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# A steroid-inducible promoter for the controlled overexpression of cloned genes in eukaryotic cells

**(glucocorticoid response elements/transcriptional activation/synergism/adenovirus 2 major late promoter)**

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ABSTRACT **Previous studies have shown that members of the steroid receptor family of transcriptional regulators can function synergistically when bound to multiple arrays of specific DNA binding sites known as hormone response elements, usually located upstream of target genes. We have constructed a mammalian expression vector containing a synthetic promoter composed of five high-affinity glucocorticoid response elements (termed GRE5) placed upstream of the adenovirus 2 major late promoter "TATA" region. In transiently transfected HeLa cells in the presence of dexamethasone, the GRE5 promoter was at least 50-fold more efficient than the mouse mammary tumor virus long terminal repeat in expressing bacterial chloramphenicol acetyltransferase activity. When the GRE5 vector was introduced stably into the HeLa cell genome, chloramphenicol acetyltransferase activity was induced from 10- to >50-fold by dexamethasone in six of eight responsive clones. The levels of both basal and induced expression varied from one clone to the next, probably due to an effect of chromosomal location on promoter activity. When propagated stably in HeLa cells in an Epstein–Barr virus episomal vector, the GRE5 promoter was >50-fold inducible and its activity was strictly dependent on the presence of dexamethasone. We also show that the GRE5 promoter stably propagated in HeLa cells is inducible by progesterone in the presence of a transiently transfected progesterone receptor expression vector. The GRE5 promoter should be widely applicable for the strictly controlled high-level expression of target genes in eukaryotic cells that contain either the glucocorticoid or progesterone receptors.**

Transcription of eukaryotic class II genes is regulated by a complex array of trans-acting transcription factors that bind to specific DNA sequences in the promoter of a target gene. On most promoters transcriptional preinitiation complexes are assembled at the "TATA" box that is located 25–30 bp 5′ to the site of initiation (1). Preinitiation complex assembly is regulated by factors that bind to DNA sequences known as enhancers often situated at various distances upstream of the TATA box/initiation site (2–4). Enhancers can be located immediately adjacent to the site of transcriptional initiation or several kilobases distant from it (5, 6). The activity of enhancer factors can be directly responsive to specific intercellular signals or indirectly via intracellular transduction pathways (7–15).

The nuclear receptors represent a family of transcriptional enhancer factors that act by binding to specific DNA sequences found in target promoters known as response elements (REs) (7–10). Specific members of the nuclear receptor family represent the primary intracellular targets for small lipid-soluble ligands (such as steroid and thyroid hormones, retinoids, and vitamin $D_3$) and as such act as ligand-inducible transcription factors. Sequence comparisons (16) and structure–function analyses (17–20) have shown that the receptors are composed of a series of conserved domains. The most highly conserved domain is the DNA binding domain located in region C (16, 20, 21) containing a 66- to 68-aa core composed of two zinc fingers (22–24), which is essential for recognition of REs. Three amino acids adjacent to the N-terminal zinc finger of the DNA binding domain, known as the P-box, are critical for DNA sequence recognition (25–27). A subfamily composed of the glucocorticoid, mineralocorticoid, progesterone, and androgen receptors contain Gly, Ser, and Val at discriminatory positions of the P-box and recognize AGAACA half-sites arranged in a palindrome with a 3-bp spacer region (25–27). The ligand-binding domain, located C-terminal to the DNA binding domain in region E, is less well conserved among the receptors and contains a ligand-inducible transcriptional-activation function (7–10, 17, 18). Transcriptional-activating domains have also been identified in the poorly conserved N-terminal A/B regions of the glucocorticoid and estrogen receptors (17, 18, 28).

