Identification and characterization of a suppressor element in the 5'-flanking region of the mouse androgen receptor gene

M.Vijay Kumar¹, Evan A.Jones^{1,+}, Michael E.Grossmann², Marceil D.Blexrud¹ and Donald J.Tindall^{1,2,*}

Departments of ¹Urology and ²Biochemistry/Molecular Biology, Mayo Foundation, Rochester, MN 55905, USA

Received June 10, 1994; Revised and Accepted July 12, 1994

EMBL accession no. X64586

ABSTRACT

Androgens play an important role in the development and maintenance of male reproductive organs through the androgen receptor (AR). In order to study the mechanism of regulation of AR at the molecular level, a 1571 bp fragment in the 5'-flanking region of the mouse androgen receptor (mAR) gene was isolated and sequenced. Transfection of 5'-deletion constructs cloned into vectors containing the chloramphenicol acetyl transferase (CAT) gene indicated the presence of a promoter in the sequence –146 to +131. These experiments also suggested the presence of a suppressor element. Further characterization indicated that the suppressor is present between –486 to –351. It is functional in the context of the natural AR promoter and the heterologous thymidine kinase promoter. Transfection of a –546/+131 construct in which the putative suppressor element (–421 to –448) had been deleted caused increased basal CAT activity suggesting that the suppressor is limited to this 28 bp element in the 5'-flanking region of the mouse AR gene.

INTRODUCTION

Androgens play a central role in the development and maintenance of male reproductive organs. The effects of androgens are mediated by an intracellular androgen receptor (AR) that shares strong homology with other steroid receptors. An understanding of the molecular pathway(s) of androgen action has been facilitated by the cloning and sequencing of cDNAs and genes for the androgen receptor (1–4). However, very little is known about the mechanism(s) which regulate its expression at the molecular level. AR has been shown to be regulated by castration (5), anti-androgens (6), androgens (5–7), epidermal growth factor (8), prolactin (9), growth hormone (9) and FSH (10). Administration of FSH to immature rats resulted in rapid, transient decrease in AR mRNA levels in the testis (11).

Furthermore, it has been shown that FSH, acting through the cAMP pathway, could stimulate AR gene transcription in Sertoli cells, but had little effect in LNCaP cells. Presumably, these effects are mediated, at least in part, by a cAMP response element which has been identified in the 5'-flanking region of the AR gene (12). Thus, the expression of the AR gene appears to be regulated by its own ligand and other metabolic pathways in a tissue-specific and cell specific manner.

As a first step in understanding the mechanism of regulation of AR at the molecular level, we have isolated the 5'-flanking region of the mouse AR (mAR) gene. In order to determine the region containing promoter activity, 5'-deletion mutants were constructed using a PCR technique. The full length and mutant mAR DNAs were cloned into the pBLCAT3 vector and these constructs were used in transient transfection assays. Transfection of the deletion constructs revealed the presence of two promoters in the 5'-flanking region of the AR gene. The results confirmed the presence of a previously characterized promoter (13) and the presence of a second promoter 3' to the first promoter (14). In this paper, we have further analyzed the 5'-flanking region of the mAR gene and have identified and characterized a suppressor element.

MATERIALS AND METHODS

Screening of the genomic library

The full length mouse androgen receptor (mAR) cDNA was used to screen a genomic library for the 5'-flanking region. The 5'-most fragment of the cDNA (2) was isolated by sequential digestion of the mAR cDNA with restriction enzymes *EcoRI* and *A₁III* (488 bp), and the fragment was separated on a 1% agarose gel. The fragment of interest was visualized with ethidium bromide staining, and the DNA was extracted from the gel using a Gene Clean kit (Bio 101). The isolated fragment was labeled with α -³²PCTP by nick translation using a multiprime DNA labeling system (Amersham Life Sciences) and used as a probe

*To whom correspondence should be addressed at: 17 Guggenheim, Mayo Foundation, Rochester, MN 55905, USA

+Present address: Department of Pediatric Endocrinology, West Virginia University Health Associates, Morgantown, WV 26506, USA

to screen an EMBL3 mouse genomic library (Clontech Laboratories). From the positive clones a 1.5 kb fragment was isolated which is located upstream of the ATG start site. This fragment was cloned into the *EcoRI* site of pBluescript vector (Stratagene Cloning Systems). Both strands of the cloned fragment were sequenced using the Sequenase enzyme (United States Biochemicals) and α -³⁵SATP. The sequences were further confirmed with a fmol sequencing system (Promega) using Taq polymerase, α -³⁵SATP, 3 μ g template DNA, 4 pmol primer for 30 cycles at 95°C (0.5 min), 50°C (0.5 min) and 72°C (1.0 min).

