The Glucocorticoid Receptor Is Tethered to DNA-bound Oct-1 at the Mouse Gonadotropin-releasing Hormone Distal Negative Glucocorticoid Response Element*  

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An element required for glucocorticoid repression of mouse gonadotropin-releasing hormone (GnRH) gene transcription, the distal negative glucocorticoid response element (nGRE), is not bound directly by glucocorticoid receptors (GRs) but is recognized by Oct-1 present in GT1-7 cell nuclear extracts or by Oct-1 purified from HeLa cells. Furthermore, purified full-length GRs interact directly with purified Oct-1 bound to the distal nGRE. Increasing the extent of distal nGRE match to an Oct-1 consensus site not only increases the affinity of Oct-1 binding, but also alters the conformation of DNA-bound Oct-1 and the pattern of protein DNA complexes formed in vitro with GT1-7 cell nuclear extracts. In addition, the interaction of purified GR with DNA-bound Oct-1 is altered when Oct-1 is bound to the consensus Oct-1 site. Mutation of the distal nGRE to a consensus Oct-1 site is also associated with reduced glucocorticoid repression in transfected GT1-7 cells. Furthermore, repression of GnRH gene transcription by 12-O-tetradecanoylphorbol-13-acetate, which utilizes sequences that overlap with the nGRE, is reversed by this distal nGRE mutation leading to activation of GnRH gene transcription. Thus, changes in the assembly of multi-protein complexes at the distal nGRE can influence the regulation of GnRH gene transcription.

Gonadotropin-releasing hormone (GnRH) is secreted by neurons in the hypothalamus and is at the top of the endocrine axis that controls reproductive function. In recent years, molecular studies of GnRH gene regulation have been facilitated by the development of the immortalized GnRH-secreting GT1 cell lines (1). In these cell lines, GnRH expression is regulated by various neurotransmitters (2–5), second messengers, and other signal transduction pathways (6–14).

Our laboratory has utilized the GT1-7 cell line to investigate the molecular mechanism responsible for glucocorticoid repression of GnRH gene expression. Glucocorticoids have been implicated in physiological regulation of GnRH, as stress-related reproductive disorders and high cortisol levels in women have been associated with reductions in circulating luteinizing hormone (15–17). Glucocorticoids can directly suppress gonadotropin secretion from the pituitary (18), but the possibility that they also act at a hypothalamic level, i.e. directly on GnRH neurons, has been suggested in a few physiological studies (19, 20). The detection of glucocorticoid receptors (GRs), a member of the steroid/thyroid hormone family of nuclear receptors (21–23), within a subset of GnRH neurons (24), provides additional support for the notion that glucocorticoids exert direct effects on GnRH gene expression. The GT1-7 cell line also contains functional GRs that, in the presence of glucocorticoid agonists dexamethasone, repress GnRH promoter activity (7). Two regions of the mouse GnRH promoter, the distal and proximal glucocorticoid response elements (nGREs), mediate glucocorticoid repression of transcription (25). GRs do not bind directly to either of these nGREs, but at the distal nGRE they are part of a multi-protein complex that also includes Oct-1 (25), a member of the POU domain family of transcription factors (26–30).

The fact that GR and Oct-1 co-occupy the distal nGRE (25) of the mouse GnRH promoter suggests that functionally relevant interactions between GR and Oct-1 may not be limited to solution as observed in other studies. We therefore have examined whether GR interacts directly with DNA-bound Oct-1. We show here that purified GR indeed can associate with Oct-1 bound to the GnRH distal nGRE. This provides the first evidence of glucocorticoid repression of transcription that is mediated by the tethering of GR to a DNA-bound transcription factor. We furthermore show that the nature of Oct-1 interactions at the distal nGRE influences transcriptional repression, not only by glucocorticoids, but also by the tumor-promoting phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA). Thus, alternative conformational states of Oct-1, which are dictated by precise DNA contacts made at different binding sites, may influence the assembly of multi-protein complexes that impart unique regulatory properties upon linked promoters.