REs are often found in multiple arrays, usually located upstream of the site of transcriptional initiation (6, 29, 30) and many studies have shown that REs can act synergistically to regulate transcription. Functional analysis of two glucocorticoid response elements (GREs) located far upstream of the rat tyrosine aminotransferase (TAT) gene has shown that they combine synergistically to mediate transcriptional activation by the glucocorticoid receptor (GR) (6). The two nonconsensus estrogen response elements (EREs) of the *Xenopus* vitellogenin B1 gene are virtually inactive in isolation but together mediate estrogen-dependent transcriptional activation in transiently transfected cells (29). The degree of synergism between paired EREs or GREs is dependent on their sequence, the spacing between them, and their distance from the TATA box of the promoter (31, 32, 49). Several studies have also shown that nuclear receptors and other classes of transcriptional regulators can combine to activate transcription synergistically (28, 32).

Promoters activated by specific inducible nuclear receptors are well suited for eukaryotic expression vectors since expression of genes can be regulated simply by controlling the concentration of ligand present in the growth medium (18, 33–35). Glucocorticoid-inducible promoters such as that of the long terminal repeat of the mouse mammary tumor virus (MMTV) have been widely used in this regard because the GR is expressed in a wide variety of cell types. The MMTV GRE is composed largely of a series of half-sites (36, 37). Interestingly, while the MMTV promoter can be induced by

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Abbreviations: Ad2MLP, adenovirus 2 major late promoter; GR, glucocorticoid receptor; GRE, glucocorticoid response element; MMTV, mouse mammary tumor virus; CAT, chloramphenicol acetyltransferase; RE, response element; TAT, tyrosine aminotransferase; ERE, estrogen RE; SV40, simian virus 40; EBV, Epstein–Barr virus; tk, thymidine kinase.

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ligand-bound receptors for both glucocorticoids and progesterone when introduced into cells by transient transfection, it is selectively responsive to glucocorticoids when propagated on an episomal vector (38). It is proposed that the nucleosome structure of the episomal DNA selectively inhibits access of the progesterone receptor to the RE. It would therefore be useful to develop a tightly controlled highly inducible expression system that responds to a wide variety of steroid hormones.

Here, we have constructed a synthetic minimal promoter composed of five high-affinity binding sites for the GR (GRE5) placed upstream of the adenovirus 2 major late promoter (Ad2MLP) TATA box/initiation site. At least 50-fold more chloramphenicol acetyltransferase (CAT) activity is induced by dexamethasone from GRE5/CAT than from MMTV/CAT in transiently transfected HeLa cells, indicating that the GRE5 promoter can direct high levels of expression of cloned genes. Moreover, the GRE5 promoter is highly inducible when propagated in stably transfected cells either when integrated in the chromosome or carried in an Epstein–Barr virus (EBV) episomal vector. Finally, we also show that the promoter is responsive to progesterone in stably transfected cells.

## MATERIALS AND METHODS

**Recombinant Plasmids.** A 35-bp oligonucleotide containing the rat TAT GRE flanked by *Bam*HI and *Bgl* II ends was concatemerized in the presence of *Bam*HI, *Bgl* II, and T4 DNA ligase, and bands corresponding to dimers and pentamers were purified from a 5% polyacrylamide gel for insertion into the *Bgl* II site (position −65) of pAL10 (31). Recombinants containing a single GRE or head-to-tail direct repeats of GREs conserving the *Bgl* II site at position −65 were isolated to create GRE/pAL10, GRE2/pAL10, and GRE5/pAL10. GRE5/pAL10 was then modified to remove unnecessary sequence and restriction sites, and a polylinker containing sites for *Eco*RI, *Kpn* I, *Sma* I, *Sac* I, *Xho* I, *Hin*dIII, and *Bgl* II was inserted in either orientation to create pGRE5-1 and pGRE5-2 (see Fig. 2). The rabbit β-globin sequence of pGRE5 runs from the naturally occurring *Bam*HI site to the *Eco*RI site and contains the second globin intron (39). To create pGRE5/CAT recombinants, the *Bgl* II–*Sma* I fragment from pBLCAT8+ (40) containing the CAT gene was excised and inserted in the pGRE5-1 or pGRE5-2 polylinker digested with *Sma* I and *Bgl* II in the sense and antisense orientations, respectively. The pGRE1tkCAT and pGRE2tkCAT plasmids were created by inserting one or two perfectly palindromic GREs upstream of the herpes simplex virus thymidine kinase (tk) promoter in pBLCAT8+. To create p220.2-GRE5/CAT recombinants, the *Xba* I–*Sal* I fragment (see Fig. 2) from pGRE5/CAT was inserted in the EBV episomal vector p220.2 (41, 42) digested with *Xba* I and *Xho* I.