Generation of deletion constructs

Deletion mutants were constructed using a polymerase chain reaction (PCR). PCR primers were synthesized on an Applied Biosystems synthesizer at the Molecular Biology Core facility, Mayo Foundation, Rochester and purified on NAP-25 Sephadex G-25 columns (Pharmacia). The PCR primers incorporated *Bam*HI restriction enzyme site at the 5'-end and an *Xho*I site on the 3'-end primer. The PCR product was digested sequentially with *Bam*HI and *Xho*I and separated on a 1.2% agarose gel. The DNA of interest was extracted using a Gene Clean kit and cloned into the *Bam*HI/*Xho*I sites in the promoter-less pBLCAT3 vector or pBLCAT2, which contains a thymidine kinase promoter upstream of the CAT gene (15). The construct -546/+131(del -421/-448) was made by using the megaprimer technique (16). An oligonucleotide 5'CTCCCTTCTGCTTGTCCTATACCT-AAGAGCAATTGG3' was synthesized in which the first 18 nucleotides (CTCCCTTCTGCTTGTCCT) was located on the 5'-end of the sequence to be deleted and the second half of the oligonucleotide (ATACCTAAGAGCAATTGG) was located downstream. The oligonucleotide was used as the 5'-end primer in a PCR reaction whereas +131 oligonucleotide was used as the 3'-end primer. After the first PCR reaction, the product was separated on a 1.2% agarose gel, and the band of interest was excised and was purified using a Gene Clean kit. The PCR product served as a 3'-end primer in second PCR reaction wherein the -546 oligonucleotide was used as the 5'-primer. The product of the second PCR was electrophoresed and the band of interest was extracted and ligated into a pCRII vector (Invitrogen). The ligation takes advantage of the activity of DNA polymerase wherein a single deoxyadenosine is added to the 3'-end of the PCR product which could be used to clone into the pCRII vector with a single 3'-T overhang. The ligated vector was transformed into One Shot competent *E. coli* (Invitrogen). As the insert contains a *Bam*HI site at the 5'-end and an *Xho*I site at the 3'-end, the construct was sequentially digested with these two enzymes, separated on 1.2% agarose gel, DNA was purified using a gene clean kit and ligated into the same site of the pBLCAT3 vector. A typical PCR reaction was carried out in a 100 μ l reaction mixture for 30 cycles at 94°C (1 min), 55°C (2 min) and 72°C (3 min) using Taq (Perkin Elmer Cetus) or pfu (Stratagene cloning systems) polymerase. All the PCR generated DNAs were sequenced using the fmol sequencing system (Promega) to screen for possible mutations generated during PCR. The constructs used in this study did not contain any PCR-induced mutations.

In order to create the construct -451/-418, complementary oligonucleotides with sequences 5'-TCGACCTGGTGGGCC-CTGGGGGAGCGGGGAGGGAATAG -3' and 5'-TCGA-CTATCCCTCCCCGCTCCCCCAGGGCCCAGGAGGG -3' were synthesized and annealed which yielded a *Sal*I site. The

oligonucleotide was cloned into *Sal*I site of pBLCAT2 vector, transformed and used for transient transfection. The construct was sequenced in order to confirm proper cloning.

Cell culture and DNA transfection

The cell lines used in these experiments were mouse hypothalamic GT1-7 (17) and mouse pituitary α T3-1 (18). α T3-1 and GT1-7 were gifts from Dr Pamela Mellon, University of California, San Diego.