EXPERIMENTAL PROCEDURES

In Vitro Mutagenesis—DNA fragments containing 3446 base pairs of the mouse GnRH promoter (25) were mutagenized to create a consensus Oct-1 binding site at the distal nGRE using the protocol supplied in the in vitro mutagenesis kit (CLONTECH). The primer used for the mutagenesis, 5'-GCTAGATATGGCATGACCG-3', contained nucleotide changes at base pairs 206 and 207 of the mouse GnRH promoter, to generate a consensus Oct-1 binding site at the mouse GnRH distal nGRE. The GnRH nGRE mutation was confirmed by deoxy sequencing (31).

The mouse GnRH promoter containing either the wild type GnRH distal nGRE or the consensus Oct-1 binding site linked to the luciferase
FIG. 1. Sequence of the L7, L7M, and L73M oligonucleotides. The sequences of the L7 (distal GnRH nGRE, −238 to −201), L73M, and L7M oligonucleotides are shown. The region of the L7 oligonucleotide that is homologous to the Oct-1 binding site is overlined and underlined, and the mismatches to the perfect Oct-1 site are shown by asterisks. The L7 exhibits a 5/8 match to a consensus Oct-1 binding sequence (5′-ATGCAAT-A3′) on the top strand and a 6/8 match on the bottom strand. Both L7M and L73M contain a consensus Oct-1 binding site (underlined), which was derived by mutating the two nucleotides at positions −206 and −207 of the mouse GnRH promoter. These nucleotides are shown by asterisks in the L7 sequence. L7M contains base pairs −237 to −201 of the GnRH distal nGRE, and L73M contains base pairs −218 to −201 of the GnRH distal nGRE.

Interaction between GR and Oct-1

reporter gene (25) was digested with HindIII and BstXI to generate two fragments, one from base pairs −3446 to −471, and the other containing the GnRH promoter up to base pair −471 linked to the luciferase reporter gene. The GnRH promoter deletion to base pair −471 was then blunt-ended and ligated to generate the −471mGnRH-Luc.

Cell Culture and Transfections—GT1–7 cells were grown as described previously (25). 5 μg of plasmid DNA along with 5 μg of herring sperm DNA carrier were transfected into GT1–7 cells on 60-mm dishes using the calcium phosphate precipitation method as described previously (25). The precipitate was allowed to sit on the cells for 12–16 h, after which the cells were washed with Tris-buffered saline and refed with fresh medium. The cells were then treated with either 10−6 M dexamethasone or 100 ng/ml TPA (Sigma) where indicated. Following a 24-h incubation, cells were harvested and assayed for luciferase activity (Luciferase kit, Promega, Madison, WI) using the same amount of total protein from each of the plates.

Statistical Analyses—The mean luciferase activity for each construct was determined from at least five independent experiments. The luciferase activity between controls and after dexamethasone or TPA treatment was compared using the Mann-Whitney rank-sum test. The percent luciferase activity for each construct was determined from at least five independent experiments. The luciferase activity for each of the plates. For EMSAs with purified Oct-1 and purified rat GR (32), 0.05 μM 32P-labeled double-stranded oligonucleotide (L7 or L7M) was incubated with 1× Oct-1 gel shift buffer (see above), 1 μg of poly(dIdC), 1 μl of 10 mg/ml BSA, 2 μl of purified Oct-1, and 0.5 μl of purified GR. The reaction was incubated for 15 min at room temperature and then 5 min on ice. Protein-DNA complexes were resolved as described above.

Purified Oct-1 Binds to the GnRH nGRE—Previous results from our laboratory showed that Oct-1 present in GT1–7 cell nuclear extracts binds to the mouse GnRH distal nGRE in vitro (25). As the Oct-1-containing complex formed on this nGRE appeared to contain multiple proteins, we wished to determine whether Oct-1 binding to this site required GT1–7 cell-specific nuclear factors. Oct-1 was purified from HeLa cells using established methods (29, 34) or from a Drosophila cell (S2) expression system (Invitrogen Corp., Carlsbad, CA). In the latter case, FLAG epitope-tagged full-length human Oct-1 was purified as described (35). Briefly, cells were lysed by sonication, and the supernatant from a 11000 × g centrifugation incubated with anti-FLAG M2 affinity gel (Sigma) at 4 °C overnight. The M2 agarose was washed five times and the bound protein eluted by incubation with the FLAG peptide (Sigma) for 1 h at 4 °C. The eluate was concentrated on a Centricon-30 centrifugal concentrator (Amicon Inc., Beverly, MA), and aliquots were frozen at −140 °C.

