© 1990 Oxford University Press

Glucocorticoid regulation of a phenobarbital-inducible cytochrome P-450 gene: the presence of a functional glucocorticoid response element in the 5′-flanking region of the CYP2B2 gene

Anil K.Jaiswal, Tapio Haaparanta[+], Phuong-Van Luc, Josephine Schembri and Milton Adesnik*
Department of Cell Biology and The Kaplan Cancer Center, New York University School of Medicine, New York, NY 10016, USA

Received December 13, 1989; Revised and Accepted May 23, 1990

ABSTRACT

The rat cytochrome P450 CYP2B2 gene encodes one of the two major phenobarbital-inducible forms of hepatic microsomal cytochrome P-450. The sequence of a 1.4 Kb DNA segment from the 5′ flanking region of this region [Jaiswal, A., Rivkin, E. and Adesnik, M. Nucl. Acids. Res. *15*: 6755 (1987)] reveals the presence of a pentadecameric oligonucleotide sequence, located approximately 1.3 Kb upstream of the transcription initiation site, which is highly similar to the sequences of glucocorticoid response elements (GREs) that mediate the hormone-dependent transcriptional activation of many other genes. The putative GRE in the CYP2B2 gene 5′ flanking region is shown to be functional by demonstrating that segments of DNA that contain it, including one that is only 25bp long, are capable of conferring dexamethasone inducibility on a chloramphenicol acetyltransfer-ase gene whose transcription is driven by the Herpes virus thymidine kinase gene promoter. Moreover, binding of a protein contained in a rat liver nuclear extract to a 25 bp synthetic DNA segment that contains the putative GRE was demonstrated in a gel mobility shift assay. This binding was specifically competed away by a DNA segment that contains the murine mammary tumor virus long terminal repeat which encompasses several well characterized GRE elements. The implications of these findings for the in vivo regulation of the P450IIB2 gene by glucocorticoids are discussed.

INTRODUCTION

The hepatic microsomal cytochromes P-450 constitute a superfamily of monooxygenases that function in the metabolism of many endogenous substrates as well as an extraordinary variety of foreign compounds (1,2). More than twenty distinct P-450s have been identified in rat-liver (3) and individual forms of the enzyme exhibit a variety of regulatory features including developmental regulation, modulation by steroid and polypeptide hormones and induction by specific drugs or xenobiotics (for a review see reference 1).

Initial studies on P-450 gene regulation focused on the induction phenomenon and identified three classes of P-450: those induced by phenobarbital (PB), by polycyclic hydrocarbons and by synthetic steroids [pregnenolone-16-alpha-carbonitrile (PCN) and dexamethasone], respectively (1). These do not truly represent distinct classes of the monooxygenases since certain polybrominated biphenyls markedly induce both the major phenobarbital and polycyclic hydrocarbon-inducible P-450s (4) and phenobarbital also induces a major PCN-inducible P-450 (5). It is also noteworthy that a gene product has been identified that is closely related (90% sequence similarity) to the major PCN-induced P-450 but is inducible only by phenobarbital and not by PCN or dexamethasone (5).

The two major phenobarbital-inducible forms of rat hepatic cytochrome P-450, P450IIB1 and IIB2, the products of the CYP2B1 and CYP2B2 genes, are 97% similar in their protein sequences (6, 7) and show similar, but not identical, regulatory properties (8, 9). Nucleic acid hybridization studies using gene-specific oligonucleotide probes demonstrated that treatment of rats with phenobarbital leads to a several hundred fold increase in the levels of the mRNAs encoding the two proteins (10) and run-on transcription studies, which do not distinguish the two gene transcripts, demonstrate that this induction results primarily, if not exclusively, from transcriptional activation of the corresponding genes (11, 12, 13). Some controversy exists as to whether these two phenobarbital-inducible P-450 genes are also inducible by dexamethasone. One rather comprehensive study (14) found that after dexamethasone injection, P450IIB1+IIB2 mRNA increased twelve fold to 50% of the phenobarbital-inducible level but this was not accompanied by any increase in transcription of the corresponding genes. Moreover, the glucocorticoid treatment actually led to a 50% decrease in the levels of the encoded proteins as measured by a radial immunodiffusion assay which doesn't distinguish the two

* To whom correspondence should be addressed

[+] Present address: La Jolla Cancer Research Foundation, 1091 North Torrey Pines Road, La Jolla, CA 92037, USA

closely related P450s. On the other hand, immunoblotting studies using a highly specific polyclonal antibody (8) demonstrated that a similar treatment with dexamethasone leads to a 20–100 fold induction of these proteins to levels which are 10–20% of those attained after PB treatment.