**Cell Transfections and Tissue Culture.** For transient transfections, 5 μg of CAT expression vector or 2 μg of pAL10 derivatives, 1 μg of pG1B internal control plasmid (18) for quantitative S1 nuclease analysis or 3 μg of β-galactosidase expression vector pCH110 for CAT assays, and 11–13 μg of BlueScribe (Stratagene) carrier DNA were transfected onto 9-cm plates of HeLa cells in Dulbecco's modified Eagle's medium containing 5% (vol/vol) fetal bovine serum at ≈50% confluency by using the calcium phosphate coprecipitation technique (43). Dexamethasone (25 nM) was added immediately after transfection and 24 hr after transfection. Cells were harvested 44–48 hr after transfection. For stable transfections, HeLa cells were transfected as above with 15 μg of pGRE5/CAT, 1 μg of neomycin-resistance gene expression vector pRc-RSV (Invitrogen), and 4 μg of BlueScribe carrier DNA. G418 (1 mg/ml) was added starting 48 hr after transfection and G418-resistant colonies were picked 3–4 weeks later. To test for dexamethasone-inducible CAT activity, cells from G418-resistant clones were split into duplicate 9-cm plates and treated as indicated with 25 nM dexamethasone for 48 hr prior to harvesting. Alternatively, HeLa cells were transfected with 15 μg of the EBV episomal vector p220.2-GRE5/CAT and 5 μg of BlueScribe carrier DNA. Hygromycin (250 μg/ml) was added 48 hr later for selection. Induction of CAT activity of the G418-resistant HeLa cell line 19-11 by progesterone was tested by transfecting cells with 1 μg of human progesterone expression vector hPRO (44), 3 μg of pCH110, and 16 μg of BlueScribe carrier. Progesterone (100 nM) was added as indicated.

**CAT Assays and Quantitative S1 Nuclease Analysis.** Cells were harvested in 250 μl of 0.25 M Tris·HCl (pH 7.5) (45). Extract concentrations varied by ±20%. CAT assays were performed as described (28). Quantities of extracts derived from transiently transfected cells were normalized for β-galactosidase activity (28). For extracts derived from stably transfected cells, volumes were used as indicated in the figures. Quantitative S1 nuclease analysis was performed as described (31).

## RESULTS

**Dexamethasone-Dependent Transcriptional Activation of Minimal Promoters Composed of Multiple GREs and the Ad2MLP TATA Region.** Previous studies with estrogen-inducible minimal promoters have shown that a TATA region and one or more EREs were sufficient for a transcriptional response to estrogen in the presence of ligand-bound estrogen receptor (31). In HeLa cells, promoters based on the Ad2MLP TATA region (positions −34 to +33) have undetectable basal activity in the absence of inducer (31) and are, therefore, potentially useful in inducible expression vectors. Here, we have constructed a series of glucocorticoid-responsive minimal promoters composed of the Ad2MLP TATA region and one or more GREs from the rat TAT gene (6) placed upstream of a rabbit β-globin reporter gene in the plasmid pAL10 (31). To test for dexamethasone-inducible transcription, recombinants were transiently transfected into HeLa cells along with pG1B, which constitutively expresses the rabbit β-globin gene (31) and acts as an internal control, and transcription was monitored by quantitative S1 nuclease analysis. No transcription was observed from the Ad2MLP recombinant lacking GRE sequences either in the absence or presence of dexamethasone or from any of the GRE-containing recombinants tested in the absence of hormone (Fig. 1, lanes 1–3, 5, 7, and 9). The recombinant GRE1