RESULTS

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For EMSAs with purified Oct-1 and purified rat GR (32), 0.05 μM 32P-labeled double-stranded oligonucleotide was incubated in 1× Oct-1 gel shift buffer (see above), 1 μg of poly(dIdC), 1 μl of 10 mg/ml BSA, 2 μl of purified Oct-1, and 0.5 μl of purified GR. The reaction was incubated for 15 min at room temperature and then 5 min on ice. Protein-DNA complexes were resolved as described above.

Protease Sensitivity Assay—0.05 μM 32P-labeled double-stranded oligonucleotide (L7 or L7M) was incubated with 1× Oct-1 gel shift buffer in a 15 μl reaction volume with 5 μg of GT1–7 cell nuclear extract for 15 min at room temperature. The reaction mix was run on native 10% polyacrylamide (75:1) gels in 1× TBE buffer. Gels were electrophoresed in 1× TBE at 180 V for approximately 30 min.

Oct-1 Purification—Oct-1 was either purified from HeLa cells using established methods (29, 34) or from a Drosophila cell (S2) expression system (Invitrogen Corp., Carlsbad, CA). In the latter case, FLAG epitope-tagged full-length human Oct-1 was purified as described (35). Briefly, cells were lysed by sonication, and the supernatant from a 11000 × g centrifugation incubated with anti-FLAG M2 affinity gel (Sigma) at 4 °C overnight. The M2 agarose was washed five times and the bound protein eluted by incubation with the FLAG peptide (Sigma) for 1 h at 4 °C. The eluate was concentrated on a Centricon-30 centrifugal concentrator (Amicon Inc., Beverly, MA), and aliquots were frozen at −140 °C.

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incubated with GT1–7 cell nuclear extract, only a single protein-DNA complex was formed (Fig. 3A, lane 4). As expected from our previous studies with purified Oct-1 (see Fig. 2), the distal nGRE did not effectively compete with the consensus Oct-1 containing oligonucleotide for binding of Oct-1 present in the GT1–7 cell nuclear extract (data not shown). A comparison of the competitive strength of the wild type nGRE versus the consensus Oct-1 sequence for GT1–7 nuclear extract binding revealed that the apparent affinity of GT1–7 nuclear extract for the consensus Oct-1 sequence is approximately 10-fold higher than for the GnRH distal nGRE (data not shown). Furthermore, the Oct-1 containing C1-L7M protein-DNA complex, formed on the consensus Oct-1 site had a slightly increased electrophoretic mobility as compared with the Oct-1-containing complex, C1-L7, formed on the distal nGRE (Fig. 3A, compare lanes 4 and 3). This apparent migration difference, although small, was noted in multiple EMSAs performed with these oligonucleotides using different GT1–7 cell nuclear extract preparations (data not shown). These large complexes migrate slowly through the 10% polyacrylamide gels and are difficult to resolve. However, we were able to resolve the distal nGRE-protein complex, C1-L7, and the consensus Oct-1 site-protein complex, C1-L7M, on a lower percentage gel (Fig. 3B, lanes 1 and 2). As will be shown below, this differential migration most likely reflects the fact that GR is present within the multi-protein C1-L7M complex, but not the C1-L7M complex (see Fig. 3C).

In addition to Oct-1, one multi-protein complex formed on the distal nGRE when using GT1–7 cell nuclear extracts also includes GR (25). To determine whether GR is also present in the complex formed at the mutant nGRE possessing a consensus Oct-1 binding site, we added the BuGR2 anti-GR antibody to a binding reaction containing the mutant nGRE and GT1–7 cell nuclear extract. As shown in Fig. 3C (lanes 1 and 2), BuGR2 does not supershift the complex formed on the consensus Oct-1 binding site. Thus, increasing the affinity of Oct-1 binding site by mutation of the GnRH distal nGRE alters the recruitment of GR into a multi-protein complex formed on this site with GT1–7 cell nuclear proteins.