We have recently reported the sequence of a 1.4Kb segment of DNA from the 5′-flanking region of the rat CYP2B2 gene (15) and recognized the presence of a pentadecanucleotide sequence approximately 1.3Kb upstream of the transcription initiation site which is highly similar (Fig. 1) to glucocorticoid response elements (GREs), such as those that are located approximately 2.5Kb upstream of the rat tyrosine-aminotransferase gene (16, 17), and function as hormone-dependent transcriptional enhancers. Moreover, this putative GRE in the CYP2B2 gene is flanked by a CAAT box, (Figure 1) a cis-acting transcriptional regulatory element which has recently been shown to act synergistically with a GRE in the glucocorticoid activation of transcription (18). In this report, we demonstrate the functionality of this GRE by showing that DNA segments from the CYP2B2 gene 5′ flanking region that contain it are capable of conferring dexamethasone inducibility on a chloramphenicol acetyltransferase (CAT) gene whose transcription is driven by the Herpes virus thymidine kinase gene promoter. Indeed, multimers of a 25 base pair segment containing the putative GRE are sufficient to confer hormone inducibility.

EXPERIMENTAL PROCEDURES

Reagents

The rat liver Charon 4A genomic library was the gift of Dr. Tom Sargent (NIH), the plasmid vectors pBLCAT2 and pBLCAT3 (19) a gift from Dr.B. Luckow (Heidelberg), the plasmid pSV2neo (20) a gift of Dr. Paul Berg (Stanford University) and the plasmid pSVOCAT with a SmaI cloning site from Dr. C. Gorman (N.I.H). Plasmids containing the human β actin gene and the Murine Mammary Tumor Virus long terminal repeat (MMTV LTR) were obtained from Dr. N. Batula (N.I.H) and Dr. K. Paturu (N.I.H), respectively. Restriction enzymes and polynucleotide kinase were obtained from Boehringer Manheim Biochemicals, [^{32}P]-alpha-UTP and [^{32}P]-gamma-ATP, and [^{14}C)chloramphenicol from New England Nuclear Corp. and G418 (Geneticin) from Gibco.

Recombinant plasmids

A genomic clone containing the 5′ portion of the CYP2B2 gene and approximately 5 Kb of 5′ flanking region was isolated from a lambda Charon 4A rat liver genomic library by the in vivo recombination screening procedure of Seed (21) using as probe a fragment from a previously described CYP2B2 genomic clone (12) that encompassed exon 2 and short flanking intronic regions.

```
               -1376
P450e-GRE  .....CCACCCCAATAATATCAGTTAGGGTACAAAGTGTTCAAAC.....
GRE Consensus                             GGTACANNNTGTTCT
```

Figure 1: Comparison of the Sequence of a Putative GRE in the CYP2B2 Gene to GRE Concensus Sequence. The sequence of a segment from the CYP2B2 gene 5′ flanking region extending downstream from residue −1376 is shown with the putative GRE and a possible CAAT box in bold letters. The GRE concensus sequence shown is from Jantzen et al. (16). The arrows indicate the two halves of the incomplete palindromic sequence characteristic of functional GREs.