GT1-7 and α T3-1 cells were transfected using an electroporation technique. The cells were grown to confluency in T-175 flasks in DMEM medium containing 5% charcoal stripped fetal bovine serum. The cells were harvested with trypsin-EDTA and washed twice. For transfection 20×10^6 cells were used per electroporation. The cells were pelleted and re-suspended in 400 μ l of PBS containing 0.1% glucose and the DNA to be transfected. The cell suspension was loaded to a cuvette and electroporated at 960 mF and 0.35 kV in a BioRad gene pulser. After electroporation the cells were incubated on ice for 7 min and 1 ml PBS containing 4% fetal bovine serum was added. After incubation for 7 min at room temperature, the cells were mixed with 42 ml of culture medium, and 4 aliquots of 10 ml each were distributed to 100 mm tissue culture petri dishes. Cells were incubated at 37°C for 48 h in the presence of 5% CO₂, washed twice with PBS and harvested in PBS containing 5mM EDTA. Cells were pelleted and resuspended in 500 μ l 100 mM Tris buffer pH 8.0 containing 0.1% Triton X-100. The suspension was vortexed and incubated on ice for 15 min and centrifuged for 10 min at 4°C. The supernatant was used for further assays.

Protein content was assayed using the Bradford (19) reagent (BioRad). β -galactosidase was assayed using o-nitrophenyl- β -D-galactopyranoside (ONPG) as a substrate (20). CAT assays were performed with a liquid phase extraction technique (21) using ³H-acetyl CoA.

RESULTS

Characterization of the 5'-flanking region of the mAR gene

In order to understand the regulation of AR at molecular level, we have isolated and sequenced the 5'-flanking region of the mouse AR. A genomic library was screened with the *EcoRI*-*Afl*III fragment of mAR cDNA (2), a 20 kb fragment was isolated and subjected to restriction map analysis. An *EcoRI* fragment of approximately 1.5 kb was isolated and cloned into pBluescript vector. Sequence analysis indicated that the 1.5 kb fragment was located upstream of the translation start site (Fig. 1). This fragment is 1571 bp long, extending from -546 to +971 with respect to the transcription initiation site (+1) identified by S-1 nuclease protection analysis (13). The nucleotides from +972 to 1025 (Fig. 1) were derived by He *et al.*, (2) with the last three nucleotides forming the translation initiation codon ATG.

Comparison of our sequence with that of Faber *et al.*, (13) showed that our sequence is 971 bp longer than the published mouse sequence. The published sequence (13) possesses extra nucleotides at -223, -224 and -270 which are not present in our sequence (data not shown). On the other hand, a C present at -51 of our sequence is missing in that of Faber *et al.*, (13). Furthermore, we identified an A instead of G at -83. At -19, -18 and -13, -12 we found GC instead of CG.