**GR Interacts with Oct-1 at the GnRH Distal nGRE—** GRs contained within a multi-protein complex on the distal nGRE do not bind DNA directly at this site (25). The only GT1–7 cell nuclear protein that we have established is directly bound to the distal nGRE is Oct-1 (25). Does GR bind Oct-1 in this complex and does GR distinguish between Oct-1 bound at either a consensus site Oct-1 site or the distal nGRE? GR and Oct-1 have been shown to interact in vitro in the absence of DNA. In fact, it has been postulated that repression of histone H2b promoter activity by glucocorticoids is brought about by the sequestration of Oct-1 by GR in solution (29). Furthermore, although GR and Oct-1 can bind simultaneously in vitro to two distinct sites on the mouse mammary tumor virus long terminal repeat (MMTV LTR; Ref. 23), GR and Oct-1 have never definitively been shown to interact with each other while DNA-bound. In order to reveal whether GR and Oct-1 can co-occupy the GnRH distal nGRE in vitro, we performed EMSAs with this nGRE using purified preparations of rat GR (32) and Oct-1. As shown in Fig. 4 (lanes 1 and 2), an Oct-1-nGRE complex (i.e. complex C1-L7) formed in vitro was supershifted (i.e. complex C1-L7) by the addition of purified GR to the binding reaction. The supershift occurs only when purified GR is added to Oct-1 and not when another protein fraction from the GR purification procedure is incubated with Oct-1 (data not shown). We also tested the ability of purified GR and Oct-1 to bind to the mutant nGRE possessing a consensus Oct-1 binding site. The binding of Oct-1 to the consensus Oct-1 binding site oligonu-

The inability of the GnRH distal nGRE to compete with the consensus Oct-1 site for Oct-1 binding was not surprising, given the limited homology of the Oct-1 sequence in this nGRE to a consensus Oct-1 site (Fig. 1). Specifically, the Oct-1 binding site in this element has only a 5/8 match on the bottom strand to a consensus Oct-1 binding site in this element has only a 5/8 match on the bottom strand to a consensus Oct-1 binding site in this nGRE (Fig. 2A). As shown previously, a 250-fold molar excess of the consensus Oct-1 site had a slightly increased electrophoretic mobility as compared with the Oct-1-containing complex, C1-L7, formed on the distal nGRE (Fig. 3A, compare lanes 4 and 3). This apparent migration difference, although small, was noted in multiple EMSAs performed with these oligonucleotides using different GT1–7 cell nuclear extract preparations (data not shown). These large complexes migrate slowly through the 10% polyacrylamide gels and are difficult to resolve. However, we were able to resolve the distal nGRE-protein complex, C1-L7, and the consensus Oct-1 site-protein complex, C1-L7M, on a lower percentage gel (Fig. 3B, lanes 1 and 2). As will be shown below, this differential migration most likely reflects the fact that GR is present within the multi-protein C1-L7M complex, but not the C1-L7M complex (see Fig. 3C).

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The complexes were resolved on an 8% (75:1) polyacrylamide gel. The protein-DNA complexes were resolved on a 10% polyacrylamide gel (75:1) at 4 °C. This gel was electrophoresed for a relatively long period of time (relative to other gels in this report) in order to maximize the resolution between closely spaced complexes. For EMSAs in the presence of the anti-GR antibody BuGR2, 2 μl of the antibody was first incubated with 2 μl of GT1–7 nuclear extract for 15 min at room temperature. 0.05 pmol of 32P-labeled oligonucleotide for 15 min at room temperature was then added and the reaction incubated for another 15 min at room temperature. The complexes were resolved on a 8% (75:1) polyacrylamide gel. C, 5 μg of GT1–7 nuclear extract was incubated with 0.05 pmol of 32P-labeled L7 oligonucleotide for 15 min at room temperature (lane 1). For EMSAs as shown in Fig. 5, treatment with increasing amounts of trypsin resulted in the appearance of trypsin-resistant fragments on both the L7 and L7M probes. However, Oct-1 bound at these different DNA elements exhibited a differential sensitivity to trypsin. Complexes labeled 1 and 2 represent intact Oct-1 bound to the L7 and L7M probes (Fig. 5A, lanes 2 and 8). When bound to the consensus Oct-1 site, predominant trypsin-resistant Oct-1-DNA complexes, i.e. designated a–e (Fig. 5A, lanes 9–12) were observed, while at the nGRE, a different set of trypsin-resistant Oct-1-DNA complexes, i.e. A and B (Fig. 5A, lanes 5 and 6), were noted. Complexes A and B on the distal nGRE exhibit different electrophoretic mobilities than complexes d and e on the consensus Oct-1 oligonucleotide. The difference in mobility is particularly evident when lanes 5 and 11 are placed next to each other (Fig. 5B, lanes 1 and 2).