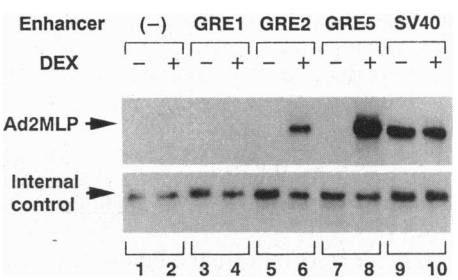


FIG. 1. Accurate dexamethasone-dependent transcriptional initiation by minimal promoters composed of one or more GREs and the Ad2MLP TATA region. Quantitative S1 nuclease analysis of RNA isolated from HeLa cells transfected with 2 μg of pAL10 (lanes 1 and 2), GRE1/pAL10 (lanes 3 and 4), GRE2/pAL10 (lanes 5 and 6), GRE5/pAL10 (lanes 7 and 8), and SV40/pAL10 (lanes 9 and 10) along with pG1B internal control (1 μg). Ad2MLP refers to transcription of pAL10 derivatives and internal control refers to pG1B transcription. Dexamethasone (DEX, 25 nM) was added as indicated.

containing a single GRE upstream of the Ad2MLP TATA region did not produce detectable transcription in the presence of dexamethasone (Fig. 1, lane 4). In contrast, dexamethasone-inducible transcription was observed from the Ad2MLP in recombinants containing two or more GREs (Fig. 1, lanes 5–10). Promoters containing five GREs were 8-fold more inducible than those containing only two elements (Fig. 1, compare lanes 6 and 8), consistent with previous observations that multiple REs can act synergistically to mediate ligand-dependent transcriptional activation by nuclear receptors (6, 29–31). The promoter of the GRE5 recombinant was also >3-fold more efficient in the presence of dexamethasone than the constitutively active promoter of an Ad2MLP recombinant containing an equivalently positioned simian virus 40 (SV40) enhancer (Fig. 1, lanes 7–10).

The above results show that introduction of five GREs upstream of the Ad2MLP TATA region creates a promoter that has very low basal activity and is highly inducible in HeLa cells by the endogenous levels of ligand-activated GR. We have therefore constructed two eukaryotic expression vectors based on this promoter, pGRE5-1 and pGRE5-2, containing polylinker sequences in opposite orientations (Fig. 2*A*). The polylinker of each vector was inserted between a fragment of rabbit β-globin gene sequence that contains an intron and a sequence from the SV40 genome containing a poly(A) addition signal. To test the capacity of these vectors to induce expression of high levels of protein, we inserted the bacterial CAT gene into pGRE5-2 and introduced the recombinant plasmid into HeLa cells by transient transfection. Inducible expression of CAT activity by pGRE5-2/CAT was compared to other