A 1.4 Kb 5′ flanking segment extending from a HindIII site at −1406 to the NcoI site at +22, one base pair upstream of the translation initiation codon, which had been rendered blunt at the NcoI site by treatment with mung bean nuclease, was cloned by a series of steps between the EcoRl and HincII sites of pUC18 so as to place the HindIII site from the vector polylinker region downstream of the insert. The 1.4 Kb HindIII fragment in this plasmid was then cloned into the HindIII site of a pUC18-CAT plasmid that had been constructed by recloning of a SmaI (5′)-BamH1 (3′) fragment, derived from a modified pSVOCAT (22) plasmid and containing the coding region of the CAT gene as well as the small t intron and polyadenylation signals of SV40 inserted between the HincII and BamH1 sites of pUC18. The pSVOCAT plasmid used as a source of the CAT gene contained a SmaI site in place of the HindIII site just upstream of the CAT gene. The final product, pUC-1.4-CAT, was nearly identical (except for a few restriction sites) to one that would have been obtained by cloning the 1.4 Kb CYP2B2 promoter fragment into the vector pBLCAT3. The latter plasmid was therefore, used as a control, promoterless CAT plasmid. The sequence of the DNA segment from the 5′ flanking region of the P450e gene that was incorporated into pUC-1.4-CAT has been previously reported (15). A 1031 bp HindIII-XbaI fragment, (−1406 to −375), a 420 bp HindIII-NcoI fragment (−1406 to −987) and an Hph1-Xba fragment (−1255 to −375) from the CYP2B2 gene 5′-flanking region were cloned in the 5′→3′ orientation just upstream of the Herpes virus thymidine kinase gene promoter segment in the plasmid vector pBLCAT2 (19). To clone the HindIII-NcoI fragment, pUC-1.4 CAT was cleaved with NcoI, and treated with Klenow polymerase to make the NcoI site blunt, before addition of HindIII linkers, digestion with HindIII, isolation of the appropriate fragment by agarose gel electrophoresis and ligation to HindIII cleaved, dephosphorylated pBLCAT2. To clone the Hph1-Xba fragment, which lacked the 149 bp from the extreme 5′ end of the other two fragments, and hence did not include the putative GRE, pUC-1.4 CAT was cleaved with Hph1, made blunt by sequential treatment with mung bean nuclease and the Klenow fragment of DNA polymerase ligated to HindIII linkers, and then cleaved with HindIII and XbaI to yield the required Hphl-XbaI fragment (containing a HindIII linker at the blunted Hph1 site). This was purified by polyacrylamide gel electrophoresis and cloned between the HindIII and XbaI sites of pBLCAT2.

The plasmid pBLCAT2-P450e(GRE^{n}) contained 9 or 10 tandemly linked copies of the putative GRE of the CYP2B2 gene inserted upstream of the basal TK promoter. This plasmid was constructed by ligating a synthetic double stranded oligonucleotide with the sequence given below that contained the GRE and cohesive BamH1 sites at its termini to the vector pBLCAT2 that was cleaved with Bg1II and dephosphorylated.

```
5′ GATCCGTTAG GGTACAAAGTGTTCA AACAG 3′
      3′ GCAATC CCATGTTTCACAAGT TTGTCCTAG 5′
```

In the above sequence the putative GRE is boxed. The number of copies of the oligomer inserted into the recombinant plasmid obtained from the ligation was estimated from the length of the fragment released by digestion with EcoR1 and HindIII, enzymes for which there are cleavage sites flanking the Bg1II sites in the pBLCAT2 vector. The individual strands of the oligomer were

synthesized on an Applied Biosystems 380A DNA synthesizer and purified by polyacrylamide gel electrophoresis. Equal amounts of the two strands of the oligomer were annealed in a buffer containing 40 mM Tris pH 7.5, 50 mM NaCl and 20 mM $MgCl_2$ by incubation for 10 minutes at 70°C and cooling to room temperature. The annealed oligomer was phosphorylated with polynucleotide kinase before ligation to the cleaved dephosphorylated vector.

DNA transfections and CAT assays

Rat Reuber hepatoma H4II cells were obtained from Dr. Mary Weiss (Institut Pasteur, Paris) and maintained in F12K medium containing 10% fetal calf serum. The cells were transfected (23) with a 40:1 ratio of each recombinant plasmid to pSV2neo (20) and permanent transformants were selected in the presence of G418 (400 μg/ml). The pooled resistant colonies were expanded in culture and analyzed for CAT activity as described (23) with autoradiograms of thin layer chromatography (TLC) plates exposed for the times indicated. For dexamethasone induction (24 hours at 0.1 μM) and G418 selection experiments, the culture medium (500 ml) containing fetal calf serum was added to 10 gm of activated charcoal, shaken overnight at 4°C, centrifuged, filtered and sterilized by filtration through a Nalgene filter.

Nuclear run-on transcription

This was carried out by a dot-blot procedure as previously described (23) using 2.0 μg of the CAT insert from pUC-1.4-CAT and 1.0 μg of the β actin gene insert immobilized on a nitrocellulose filter. Each filter was incubated in hybridization buffer containing 10^6 cpm/ml of RNA.