**Fig. 4. Purified GR interacts with Oct-1 in vitro on the mouse GnRH distal nGRE.** 2 μl of purified Oct-1 was incubated with 0.05 pmol of 32P-labeled L7 or L7M oligonucleotide (lanes 1 and 3, respectively) in 1X Oct-1 binding buffer for 15 min at room temperature as described under “Experimental Procedures.” For interaction of purified GR with Oct-1, 0.5 μl of purified GR was first incubated with 2 μl of pure Oct-1 for 15 min at room temperature and then for 5 min on ice. 0.05 pmol of 32P-labeled L7 or L7M oligonucleotide (lanes 2 and 4, respectively) was then added and then reaction continued for another 15 min at room temperature. The protein-DNA complexes were resolved on a 10% polyacrylamide gel (75:1) at 4 °C. This gel was electrophoresed for a relatively long period of time relative to other gels in this report in order to maximize the resolution between closely spaced complexes. Complexes C1-L7 and C1-L7M, shown by arrows, represent Oct-1-bound L7 and L7M oligonucleotides, respectively. The supershifted complexes on the L7 oligonucleotide, S1-L7 and S2-L7, respectively, are shown by arrows. The supershifted complex on the L7M oligonucleotide, S1-L7M, is also shown by an arrow.

**Fig. 3. In vitro binding of GT1–7 nuclear proteins to the consensus Oct-1 binding site oligonucleotide.** A, 5 μg of GT1–7 nuclear extract was incubated with 0.05 pmol of 32P-labeled L7 or L7M oligonucleotides (lanes 3 and 4, respectively) for 15 min at room temperature as described under “Experimental Procedures.” The protein-DNA complexes were resolved on a 10% polyacrylamide gel (75:1) gel. The protein-DNA complex (i.e. C1-L7), which was previously found to include GR and Oct-1 (25), is shown by an arrowhead, and the protein-DNA complex, C1-L7M, formed by consensus Oct-1 site oligonucleotide is shown by an arrow. Lanes 1 and 2 contain L7 and L7M probes, respectively, incubated in the absence of GT1–7 nuclear extract. B, 3 μl of GT1–7 nuclear extract was incubated with 0.5 pmol of 32P-labeled L7 (lane 1) or L7M (lane 2) for 15 min at room temperature. The complexes were resolved on a 8% (75:1) polyacrylamide gel. Arrow. The supershifted complex on the L7M oligonucleotide, S1-L7M and S2-L7M, respectively, are shown by arrows. The major protein-DNA complex C1-L7M formed by GT1–7 nuclear extract with the L7M oligonucleotide is shown by an arrow.

**Complexes C1-L7M and C1-L7** represent Oct-1 bound to either an nGRE or a consensus Oct-1 site was subjected to a limited trypsin digestion (38, 39) and resulting protein-DNA complexes examined by EMSAs. As shown in Fig. 5, treatment with increasing amounts of trypsin resulted in the appearance of trypsin-resistant fragments on both the L7 and L7M probes. However, Oct-1 bound at these different DNA elements exhibited a differential sensitivity to trypsin. Complexes labeled 1 and 2 represent intact Oct-1 bound to the L7 and L7M probes (Fig. 5A, lanes 2 and 8). When bound to the consensus Oct-1 site, predominant trypsin-resistant Oct-1-DNA complexes, i.e. designated a–e (Fig. 5A, lanes 9–12) were observed, while at the nGRE, a different set of trypsin-resistant Oct-1-DNA complexes, i.e. A and B (Fig. 5A, lanes 5 and 6), were noted. Complexes A and B on the distal nGRE exhibit different electrophoretic mobilities than complexes d and e on the consensus Oct-1 oligonucleotide. The difference in mobility is particularly evident when lanes 5 and 11 are placed next to each other (Fig. 5B, lanes 1 and 2).