Analysis of the 5'-flanking region of the mAR gene revealed neither TATA nor CCAAT boxes. Instead, the promoter region

```

-550   CCAGCT ATCCTACAGG AGGATCTCAA AGGTTTCAGC AAGAGTTGCT TTGACTGCAG CTTGTTCTTT AATGTCAGGA GACTCTCCCT TCTGCTTGTC
-450 CTGTTGGGCC CTGGGGGGAG CGGGGAGGGA ATACCTAAGA GCAATTGGTA GCTGGTACTT CTAATGCCTC TTCCTCCTCC AACCTCCAAG AGTCTGTTTT
-350 GGGATTGGGT TCAGGAATGA AATTCTGCCT GTGTCTAACCT CCTGGGGAGC CGGTAGACTT GTCTGTTAAA AATCGCTTCT GCTTTGGAGC CTAAAGCCCG
-250 GTTCCGAAAA ACAAGTGGTA TTTAGGGAAG AGGGGTCTTC AAAGGCTACA GTAGTCAATT CCAGCCTTCA ACCATACTAC GCCAGCACTA CGTTCTCTAA
-150 AGCCACTCTG CGCTAGCTTG CGGTGAGGGG AGGGGAGAAA AGGAAAGGGG AGGGGAGGGA GAAAGGAGGT GGAAGGCGAG AGAGGCCGGC
-50   CTGCGGGGGC GGGACCGACT CACAACTGT TGCATTGTCT TTCCACCTCC +1 CAGCGCCCCC TCGGAGATCC CTAGGAGCCA GCCTGCTGGG AGAACCAGAG
      Sp1
51   GGTCCGGAGC AAACCTGGAG GCTGAGAGGG CATCAGAGGG GAAAGACTG AGCTAGCCAC TCCAGTGCCA TACAGAAGCT TAAGGAGCGC ACCACGCCAG
151  CCCCAGCCCA GCGACACGCA ACGCCTGTTG CAGAGCGGGC GCTTCGAAGC CGCCCAGGAG CTGCCCTTTC CTCTTCGGTG AAGTTTCTAA AAGCTGCGGG
251  AGACTCAGAG GAAGCAAGGA AAGTGTCCGG TAGGCATACG GCTGCCTTTG TCCTCTTCCC CTCTACCTCT ACCCCCTCCT GGGTCCCTTC TCCAGGAGCT
351  GACTAGGCAG GCTTCTTGCC CAACCTCTC CCCTACACCC CCAGCTCTGC CAGCCAGTTT GCACAGAGGT AAACCTCCCT TGGCTGAGAG TAGGGGAGCT
451  TGTTCACAT  TGCAAGGAAG GCTTTTGGGA GCCCAGAGAC TGAGGAGCAA CAGCAGCCCC AGGAGAGTCC CTGGTTCCAG GTTCTCGCCC CTCGACCTCC
551  TCCTCCCGCC CCTCACCCT GTGTGTGGTG TTAGAAATGA AAAGATGAAA AGGCAGTAG GGTTCAGTA GTCGAAAGCA AAACAAAGC TAAAGAAAAA
651  CAAAAAGAAA ATAGCCAGT TCTTATTGCG ACCTGCTTCA GTGCACTTTG AATTGGAAG GCAGAGGATT TCCCCTTTTC CCTCCCGTCA AGGTTTGAGC
      CRE
751  ATCTTTTAAT CTGTTCTTCA AGTATTAGA GACAACTGT GTAAGTAGCA GGCAGATCC TGTCTTGCAG GTGCCTTCTT TACTTGAGGA CTTTGAGGTT
851  ATCTGGGCAC TCCCCCACC CACCCCCCT CCTGCAAGTT TTCTTCCCG GAGCTTCCCG CAGGTGGGCA GCTAGCTGCA GATACTACAT CATCAGTCAG
951  GAGAACTCTT CAGAGCAAGA GACGAGGAGG CAGGATAAGG GAATTCGGTG GAAGCTACAG ACAAGCTCAA GGATG

```

Figure 1. Sequence of the mouse androgen receptor 5'-flanking region. An EMBL3 genomic library was screened with the mouse androgen receptor (mAR) cDNA and a fragment of 1.5 kb was isolated and sequenced. The transcription start site of the mAR is indicated as +1 (20). The numbers on the left indicate the sequence of mAR flanking region with nucleotides upstream of the transcription start site shown as negative and those downstream shown as positive. Analysis of the sequence indicated no TATA or CCAAT boxes. The sequence is rich in GC sequences including a homopurine region of GGGGA which is underlined. The translation start site ATG is indicated in italics.

is purine-rich containing a stretch of six copies of GGGGA sequences upstream of a putative Sp1 binding site GGGCGG, -39 to -44 (Fig. 1 and Table 1). The 5'-flanking region of the AR gene possess several other sequences similar to known response elements (Table 1). Important among these are a functional cyclic AMP response element (CRE) which is located about 300 bp upstream of the translational start site, +733 to +740 (12) and a Jun/Fos binding site which is located about 150bp upstream of the Sp1 binding site, -193 to -199 (22).