Gel mobility-shift assay

A nuclear extract from livers of male Sprague-Dawley rats, weighing 250–300 gm, was prepared essentially as described (24). Aliquots of the extract were quick frozen in liquid nitrogen and stored at −70°C. The probe for the gel mobility-shift assay was the synthetic double stranded oligomer used to construct pBLCAT2-P450e(GRE^n). 0.5 μg of the annealed oligomer was phosphorylated with polynucleotide kinase and ^{32}P-gamma-ATP to a specific activity of 10^9 cpm/μg. The binding reaction was carried out in a total volume of 10 μl containing 8000 cpm of labeled oligomer and 16 μg of nuclear protein in a solution that was 20 mM Hepes, pH 7.5; 20% glycerol; 0.1M KCl; 0.2 mM EDTA; 0.5 mM DTT; 0.5 mM PMSF and contained 4 μg of poly dI:dC. After a 30 min incubation at room temperature, 2 μl of 10× gel loading solution (0.42% bromphenol blue in 50% glycerol) was added, and half of each reaction mixture was loaded on a 4% polyacrylamide gel (acrylamide:bisacrylamide = 25:1). A 1.4 Kb fragment containing the MMTV-LTR cloned in the pUC18 plasmid or the pUC18 plasmid DNA itself were used as competitor. After electrophoresis, the gel was dried and subjected to autoradiography for 24 hours using Kodak XR-5 film and an intensifying screen.

RESULTS

A 1.4Kb DNA segment from the 5′-flanking region of the CYP2B2 gene, that extends at its 3′-end to position + 22, one residue upstream of the translation initiation codon, was inserted into a pUC18-based promoterless CAT plasmid and the resultant plasmid, pUC-1.4-CAT, was transfected into rat H4II hepatoma cells together with the plasmid pSV2neo. Pooled permanent transformants selected for G418 resistance were analyzed for CAT expression with and without pretreatment of the cultures for 24 hours with dexamethasone. In the absence of glucocorticoid, a low level of CAT expression was observed that did not significantly differ from the level obtained in control cells transfected with the promotorless pBLCAT3 vector (Fig. 2, table I). In the presence of dexamethasone, the CAT expression was increased 3–4 fold (Fig 2, table I) whereas no increase was

TABLE I. Chloramphenicol acetyltransferase activity (CAT) in rat hepatoma H4-II cell lines stably transformed with P450-CAT and P450-TK-CAT recombinant plasmids.

Plasmid	No. of experiments	CAT activity: pmoles of chloramphenicol acetylated/ min./mg of protein	
		−Dex	+Dex
pBLCAT3	2	0.16±0.02	0.21±0.04
pUC-1.4-CAT	3	0.22±0.06	0.81±0.05
pBLCAT2	2	0.88±0.10	0.46±0.09
pBLCAT2-P450e (HindIII-XbaI)	4	1.22±0.06	22.62±3.90
pBLCAT2-P450e (HindIII-NcoI)	4	2.14±0.11	46.87±5.86
pBLCAT2-P450e (HphI-XbaI)	2	1.78±0.39	1.91±0.28
pBLCAT2-P450e(GRE^n)	2	2.59±0.85	49.70±8.6

Permanent transformants were obtained after cotransfection of H4II cells with pSV2neo and each of the indicated plasmids. These were assayed for CAT activity, with (+Dex) and without (−Dex) prior exposure to dexamethasone for 24 hours.

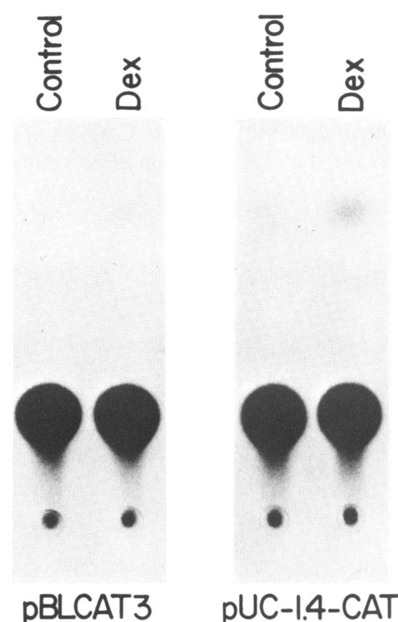

Figure 2: Dexamethasone Induction of CAT Activity in Permanent H4II Transformants Obtained After Transfection with a Plasmid Containing a CAT Gene whose Expression is Driven by a 1.4 Kb 5′ Flanking Segment of the CYP2B2 Gene. Permanent transformants of H4II cells were obtained after transfection with the promoterless vector pBLCAT3 (left) or with the vector which contained the 1.4 Kb 5′ flanking segment of the CYP2B2 gene cloned in the 5′ 3′ orientation upstream of the reporter CAT gene (right). CAT activity was measured in extracts of cultures incubated in the presence (Dex) or absence (control) of dexamethasone. The autoradiograms shown of the thin layer chromatograms of the CAT reaction mixtures were obtained after 3 days of exposure to Kodak XR-5 x-ray film.

observed in cells transfected with the promoterless vector (Fig 2, Table I).