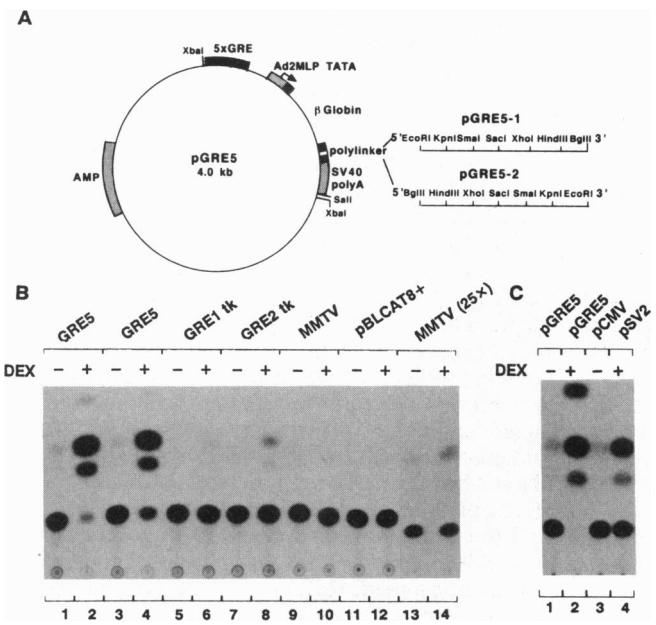


FIG. 2. Dexamethasone-dependent activity of pGRE5/CAT expression vector. (*A*) Schematic representation of the pGRE5 expression vectors. All restriction sites indicated (except for *Xba* I) are unique. (*B*) CAT activity of extracts of HeLa cells transiently transfected with GRE-containing expression vectors. HeLa cells were transfected with 5 μg of CAT expression vector pGRE5-2/CAT (lanes 1–4; two isolates), GRE1tkCAT and GRE2tkCAT (lanes 5 and 6 and lanes 7 and 8, respectively), MMTV/CAT (lanes 9, 10, 13, and 14), and pBLCAT8+ (lanes 11 and 12), along with 3 μg of pCH110 β-galactosidase expression vector as an internal control. All CAT assays contained extract corresponding to a constant amount of β-galactosidase activity except for lanes 13 and 14 where 25-fold more extract was used. (*C*) CAT activity of HeLa cell extracts transfected as described in *B* with pGRE5-2/CAT (lanes 1 and 2), pCMVCAT (lane 3), and pSV2CAT (lane 4). Dexamethasone (DEX, 25 nM) was added as indicated.

dexamethasone-inducible CAT expression vectors containing synthetic promoters and to MMTV/CAT, by using quantities of extract normalized to the activity of the β-galactosidase internal control (Fig. 2*B*). No induction of CAT activity by dexamethasone was observed in extracts of cells transfected with pBLCAT8+, which contains the herpes simplex virus tk promoter but no GREs (Fig. 2*B*, lanes 11 and 12). Insertion of one or two GREs upstream of the tk promoter gave rise to dexamethasone-inducible CAT activity (Fig. 2*B*, lanes 5–8). The induced activity of the GRE2tk promoter was 3-fold higher than that observed with the GRE1tk promoter. Strikingly, transfection of either of two independent preparations of GRE5-2/CAT into HeLa cells in the presence of dexamethasone gave rise to >10- and 30-fold more CAT activity than observed with GRE2tkCAT and GRE1tkCAT, respectively (Fig. 2*B*, lanes 2 and 4). No induction of activity was observed when the CAT gene was inserted in pGRE5-1 in the antisense orientation (data not shown). Virtually no dexamethasone-inducible CAT activity was observed in extracts of cells transfected with MMTV/CAT (Fig. 2*B*, lanes 9 and 10). However, activity was clearly observed if 25-fold more extract was used (Fig. 2*B*, lanes 13 and 14). Under these conditions, the activity of pGRE5-2/CAT in the presence of dexamethasone was at least 50-fold higher than that of the MMTV/CAT. Moreover, whereas the inducible activity of the MMTV promoter was enhanced >5-fold by cotransfection of a GR expression vector, the activity of the GRE5 promoter was not significantly affected (data not shown), indicating that the GRE5 promoter is efficiently inducible by endogenous levels of GR in HeLa cells. We have also compared the activity in HeLa cells of pGRE5-2/CAT to the constitutively active SV40 and cytomegalovirus (CMV) enhancer-based promoters of pSV2-CAT and CMV-CAT and observed that, in the presence of dexamethasone, the pGRE5 promoter was much more efficient than either the pSV2 or pCMV promoters (Fig. 2*C*). Under these conditions the CMV promoter was as efficient as the induced MMTV promoter (data not shown). These results demonstrate that high levels of induction by dexamethasone can be obtained when using a promoter composed solely of multiple GREs and a TATA region.