Suppressor activity is not mediated through a putative ARE/GRE

The initial transfection experiments with 5'-deletion constructs suggested the presence of a suppressor element in the region -546 to -351 (data not shown). It is well known that AR mRNA levels are negatively regulated by androgens, wherein castration results in up-regulation of the mRNA levels and treatment of castrated rats with testosterone resulted in down-regulation of the AR mRNA (7). Computer analysis of this region indicated the presence of two ARE/GRE-like half-sites, TGTCT and TGTCTCT at -488 and -454 respectively (Table 1). Therefore, in order to determine whether the negative activity were mediated by these sequences, the construct -546/+131 was co-transfected with 250 ng of mAR cDNA in the GT1-7 cells. Treatment with DHT did not induce CAT activity which was similar to that of the ethanol treated controls (Fig. 2). In contrast co-transfection of an MMTV-CAT construct which contains known ARE/GRE sequence and an ARE-tk-CAT construct with 250 ng of mAR

Table 1. Consensus response elements in the 5'-flanking region of the mouse

Putative Element	Consensus Sequence	mAR Sequence	Location
TATA box	TATAA	Not found	
CAAT box	CCAAT	Not found	
Sp1	GGGCGG ⁴⁰ GGGCGG	GGGCGG GGGCGG GGGCGG	-39 to -44 +200 to +205 +557 to +562
Ap1	TGAC/GTCA ⁴¹	TGAGTCA	-193 to -199
Ap2	CCCCAGGC ⁴⁰	CCCTGGGGGG GGGGGGGGGG CCAGGCC CCCCAGGC	-433 to -442 -39 to -48 +151 to +158 +864 to +871
CRE	TGACGTCA ⁴²	TCCCGTCA	+733 to +740
ARE/GRE/ PRE half site	TGTCT ⁴³	TGTCT TGTCT TGTCT TGTCT	-483 to -488 -449 to -454 +299 to +304 +762 to +767
OCT	ATTTGCAT ⁴⁰	ATTTGCAC	+675 to +682

The Table describes the response elements present in the 5'-flanking region of the mouse AR. The sequence has been compared to consensus described and the location in the sequence has been indicated (see Fig. 1).

Figure 2. Co-transfection of the mAR 5'-flanking region construct with an mAR cDNA expression construct in GT1-7 cells. The construct -546/+131 and the vector pBLCAT3 was co-transfected with 250 ng/plate of mAR cDNA (2). The vector pBLCAT3 was used as a negative control and MMTV-CAT construct and ARE-tk-CAT were the positive controls. Cells were cultured with either vehicle alone or dihydrotestosterone (10 nM) in ethanol for 48 h at 37°C and harvested.

Figure 3. Analysis of mAR 5'-flanking region deletion constructs in αT3-1 and GT1-7 cells. The construct -546/+131 extends from the 5'-most sequence to the 3'-end of the promoter, whereas -486/+131 and -351/+131 are deletions of approximately 60 bp and 195 bp respectively from the 5'-end. The schematic diagram on the left indicates the portions of mAR flanking region cloned into CAT vectors. CAT denotes the chloramphenicol acetyl transferase gene. The transcription start site is shown as +1 which is indicated by an arrow.

cDNA resulted in significant induction of CAT activity upon treatment with DHT. We have also observed no change in CAT activity when the -546/+131 construct was transfected with concentrations of up to 2 μg mAR (data not shown). These data demonstrate that this region is not androgen responsive and therefore is unlikely to be responsible for the androgen regulation of AR mRNA.

Identification of a suppressor sequence

We have reported previously (23) the presence of a suppressor element in the region +864 to +887 which affects the basal activity of the AR promoter. The sequence of the suppressor in the region +864 to +887 show similarities to the complementary sequence of the suppressor in the 5'-flanking region. Therefore,

Figure 4. Transfection of deletion constructs in GT1-7 cells. The construct -546/+131 extends from the 5'-most sequence to the 3'-end of the promoter, whereas the construct -546/+131(del -421/-448) is similar to -546/+131 except that the sequences between -421 and -448 were deleted using a PCR technique. The schematic diagram on the left indicates the portions of mAR flanking region cloned into CAT vectors. The graph on the right indicates fold CAT activity after transfection. CAT denotes the chloramphenicol acetyl transferase gene.