Although the results suggested that a functional glucocorticoid response element was present in the CYP2B2 gene 5′-flanking segment, the low level of expression did not really permit an assessment of the extent of induction. This low level of expression presumably resulted from the inefficiency of the CYP2B2 gene promoter in H4II cells which express their endogenous CYP2B1 and CYP2B2 genes at much lower levels than in hepatocytes in vivo (24). To better test for the presence of a functional GRE in the CYP2B2 5′-flanking region, portions of that DNA region were, therefore, incorporated into the vector pBLCAT2 that contains a 'basal' thymidine kinase gene promoter, that includes a TATA element but no other upstream promoter elements. For these studies a 1031 bp fragment extending from the HindIII site at −1406 to an Xba site at −375, and a 420 bp fragment extending from the HindIII site to a NcoI site at −987 were inserted into pBLCAT2. The basal level of CAT expression in cells transfected with pBLCAT2 was approximately 5−6 times higher than in cells transfected with the promoterless pBLCAT3 plasmid (Table I). Again, however, dexamethasone treatment did not lead to increased CAT expression (Table I). Transformants obtained with the recombinant plasmids in which the CYP2B2 gene segments were placed upstream of the basal TK promoter showed only slightly increased CAT levels in the absence of dexamethasone. Addition of dexamethasone to the medium, however, led to approximately a 20 fold increase in CAT expression (Fig 3, Table I). Both basal and induced levels were two fold higher when the shorter HindIII-NcoI CYP2B2 gene segment was used as compared to the longer HindIII-XbaI segment (Table I). The elements in these segments that mediate the glucocorticoid induction were restricted to the 149 bp at the 5′ end of these fragments (i.e. these 149 bp are necessary for the dexamethasone responsiveness) since a fragment extending from the Hph1 site at −1255 to the XbaI site at −375 did not confer hormone inducibility on the CAT gene in pBLCAT2 (Table I). A dexamethasone induction of comparable magnitude was also obtained with the plasmid pBLCAT2-P450e (GRE^n) which only contained 9 to 10 copies of a synthetic 25bp DNA segment that corresponded to bases −1355 to −1339, encompassing the putative GRE, inserted upstream of the TK promoter (Fig 3, Table I). A run-on transcription experiment using nuclei isolated from the cells transformed with the pBLCAT2-P450e (HindIII-XbaI) plasmid demonstrated that the dexamethasone treatment led to a marked increase in the transcription of the exogenous CAT gene but had no effect on the transcription of the endogenous actin genes (Fig 4).

Binding of a rat liver nuclear protein to the putative CYP2B2 gene GRE

As an additional test for the functionality of the putative GRE in the CYP2B2 gene 5′ flanking region, the 25 bp synthetic DNA segment that encompasses the 15 bp GRE was end-labelled with [^{32}P]-gamma-ATP and used as a probe in a gel mobility shift assay with a rat liver nuclear extract that was active in *in vitro* transcription. A gel-shifted band was observed that was competed away, in a concentration dependent fashion, by a DNA segment containing the MMTV LTR that encompasses several well characterized GRE elements (26, 27, 28) but not at all by pUC18 plasmid DNA (Fig. 5).

DISCUSSION

The transfection experiments presented in this paper establish the presence of a functional GRE between residues 1339−1359 upstream of the CYP2B2 gene transcription start site and a gel mobility shift experiment confirms this finding. The element almost certainly corresponds to a 15bp segment located at residues −1357 to −1343 which is highly similar to a GRE concensus sequence deduced by comparison of a set of ten GRES, from a variety of genes, that were identified by functional tests (16). The consensus GRE contains partial dyad symmetry with an inverted repeat of hexameric sequences separated by a nonspecific trinucleotide. The 3′ hexanucleotide on the coding strand of the