**The pGRE5 Promoter Is Functional in Stably Transfected Cells.** Since the pGRE5 vectors contain a synthetic promoter, it is important to verify that they are functional when stably propagated in cells. The pGRE5/CAT plasmid was, therefore, introduced into HeLa cells by cotransfection with the pRcRSV vector, which expresses the neomycin-resistance gene. Of 22 neomycin-resistant clones, 8 expressed CAT activity. These 8 clones displayed variable levels of background activity and dexamethasone inducibility (Fig. 3*A*). The quantities of extract tested correspond to 0.4% (1 μl) and 4% (10 μl) of the total extract from 9-cm plates of cells harvested at 70% confluency (≈2.5 μg and ≈25 μg of protein, respectively). Six clones (17-4, 18-5, 18-7, 18-8, 19-11, and 20-5) showed a combination of little background activity and high levels of induction in the presence of dexamethasone (Fig. 3*A*, lanes 5–12, 21–24, and 29–32). Indeed, no CAT activity was visible with extracts of the clone 19-11 not treated with dexamethasone under these conditions (Fig. 3, lanes 21 and 23). We have also found (Fig. 3*B*) that the GRE5 promoter was strongly inducible by dexamethasone when propagated in the EBV episomal vector p220.2 (41, 42). No CAT activity was observed in extracts of cells transfected with p220.2-GRE5-1/CAT, which carries the CAT gene in the antisense orientation (Fig. 3*B*, lanes 1 and 2). In contrast, very low background activity and high dexamethasone inducibility were observed in extracts of cells transfected with p220.2-GRE5-2/CAT, which expresses the CAT gene (Fig. 3*B*, lanes 3–10). These results are typical of other clones tested (data not shown). The levels of activity observed in