Figure 5. The mAR suppressor affects CAT activity of the heterologous thymidine kinase promoter. A 213 bp (-546/+333), a 423 bp (-546/-120) segment from the 5'-end of the flanking region of mAR and the sequence -418 to -451 (construct -451/-418) were cloned into the pBLCAT2 vector which contains the thymidine kinase promoter. These constructs were used to transfect GT1-7 cells. The schematic diagram on the left indicates the portions of mAR flanking region cloned into CAT vectors. CAT denotes the chloramphenicol acetyl transferase gene.

in order to eliminate the suppressor element at +864 to +887 from our studies two mutants were constructed (constructs -546/+131 and -351/+131). When these constructs were transfected in GT1-7 cells the construct -546/+131 showed a 7-fold induction of CAT activity compared to the pBLCAT3 vector, whereas removal of approximately 195 bp from the 5'-end (construct -351/+131) resulted in an 18-fold induction of CAT activity compared to the vector alone (Fig. 3). Removal of approximately 60 nucleotides from the 5'-end (construct -486/+131) did not further alter the CAT activity significantly compared to the -546/+131 construct suggesting that the suppressor activity resided downstream of -486. When these constructs were transfected into the αT3-1 cells, a similar response was observed, albeit at a lower activity (Fig. 3).

Computer analysis of the 5'-flanking region revealed the presence of a sequence TGGGGGGA at position -432 to -439 which is a perfect match of the suppressor described in the catalase gene (24). Interestingly, immediately downstream of TGGGGGGA is the sequence GCGGGG which has been shown to be a consensus sequence for the binding of a nuclear factor which suppresses transcription (25) suggesting the presence of putative suppressor sequence GGGGGGAGCGGGG at -426 to -438. If this were a functional suppressor element, then its

deletion should enhance basal transcription activity. Therefore, a PCR technique was used to generate a construct in which the putative suppressor region with additional flanking nucleotides were deleted [the construct -546/+131(del -421/-448)]. Transfection of this construct into GT1-7 cells indicated that the basal CAT activity of this construct increased 14-fold compared to pBLCAT3 (Fig. 4), whereas CAT activity of -546/+131 increased 4-fold, suggesting the presence of a suppressor in the deleted region.

Function of the suppressor in a heterologous promoter

In order to determine whether the suppressor was functional in the context of a heterologous promoter, DNA sequences containing the suppressor were cloned in a pBLCAT2 vector which contained the thymidine kinase (tk) promoter upstream of the CAT gene. A PCR technique was used to generate 213 bp and 426 bp DNA fragments extending from the nucleotide -546 and these were cloned upstream of the tk promoter. When the 213 bp (-546/-333) and 423 bp (-546/-120) constructs were transfected, the CAT activity of the wild type pBLCAT2 was suppressed by about 74% and 70% in GT1-7 cells (Fig. 5). In order to further define the suppressor activity, complementary oligonucleotides were synthesized extending from -418 to -451 and was cloned into pBLCAT2 vector. Transfection of this construct inhibited the CAT activity by 68% suggesting the presence of a suppressor in this region.

DISCUSSION

This paper describes the isolation of a 1571 bp fragment in the 5'-flanking region of the mouse AR gene. This is a longer sequence than that published for mouse, 600 bp (13), rat, 670 bp (26) and human, 1.4 kb (27). A comparison of the mouse and human 5'-flanking region showed 80% sequence similarity between the two sequences, including binding sites for Sp1, NF1, Oct and AP2 at similar positions; although no binding site was found for AP1 upstream of the transcription start site in the human sequence. Comparison of our sequence with that of Faber *et al.*, (13) showed that our sequence is 971 bp longer and includes several consensus regulatory elements which may be involved in the regulation of the gene. Furthermore, several differences were detected in our sequence when compared to that published (13). It is difficult to explain these discrepancies as we and Faber *et al.*, (13) both used genomic libraries from the same source (viz. Clontech Laboratories). The sequence presented in this paper was originally obtained by sequencing both the DNA strands using Sequenase and was confirmed with a PCR based fmol sequencing technique using deaza dNTPs. One of the main advantages of this system is that the sequencing reaction is carried out at 70°C which helps resolve sequences that are rich in secondary structures. Furthermore, the sequence was confirmed when the deletion constructs were sequenced. Consequently, each of these strands has been sequenced several times.