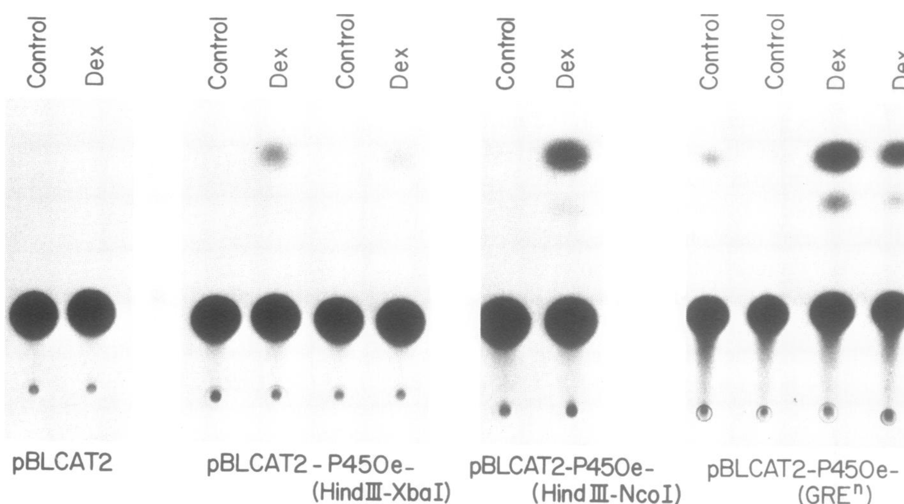

Figure 3: Dexamethasone Induction of Cells Transformed with P-450-Tk-CAT Recombinant Plasmids. H4II hepatoma cells were transfected with pBLCAT2 (l panel) in which the CAT gene was preceded by only a basal TK promoter or with plasmids which also contained the indicated CYP2B2 flanking segment (second and third panel) or multimers (GRE^n) of a 25bp segment containing the putative GRE (fourth panel) upstream of the TK promoter. Permanent transformants obtained after *G418* selection were assayed for CAT activity with (Dex) and without (control) pretreatment of the cells with dexamethasone. The duplicate samples in the second and fourth panels correspond to independent pools of permanent tranformants. The autoradiograms of the thin layer chromatograms were exposed for one (first and second panels) or two (third and fourth) days.

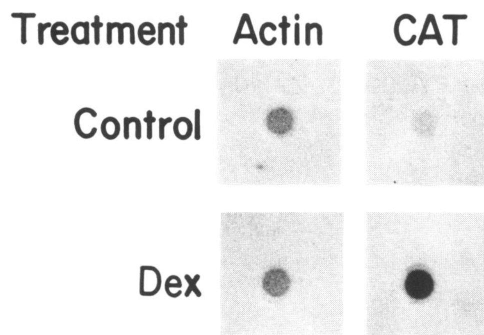

Figure 4: Dexamethasone Activation of CAT Gene Transcription in H4II Cells Transformed with pBLCAT2-P450e (HindIII-XbaI). [^{32}P]UTP-labelled run-on transcripts were prepared from H4II cells permanently transformed with pBLCAT2-P450e (HindIII-XbaI) with (Dex) and without (control) pretreatment of the cultures for 24 hours with the hormone. The RNA samples were hybridized to filters containing either actin or CAT DNA as indicated which were then washed and exposed to Kodak XR5 x-ray film, for 24 hours with an intensifying screen.

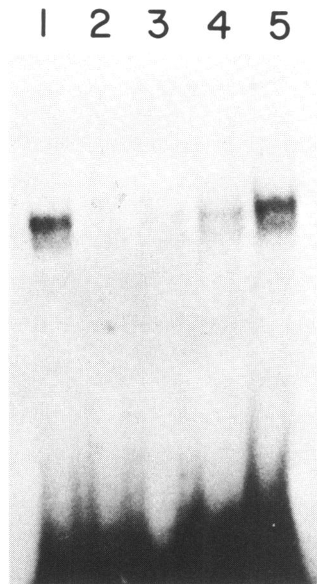

Figure 5: Binding of a Rat Liver Nuclear Protein to the CYP2B2 GRE: Competition by the MMTV LTR. A [^{32}P] labelled oligomer containing the P450 GRE was incubated with a rat liver nuclear extract in the absence (lane 1) or in the presence of competitor DNA (lanes 2–5). Lanes 2 to 4: 0.1, 0.05 and 0.01 ug of pUC18 plasmid containing the MMTV LTR was added; lane 5: 1.0 ug of pUC18 was added. The reaction mixtures were analyzed by gel electrophoresis and autoradiography.

GRE DNA, TGTTCT, is conserved in all the GRE elements compared by Jantzen et al. (16) whereas the CYP2B2 gene GRE contains the sequence TGTTCA (Fig 1). There are no data in the literature that indicate whether the T to A substitution would have any effect on the efficacy of the GRE.