**The Nature of the Oct-1 Recognition Sequence within the Distal GnRH nGRE Influences Glucocorticoid and TPA Effects on GnRH Promoter Activity**—The GnRH distal nGRE contributes to glucocorticoid repression of GnRH promoter activity (25). As this repression is mediated by GR recruitment to this site, we set out to examine whether subtle differences in GR interaction with the nGRE generated by altering Oct-1 binding affinity and conformation affected glucocorticoid regulation of
GnRH promoter activity in vivo. A 5’-deletion of the GnRH promoter to −471 was used in transient transfection assays since this deletion had previously been shown to retain full repression by dexamethasone (25). Both a wild type distal nGRE (−471GnRH) and a mutant nGRE containing a consensus Oct-1 sequence (−471MSGnRH) were used in these analyses. GT1–7 cells, transiently transfected with luciferase reporter plasmids possessing these nGREs, were treated with dexamethasone and promoter activity assessed by measuring luciferase activity. As shown in Fig. 6, dexamethasone treatment significantly decreased luciferase activity generated from the wild type −471GnRH promoter (p = 0.03; Fig. 6) and the mutant −471MSGnRH promoter (p = 0.06). The differences in the extent of dexamethasone repression mediated by the wild type promoter (i.e. 60% repression; Fig. 6) versus the mutant promoter (i.e. 29% repression; Fig. 6) was statistically significant (p = 0.004).

Transcription from the mouse and rat GnRH promoters is also repressed by TPA (a tumor-promoting phorbol ester; Refs. 8–10). The elements responsible for this repression (i.e. the negative TPA-responsive elements or nTREs) have been mapped to a region of the promoter that coincides with the nGREs (40). The mechanism of this repression has not been definitively established, although in one model down-regulation of protein kinase C has been implicated as being responsible for TPA effects (40). Given the effect of the Oct-1 mutation in the distal nGRE on glucocorticoid repression, we examined whether effects of TPA on the promoter would also be altered by conversion of the Oct-1 binding site at the distal nGRE to a consensus Oct-1 binding site. As shown in Fig. 6, mutation of the Oct-1 site within the distal nGRE to a consensus Oct-1 binding site reversed TPA effects on promoter activity, i.e. TPA treatment led to induction of GnRH promoter activity. Specifically, TPA treatment significantly decreased luciferase activity generated from the wild type −471GnRH promoter (p = 0.06; Fig. 6) but increased luciferase activity generated from the mutant −471MSGnRH promoter (p = 0.06; Fig. 6). The differences in TPA effects mediated by the wild type promoter (i.e. 35% repression; Fig. 6) versus the mutant promoter (i.e. 1.5-fold induction; Fig. 6) was statistically significant (p = 0.007). Thus, TPA and glucocorticoid regulation converge at a site within the GnRH promoter that is recognized by Oct-1. Altering the strength of Oct-1 binding at the nGRE+nTRE elements dramatically different effects on these distinct signaling pathways.

DISCUSSION

Our results provide the first demonstration of GR co-occupancy on an nGRE that does not involve direct DNA binding by GR (41), but rather protein-protein interactions between GR and a DNA-bound transcription factor. Furthermore, the mechanism of glucocorticoid repression of GnRH that we have elucidated is distinct from other examples of repression that are mediated by either direct DNA binding of GR (42) or the association of GR with other transcription factors or coactivators in solution (29, 43, 44). Glucocorticoid repression of GnRH gene transcription in GT1–7 cells is dictated by two distinct nGREs that lie close to the GnRH promoter (25). Although GRs do not bind directly to these nGREs, they appear to be recruited to these elements by virtue of their interaction with Pou domain transcription factors (25). We had previously suggested that Oct-1 could be responsible for directing GR to the distal GnRH nGRE since both GR and Oct-1 in GT1–7 nuclear extracts were components of a unique protein-DNA complex formed in vitro on this nGRE (25). In this report, we demonstrate that the in vitro binding of purified Oct-1 to the distal nGRE can occur in the absence of any other GT1–7 cell-specific nuclear factors. Furthermore, purified GR can bind directly to nGRE-bound Oct-1 in vitro, also in the absence of any GT1–7 cell specific nuclear factors.