A comparison of the DNA sequence of the mAR gene with suppressor elements described in the literature indicated the presence of a suppressor described in the catalase gene (24) and a consensus sequence for the binding of a factor which suppresses transcription (25). Therefore, in order to determine the involvement of these sequences in the suppressor activity of the 5'-flanking region of mAR, the sequence -421 to -448 was deleted [construct -546/+131 (del -421/-448)] which resulted in increased CAT activity compared to -546/+131. The results

suggest that the suppressor activity in the 5'-flanking region is due to the sequences TGGGGGAGCGGGG which is similar to the suppressor in the catalase gene (24) and also the binding site for human DNA binding factor (25). Transient transfection of constructs containing the first 213 bp and 423 bp upstream of a heterologous promoter confirmed the presence of a suppressor in that region. The construct -451/-418 further narrowed the suppressor activity to the region which includes the sequence TGGGGGAGCGGGG.

Although the role of enhancer elements in initiation of gene transcription has been well studied, very little is known about the mechanisms by which DNA binding proteins suppress transcription (25,28-33). Two principal systems have been recognized in causing transcriptional suppression, one being protein-protein interaction and a second being protein-DNA interaction. A recent example of the first system is the mutual inhibition of transcriptional activity by cJun and the glucocorticoid receptor (34-39).

One model of protein-DNA interaction suggests that when a suppressor binding sequence overlaps the enhancer sequence(s), the suppressor protein blocks the binding of the enhancer protein to the DNA or interferes with the formation of the transcription complex (30,37,38). Thus, the suppressor effect is due to the steric hindrance or competition with positively acting transcription factors. Another model is called 'quenching' (30) wherein the suppressor and activator bind to different DNA sequences but the enhancer activity is affected by the binding of a downstream suppressor. The suppression of the basal transcriptional activity of mAR gene cannot be explained by the above two models. A third model which could explain the mechanism of suppressor activity is the 'direct' suppression model (30,38,39). According to this, protein could bind to the sequence TGGGGGGA and suppress activity as shown in the case of catalase gene (24). Alternatively, a protein similar to human DNA binding factor (25) could bind the sequence GCGGGG and suppress promoter activity. It is possible that the DNA TGGGGGAGCGGGG could be bound by two or more proteins resulting in suppressor activity.

In summary, we have shown that the removal of the sequence -546 to -351 results in increased CAT activity irrespective of the length of the 3'-end of the construct suggesting the presence of a suppressor in this region. The suppressor function is not mediated through ARE/GRE-like sequences present in this fragment. The suppressor is functional in the context of the natural and heterologous promoter. Deletion of the sequence TGGGGGAGCGGGG present in the fragment abolishes the suppressor function suggesting that this sequence is the suppressor. Further experiments are being conducted to identify the protein(s) responsible for the suppressor activity.

ACKNOWLEDGEMENTS

This research was supported by NIH grants CA 32387 and HD 09140 (to DJT) and a grant from the American Foundation for Urologic Diseases in cooperation with The Connaught Foundation (to MVK).

REFERENCES

1. Chang, C., Kokontis, J., and Liao, S. (1988) Proc. Natl. Acad. Sci. USA 85, 7211-7215.
2. He, W.W., Fisher, L.M., Sun, S., Bilhartz, D.L., Zhu, X., Young, C.Y.-