It is worth noting that of the two GREs that mediate glucocorticoid induction of the TAT gene, one can act independently to stimulate transcription whereas the second can only act to synergistically enhance induction mediated by the first GRE (16). The CYP2B2 GRE is much more closely related to the former GRE, with 12 out of 15 matched residues as compared to 7 out of 15 residues matching the second GRE. It is conceivable, therefore, that the P450 GRE could act independently to mediate glucocorticoid activation of an adjacent transcription unit. On the other hand there is a CAAT box (CCAAT) approximately 15bp upstream of the CYP2B2 GRE that may serve, like other transcription factor binding sites, to markedly enhance the hormone-dependent transcription mediated by an adjacent GRE of only moderate potency (18). A synergistic action of CCAAT element may possibly compensate, to some extent, for the long distance of the GRE from the promoter: for the TAT gene, movement of the GRE from approximately −2500 to −350 enhanced the potency of the GRE approximately four fold (16).

The simple presence of a GRE shown to be functional in mediating the hormonal induction of a linked transcription unit in an in vitro transfection assay, does not indicate whether that element serves the same role in vivo. It is conceivable that it may act in vivo only in a particular organ at a particular developmental stage. In fact, it could even serve to repress transcription of the linked gene (29, 30, 31), in a regulatory process that may be mediated either by the glucocorticoid receptor that serves to activate other genes (30) or by a different trans-acting factor (31).

Given the conflicting data available on whether dexamethasone induces the P450IIB1+IIB2 proteins in vivo (8, 14), the results in this paper would suggest that this question is worthy of reexamination. Indeed, the failure to observe an increase in run-on transcription after dexamethasone treatment (14) may simply result from the high background of that assay which may be many fold higher than the true basal level of transcription. One must still reconcile, however, the conflicting observations from the two laboratories on whether chronic dexamethasone treatment of four successive daily injections of the hormone, at nearly identical dosages, leads to a marked increase (20–100 fold) (8) or a slight decrease (14) in microsomal levels of the P450IIB1+IIB2 proteins. The former finding was obtained in a study that employed the sensitive method of immunoblotting with antibody pre-absorbed with solubilized liver microsomes from 3-methylcholanthrene treated rats whereas the second investigation employed a less sensitive radial immunodiffusion assay with nonadsorbed antibody and the extent of phenobarbital induction of these proteins which would give some idea of the background of the assay, was not reported. It is conceivable, of course, that the dexamethasone induction of the proteins could be mediated at the post-transcriptional level (32). Indeed, Simmons et al, (14) found a substantial increase in P450IIB1+IIB2 mRNA after dexamethasone treatment, despite the absence of a detectable increase in the transcription rates of the genes, or in the microsomal level of the corresponding proteins. In this regard, it should be mentioned that a small but significant increase in mRNA hybridizable to a CYP2B2 cDNA probe was observed after treatment of several different hepatoma cell-lines, including the H4II cells used in this work, with dexamethasone (25). In that case, transcription rates and protein levels were not assessed.

Although the marked enhancement in P450IIB1+IIB2 apoprotein levels observed after dexamethasone treatment (8) could be a result of transcriptional activation mediated by interactions of the P450 GRE with the glucocorticoidreceptor, it must be pointed out that the authors of that report believed it to be an indirect effect of the marked decrease in growth hormone secretion that is known to be caused by treatment with this steroid hormone (33). It is conceivable, however, that

dexamethasone acts by two independent mechanisms since it causes a 50–70% higher level of P450IIB1+IIB2 protein expression than does hyphophysectomy and dexamethasone treatment of hypophysectomized animals did lead to a small increase in the apoprotein levels.

ACKNOWLEDGEMENTS

We thank Drs. C. Gorman, B. Luckow, P. Berg, N. Batula and K. Paturu for recombinant plasmids, Mary Weiss for hepatoma cell lines, Heidi Pleskin for art work, Brian Zeitlow and Judy Culkin for photography and Myrna Cort and Bernice Rosen for typing the manuscript. This work was supported by grant GM 30701 from the National Institutes of General Medical Sciences.