Oct-1 and GR have previously been shown to participate in both transcriptional activation (21–23, 33, 45–51) and repres-
Glucocorticoid activation of transcription from the MMTV LTR promoter requires the binding of GR and Oct-1 to separate sites (23). The simultaneous direct binding of GR and Oct-1 has been observed on the relevant sites within MMTV LTR DNA in vitro (55), but there is no evidence for direct interaction between these two DNA-bound factors. Glucocorticoid repression of histone H2b gene transcription is apparently brought about by the association of GR with Oct-1 in solution, which eliminates Oct-1 binding to the histone H2b promoter (29). It is important to note that the Oct-1 binding site in this case does not appear to be co-occupied by GR and Oct-1, as we have shown for the GnRH distal nGRE. The interaction between Oct-1 and GR in solution requires the receptor DNA-binding domain (29). The identification of GR domains involved in interactions with DNA-bound Oct-1 at the GnRH distal nGRE will be the subject of future studies.

How do we account for the clear distinctions between GR repression of transcription of histone H2b that involves GR-Oct-1 interactions in solution (29) versus repression of GnRH gene transcription where GR associates with DNA-bound Oct-1? As we have demonstrated in this report, Oct-1 binds with relatively low affinity to the GnRH distal nGRE, which was expected given the limited degree of homology between this nGRE and an Oct-1 consensus sequence (25). However, the relatively weak binding of Oct-1 to the distal nGRE appears to be an essential feature of the mechanism of glucocorticoid repression. When the distal nGRE is mutated to increase Oct-1 binding capacity, glucocorticoid repression in transfected GT1–7 cells is affected. Furthermore, GR does not associate as effectively in vitro with tightly bound Oct-1 at the Oct-1 consensus site nGRE. This was shown in examinations of GR-Oct-1 interactions on this consensus site nGRE with both purified GR and Oct-1 and GT1–7 cell nuclear extracts.

As discussed by others (for review, see Ref. 27), we hypothesize that DNA-bound Oct-1 may adopt different conformations depending upon the precise nature of its recognition sequence. In fact, using protease sensitivity of Oct-1-DNA complexes as an assay, we provide direct evidence for alternative Oct-1 conformations at the distal GnRH nGRE versus a consensus Oct-1 site. As diagrammed in Fig. 7A, the conformation adopted by Oct-1 upon its binding to the GnRH distal nGRE may allow efficient tethering of GR (or other factors) and lead to glucocorticoid-dependent repression of transcription. Altering the Oct-1 recognition sequence within the nGRE may still allow Oct-1 binding, but generate a different Oct-1 conformation that recruits a distinct set of co-factors to this element (Fig. 7B).

In some cases where POU domain transcription factors participate in steroid receptor-mediated transcriptional regulat-
tion, altering the POU domain protein target site has been found to influence hormonal responsiveness. Appropriate androgen receptor function within the Sex-limited protein (Slp) gene enhancer, appears to require Oct-1 binding to a nonconsensus site (56). Analogous to our results with the GnRH distal nGRE, increasing Oct-1 affinity within the Slp enhancer alters androgen responsiveness in transfected cells. As direct interactions between Oct-1 and the androgen receptor at the Slp enhancer has not been demonstrated, the mechanism responsible for altered hormone response is unknown. DNA sequences which flank those directly contacted by Oct-1, or other POU domain proteins also appear to influence the association of accessory factors. For example, sequences that flank an Oct-1 recognition site influence the ability of DNA-bound Oct-1 to discriminate between the VP16 protein of herpes simplex virus and its bovine homologue (36, 37). Furthermore, the POU domain transcription factor Pit-1 interacts with the estrogen receptor only at certain DNA sites and not others (57).

The consequences of altering the strength of Oct-1 interactions and its conformation at the distal nGRE are even more dramatic when TPA repression mediated by this sequence was examined. A fully functional GnRH promoter segment containing the mutant distal nGRE with the consensus Oct-1 site strongly repressed transcription in transfected GT1–7 cells upon TPA treatment. The mechanism of TPA repression of GnRH promoter activity remains controversial. Evidence that supports and refutes an involvement of AP-1 transcription factors in this repression has been presented (40, 58). The nTRE of the GnRH promoter, which involves AP-1 transcription factors in this repression, has not been demonstrated to be repressed by TPA in GT1–7 cells (8–10, 40). The nTRE of the GnRH promoter, which has been presented (40, 58). The nTRE of the GnRH promoter, which involves AP-1 transcription factors in this repression, has not been demonstrated to be repressed by TPA in GT1–7 cells (8–10, 40). The nTRE of the GnRH promoter, which involves AP-1 transcription factors in this repression, has not been demonstrated to be repressed by TPA in GT1–7 cells (8–10, 40).