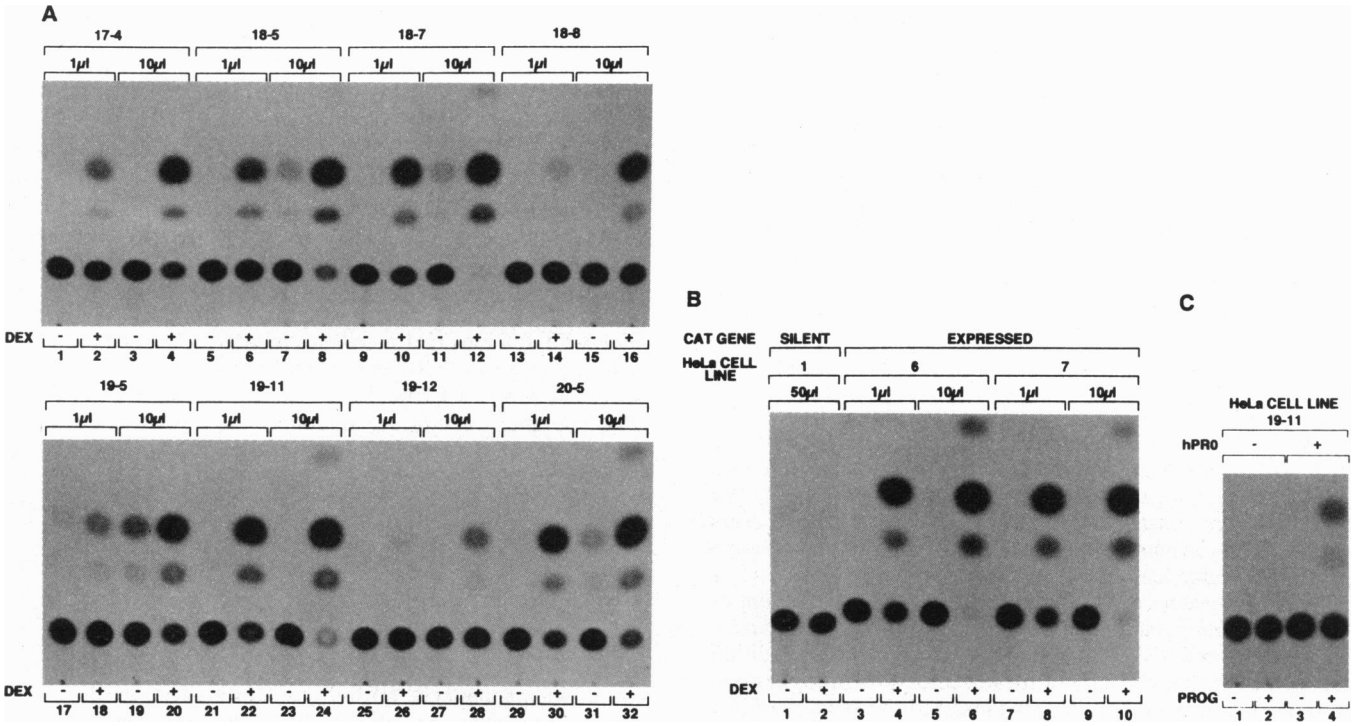


FIG. 3. GRE5 promoter functions in stably transfected cells. (*A*) HeLa cells were stably transfected with pGRE5/CAT and pRcRSV, which expresses the neomycin-resistance gene. CAT assays of G418-resistant clones expressing dexamethasone-dependent CAT activity are shown. Volumes of extract (1 or 10 μl) used are indicated. Note that extract concentrations varied by ±20%. Dexamethasone (DEX, 25 nM) was added as indicated. (*B*) Dexamethasone-dependent CAT activity of two independent clones of hygromycin-resistant HeLa cells carrying the GRE5/CAT expression cassette in the EBV expression vector p220.2 (42). Volumes of extract and dexamethasone addition are as indicated. Lanes: 1 and 2, the CAT gene was inserted in pGRE5-1 in the antisense orientation; 2–10, the CAT gene was inserted in pGRE5-2 in the sense orientation. (*C*) Induction of the GRE5 promoter by progesterone. The HeLa cell line 19-11 which contains GRE5/CAT (see *A*, lanes 21–24) was treated with 100 nM progesterone (lanes 2 and 4) for 48 hr prior to harvesting. Extracts used in lanes 3 and 4 were made from cells transfected with 1 μg of the human progesterone expression vector hPRO (43) whereas those used in lanes 1 and 2 were made from untransfected cells.

clones 6 and 7 were comparable to that observed with G418-resistant HeLa clone 19-11 (Fig. 3*A*, lanes 21–24).

**Induction of the GRE5 Vector by Progesterone in Stably Transfected Cells.** The TAT GRE can confer responsiveness to both glucocorticoids and progesterone in transiently transfected cells when placed upstream of an unresponsive promoter (46), and the progesterone receptor binds to the element *in vitro* (46). We used the HeLa cell line 19-11 (Fig. 3*A*) to determine whether the GRE5 promoter was progesterone-responsive in stably transfected cells. No CAT activity was observed in 19-11 cells treated or not with progesterone (Fig. 3*C*, lanes 1 and 2). However, upon transient transfection with the human progesterone receptor expression vector hPRO, progesterone-responsive CAT activity was observed (Fig. 3*C*, lanes 3 and 4). These results demonstrate that the GRE5 promoter is progesterone-responsive in the presence of the progesterone receptor when stably integrated in the HeLa cell genome.

