Gc is an important regulatory component of the HPA axis, and disregulation of this negative feedback loop has physiological and behavioral consequences, as exemplified by patients with Cushing’s disease or by patients or animals with chronic stress. In vivo studies in both humans and...
animal models have indicated that the sensitivity of the HPA axis to Gc can vary enormously depending on the physiological status. The molecular basis for this modulation is still poorly understood. The POMC gene has offered a model gene to define the mechanisms of Gc repression and potentially to define mechanisms involved in modulation of Gc responsiveness in the HPA axis. Previous studies have implicated up to three targets of the POMC promoter in Gc repression. They include the nGRE, located in the proximal region of the promoter and shown to form unique complexes with three molecules of GR (9, 11). Other studies have implicated the nGRE, together with upstream promoter targets, in Gc repression (38). Finally, it has been suggested that a fos-dependent mechanism may be involved under some conditions (2); fos and related members of the AP-1 family of transcription factors exert their transcriptional effect on POMC transcription through a binding site in the first exon of the gene (element PE1 of the promoter [43]). The present work has defined the upstream promoter target for Gc repression as the NurRE (Fig. 1 to 3). At this time, it is not clear what mechanism may control the contribution of each of these promoter targets to Gc repression. However, the fos-mediated pathway may operate under unusual conditions, since it was entirely defined in serum-starved cells and since deletion of the POMC promoter AP-1 target site (Fig. 1A, construct 5) does not alter Dex repression significantly. Whatever the role of AP-1 in POMC expression, it is clear that it is not sufficient to mediate Gc repression. The NurRE pathway may be dependent on corticotroph-specific recognition of the promoter, which requires NeuroD heterodimers acting at the DE2C subelement and Ptx1 acting on the CE3 element (30, 44). Indeed, our original promoter analyses had indicated that the DE2AB subelement, which includes the NurRE, is mostly active when the DE2C/CE3 elements are active in synergism (43, 44). Thus, in non-corticotroph cells or in corticotroph cells in which the activity of Ptx1, NeuroD, or its dimerization partner is reduced through signaling events, the NurRE target may be inoperative. This could be a mechanism through which desensitization of corticotroph cells to Gc repression might be achieved under conditions such as chronic stress.

The nGRE is a potential target for both GR and Nur77, since it contains a NBRE sequence (32, 45). Under the experimental conditions used in this study for AtT-20 cells, POMC promoter activity was highly dependent on upstream cell-specific sequences (Fig. 2), and under these conditions, the nGRE/NBRE was not responsive to Nur77 overexpression, to CRH, or to Gc repression (Fig. 2). Under other conditions, the nGRE was shown to contribute as a target for Gc repression (9, 11, 32, 38) and, in isolation on a minimal promoter (Fig. 3), a NBRE reporter was responsive to Nur77 overexpression as well as to Dex, albeit at much reduced magnitude compared to the NurRE. In these cases (where the distal promoter sequences may have been inoperative), two mechanisms might have involved Nur77. First, protein–protein interactions of the same nature as those involved in GR antagonism at the NurRE could be implicated (discussed below). Second, mutually exclusive binding of GR and Nur77 at the nGRE is also possible. Irrespective of the mechanism, such antagonism could be operationally only under conditions where a positive transactivator like Nur77 activated transcription through interaction with nGRE or NBRE. Consequently, the nGRE would not be expected to behave as a target for Gc repression under basal conditions, since prior work showed that mutagenesis of nGRE sequences did not affect basal POMC promoter activity in AtT-20 cells (43). In contrast, since Gc do repress basal POMC transcription in AtT-20 cells (9, 11, 12, 15) and in primary cultures of pituitary cells (15), the primary target for Gc repression is most likely to be the NurRE, which is tightly linked to cell-specific recognition of the promoter in corticotroph cells. However, under some conditions, such as chronic stress, the relative contribution of proximal promoter sequences (which include the nGRE) to basal control of transcription might be enhanced (by a mechanism yet to be identified) and thus the nGRE might become an effective target for Gc repression.

Mechanism of GR-Nur77 antagonism. The present data suggest that GR repression of Nur77-dependent transcription is mediated by protein–protein interactions either between these two proteins (Fig. 6) or between these two and a common target. Such a common target could be one of the many coactivators or corepressors that have been associated with the transcriptional activity of nuclear receptors (21, 34). In particular, CBP/p300 has been implicated in mediating the transcriptional effects of nuclear receptors and also of the jun/AP-1 family of factors (25). Since Nur77 and GR both belong to the same structural family of nuclear receptors, it will be interesting to determine whether they both interact with CBP at the N terminus as shown for GR (25) or whether they interact at different sites on CBP.

A parallel can be drawn between the present data (Fig. 5 to 7) and the antagonism between GR and AP-1 (24, 26, 41, 48), and possibly also with relA/NFκB-induced transcription (1, 3, 39, 40). Indeed, in these cases, GR-mediated repression is ligand dependent and does not require DNA binding, although the DBD is involved. The mechanism of this ligand-dependent repression is presumably different from that recently described for unliganded receptors, which involves the hinge regions of the thyroid hormone receptor and the retinoic acid receptor (18, 21, 33). Since GR is not known to repress transcription in its unliganded state and since this hinge region is different in GR, another mechanism of repression is likely. Although it is possible that repression and activation proceed through different intermediate complexes containing either histone deacetylase or acetyltransferase (46), another possibility is that the activator effect of Nur77 and the repressor activity of GR are both integrated at the level of CBP. In this case, it would be more likely that the two factors interact with different sites on CBP rather than compete for the N terminus (25). This is supported by the fact that the DBD of GR appears to play the primary role in the repressor function whereas the C terminus of GR and other nuclear receptors was involved in interaction with the CBP N terminus in a ligand-dependent fashion (25). This may be interpreted to suggest that the receptor C-terminus–CBP N-terminus interaction mediates activator function and that when the same receptors act as DNA-independent repressors, they target another domain of CBP either directly and/or by association with a corepressor protein. In this model, DNA binding by the nuclear receptor will determine whether it behaves as an activator or repressor of transcription. The POMC nGRE might be an exception, since the three GR molecules bound to this element are unable to activate transcription (9): the unique conformation of GR bound to this element for unliganded receptors, which involves the hinge regions of the thyroid hormone receptor and the retinoic acid receptor (18, 21, 33). Since GR is not known to repress transcription in its unliganded state and since this hinge region is different in GR, another mechanism of repression is likely. Although it is possible that repression and activation proceed through different intermediate complexes containing either histone deacetylase or acetyltransferase (46), another possibility is that the activator effect of Nur77 and the repressor activity of GR are both integrated at the level of CBP. In this case, it would be more likely that the two factors interact with different sites on CBP rather than compete for the N terminus (25). This is supported by the fact that the DBD of GR appears to play the primary role in the repressor function whereas the C terminus of GR and other nuclear receptors was involved in interaction with the CBP N terminus in a ligand-dependent fashion (25). This may be interpreted to suggest that the receptor C-terminus–CBP N-terminus interaction mediates activator function and that when the same receptors act as DNA-independent repressors, they target another domain of CBP either directly and/or by association with a corepressor protein. In this model, DNA binding by the nuclear receptor will determine whether it behaves as an activator or repressor of transcription. The POMC nGRE might be an exception, since the three GR molecules bound to this element are unable to activate transcription (9): the unique conformation of GR bound to this element might maintain it in a repressor status despite its interaction with DNA.

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