REFERENCES

1. Gonzalez, F.J. (1989) Pharmacol. Revs. *40*:243–288.
2. Nebert, D.W., Nelson, D.R., Adesnik,M., Coon, M.J., Estabrook, R.W., Gonzalez, F.J., Guengerich, F.P., Gunsalus, I.C., Johnson, E.J., Kemper, B., Levin, W., Phillips, I.R., Sato, R., and Waterman, M.R. (1989) DNA *8*:1–13.
3. Guengerich, F. P. (1987) Prog. In Drug Metab. *10*: 1–54.
4. Dannan, G. A., Guengerich, F. P., Kaminsky, L. S. and Aust, S. D. (1983) J. Biol. Chem. *258*:1282–1288.
5. Gonzalez, F.J., Song, B.-J. and Hardwick, J.P. (1986) Mol. Cell Biol.*6*:2926–2976.
6. Suwa, Y., Mizukami, Y., Sogawa, K. and Fujii-Kuriyama, Y. (1985)J. Biol. Chem. *260*:7980–7984.
7. Yuan, P. M., Ryan, D. E., Levin, W. and Shively, J. R. (1983) Proc.Natl. Acad. Sci. U.S.A. *80*: 1169–1173.
8. Yamazoe, Y, Shimada, M., Murayama, N., and Kato, R. (1987) J. Biol. Chem. *262*:7423–7428.
9. Omiecinski, C. J., Walz, F. G.Jr., and Vlasuk, G. P. (1985) J. Biol. Chem. *260*:3247–3250.
10. Omiecinski, C. J. (1986) Nucl. Acids. Res. *14*:1525–1539.
11. Adesnik, M., Rivkin, E., Kumar, A., Lippman, A., Raphael, C. and Atchison, M. (1982) In Biochemistry, Biophysics and Environmental Implications, Hietanan, Y. E., Laitenen, M. and Hanninen, O., eds. Elsevier, Amsterdam, pp. 143–148.
12. Atchison, M. and Adesnik, M. (1983) J. Biol. Chem. *258*:11285–11295.
13. Hardwick, J., Gonzalez, F. J. and Kasper, C. B. (1983) J.Biol. Chem. *258*:8081–8085.
14. Simmons, D.L., McQuiddy, P. and Kasper, C.B. (1987) J. Biol. Chem. *262*:326–332.
15. Jaiswal, A., Rivkin, E., and Adesnik, M. (1987) Nucl. Acids, Res. *15*:6755.
16. Jantzen, H.-M., Strahle, U., Gloss, B., Stewart, F., Schmidt, W., Boshart, M., Miksicek, R. and Schutz, G. (1987) Cell *49*:29–37.
17. Strahle, U., Klock, G. and Schutz, G. (1987) Proc. Natl. Acad. Sci. USA *84*:7871–7875.
18. Schule, R., Muller, M., Kaltschmidt, C. and Renkowitz, R. (1989) Science *242*:1418–1420.
19. Luckow, B. and Schutz, G. (1987) Nucl. Acids Res. *15*: 5490.
20. Southern, P. J. and Berg, P. (1982) J. Mol. Appl. Genet. *1*:327–341.
21. Seed, B. (1983) Nucl. Acids Res. *11*: 2427–2445.
22. Gorman, C. M., Moffat, L.F. and Howard, B. A. (1982) Mol. Cell Biol. 2: 1044–1051.
23. Jaiswal, A. K., Gonzalez, F. J. and Nebert, D. W. (1987) Molec. Endocrin. *1*: 312–320.
24. Gorski, K., Carniero, M. and Schibler, U. (1983) Cell *47*: 767–776.
25. Corcos, L. and Weiss, M. C. (1988) FEBS Letts. *233*:37–40.
26. Majors, J. and Varmus, H.E. (1983) Proc. Natl. Acad. Sci. USA *80*: 5866–5870.
27. Payvar, F., DeFranco, D., Firestone, G.L., Edgar, B., Wrange, O., Oket, S., Gustafsson, J.-A., and Yamamoto, K.R. (1983) Cell *35*:381–392.
28. Scheidereit, C., Geisse, S., Westphal, H., and Beato, M. (1983) Nature (London) *304*:749–752.
29. Weiner, F. R., Czaja, M. J., Jefferson, D. M., Giambrone, M-J., Tur-Kaspa, R., Reid, L. M. and Zern, M. (1987) J. Biol. Chem. *252*:6955–6958.
30. Akerblom, I. F., Slater, E. P., Beato, M.B., Baxter, J. D. and Mellon, P. L. (1988) Science *241*:350–353.
31. Langer, S. J. and Ostrowski, M. C. (1988) Mol. Cell Biol. *8*:3872–3881.
32. Dong, Y., Poellinger, L., Okret, S., Hoog, J.-O., von Bahr-Lindstrom, H., Jornvall, H. and Gustafsson, J. A. (1988) Proc. Natl. Acad. Sci. USA *85*:767–771.
33. Oosterom, R., Verlum, T., Zuiderwijk, J. and Lamberts, S.W.J. (1983) Endocrinology *113*:735–741.