## DISCUSSION

Previous experiments (31) with the estrogen receptor have demonstrated that a minimal promoter composed of one or more EREs and a TATA region is estrogen-inducible in transiently transfected cells. Several studies have shown that multiple hormone REs can act synergistically to mediate a transcriptional response to steroid hormones (6, 29–31). We have constructed here a dexamethasone-inducible minimal promoter composed of five high-affinity rat TAT GREs placed upstream of the Ad2MLP TATA region. Experiments in transiently transfected HeLa cells indicated that the five REs act synergistically. The GRE5 promoter is highly inducible by dexamethasone whereas analogous promoters containing one or two REs are not or are only weakly responsive to hormone (Figs. 1 and 2). The GRE5 promoter is at least 50-fold more inducible by dexamethasone than the MMTV promoter in transiently transfected HeLa cells (Fig. 2), and at least 3-fold stronger than the constitutive activity of an analogous promoter containing an equivalently positioned SV40 enhancer (Fig. 1). The GRE5 promoter is also 23-fold more inducible than the MMTV long terminal repeat in NIH 3T3 cells, which express low levels of GR (data not shown). Studies with model promoters have shown that the presence of the binding site for a wide variety of upstream or enhancer factors can significantly augment the hormonal response of promoters containing a single ERE or GRE (31, 32). Our experiments have shown that introduction of a binding site for the Ad2MLP upstream element factor between the multiple GREs and the TATA box of the GRE5 promoter has only a slight (≈1.3-fold) effect on the response of the GRE5 promoter to dexamethasone in HeLa cells (data not shown).

The GRE5 promoter is strongly inducible when stably integrated in the HeLa genome or propagated in an EBV episomal vector (Fig. 3), indicating that under these conditions GREs and a TATA region are sufficient for an efficient response to hormone. Variable levels of dexamethasone-independent CAT activity are observed in HeLa cell clones carrying the GRE5/CAT recombinant incorporated into the genome (Fig. 3). The degree of hormone-independent activity is probably dependent on the proximity of the promoter to constitutively active enhancers that activate transcription from the Ad2MLP or its insertion downstream from dexamethasone-independent promoters. Based on these results, it is difficult to determine the basal activity of the GRE5 promoter in a transcriptionally silent chromosomal background. To control the transcriptional environment as much as possible the GRE5 promoter was inserted in the EBV

episomal vector p220.2 (41, 42), which replicates autonomously in human cells. Under these conditions, the promoter is highly inducible, and very low levels of CAT activity are detected only with large amounts of extracts of cells cultured in the absence of dexamethasone (Fig. 3 and data not shown). GRE5 promoter activity in the EBV vector resembles closely that of the HeLa cell clone 19-11 (Fig. 3), created by the cotransfection of pGRE5/CAT and a vector expressing neomycin resistance, suggesting that the basal activity of GRE5 in clone 19-11 is not influenced by nearby promoters or enhancer elements. Thus these results indicate that, when propagated stably in a controlled transcriptional environment, GRE5 promoter activity is highly inducible and is strictly dependent on the presence of inducer. We also note that the high levels of induction observed raise the possibility that the GRE5 promoter/expression system can be used as a straightforward method for the controlled overproduction of proteins for purification and physical characterization.

Recent studies with the steroid-inducible MMTV promoter have suggested that chromatin structure may control the access of nuclear receptors to REs (38). While the MMTV promoter is responsive to progesterone and glucocorticoids in transiently transfected cells (47), it is inducible by glucocorticoids but not progesterone when stably propagated in an episomal vector (38, 48). Our results show that the GRE5 promoter is inducible by progesterone in the HeLa 19-11 clone transiently transfected with the human progesterone receptor expression vector hPRO (Fig. 3*C*). These results indicate that the GRE5 promoter can be activated by progesterone and glucocorticoids thus extending the range of cell types where the expression system is functional. It is also possible that ligand-activated receptors for mineralocorticoids and androgens can activate the GRE5 promoter given that these receptors recognize GREs.

In summary, we have developed a fully defined synthetic steroid-inducible promoter–expression system that should be widely applicable for the tightly controlled expression of cloned genes in a wide variety of cell types expressing GRs or progesterone receptors and possibly in cells expressing androgen or mineralocorticoid receptors.

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