- F., Kelley, D.B., and Tindall, D.J. (1990) *Biochem. Biophys. Res. Commun.* 171, 697–704.
3. Lubahn, D.B., Joseph, D.R., Sullivan, P.M., Willard, H.F., French, F.S., and Wilson, E.M., (1988) *Science* 240, 327–330.
4. Tan, J.-A., Joseph, D.R., Quarmby, V.E., Lubahn, D.B., Sar, M., Larson, R.E., French, F.S., and Wilson, E.M. (1988) *Mol. Endocrinol.* 2, 1276–1285.
5. Shan, L.-X., Rodriguez, M.C., and Janne, O.A. (1990) *Mol. Endocrinol.* 4, 1636–1646.
6. Wolf, D.A., Herzinger, T., Hermeking, H., Blaschke, D., and Horz, W. (1993) *Mol. Endocrinol.* 7, 924–936.
7. Quarmby, V.E., Yarbrough, W.G., Lubahn, D.B., French, F.S., and Wilson, E.M. (1990) *Mol. Endocrinol.* 4, 22–28.
8. Mizokami, A., Saiga, H., Matsui, T., Mita, T., and Sugita, A. (1992) *Endocrinol. Jpn.* 39, 235–243.
9. Reiter, E., Bonnet, P., Sente, B., Dombrowicz, D., de-Level, J., Closset, J., and Hennen, G. (1992) *Mol. Cell. Endocrinol.* 88, 77–87.
10. Blok, L.J., Mackenbach, P., Trapman, J., Themmen, A.P.N., Brinkmann, A.O., and Grootegoed, J.A. (1989) *Mol. Cell. Endocrinol.* 63, 267–271.
11. Blok, L.J., Themmen, A.P.N., Peters, A.H.F.M., Trapman, J., Baarends, W.M., Hoogerbrugge, J.W., and Grootegoed, J.A. (1992) *Mol. Cell. Endocrinol.* 88, 153–164.
12. Lindzey, J., Grossmann, M., Kumar, M.V., and Tindall, D.J. (1993) *Mol. Endocrinol.* 7, 1530–1540.
13. Faber, P.W., King, A., van Rooij, H.C.J., Brinkmann, A.O., de Both, M.J., and Trapman, J. (1991) *Biochem. J.* 278, 269–278.
14. Grossmann, M.E., Lindzey, J., Blok, L.J., Perry, J.E., Kumar, M.V., and Tindall, D.J. (1994) *Biochemistry*, Submitted.
15. Luckow, B., and Schutz, G. (1987) *Nucleic Acids Res.* 15, 5490.
16. Sarkar, G., and Sommer, S.S. (1990) *Biotechniques* 8, 404–407.
17. Mellon, P.L., Windle, J.J., Goldsmith, P.C., Padula, C.A., Roberts, J.L., and Weiner, R.I. (1990) *Neuron* 5, 1–10.
18. Windle, J.J., Weiner, R.I., and Mellon, P.L. (1990) *Mol. Endocrinol.* 4, 597–603.
19. Bradford, M.M. (1976) *Anal. Biochem.* 72, 248–254.
20. Rosenthal, N. (1987) *Methods in Enzymology* 152, 704–720.
21. Neumann, J.R., Morency, C.A., and Russian, K.O. (1987) *Biotechniques* 5, 444–447.
22. Jones, E.A., Kumar, M.V., Blexrud, M.D., Grossmann, M.E., and Tindall, D.J. (1994) *Biochemistry*, Submitted.
23. Grossmann, M.E., Lindzey, J., Kumar, M.V., and Tindall, D.J. (1994) *Mol. Endocrinol.* 8, 448–455.
24. Sato, K., Ito, K., Kohara, H., Yamaguchi, Y., Adachi, K., and Endo, H. (1992) *Mol. Cell. Biol.* 12, 2525–2533.
25. Kageyama, R., and Pastan, I. (1989) *Cell* 59, 815–825.
26. Baarends, W.M., Themmen, A.P.N., Blok, L.J., Mackenbach, P., Brinkmann, A.O., Meijer, D., Faber, P.W., Trapman, J., Grootegoed, J.A. (1990) *Mol. Cell. Endocrinol.* 74, 75–84.
27. Tilley, W.D., Marcelli, M., and McPhaul, M.J. (1990) *J. Biol. Chem.* 265, 13776–13781.
28. Kingsley, C., and Winoto, A. (1992) *Mol. Cell. Biol.* 12, 4251–4261.
29. Kutoh, E., Stromstedt, P.-E., and Poellinger, L. (1992) *Mol. Cell. Biol.* 12, 4960–4969.
30. Levine, M., and Manley, J.L. (1989) *Cell* 59, 405–408.
31. Thompson, A.N., Lee, E., Lawe, D., Gizang-Ginsberg, W., and Ziff, E.B. (1992) *Mol. Cell. Biol.* 12, 2501–2513.
32. Tran, P., Zhang, X.-K., Silbert, G., Hermann, T., Lehmann, J.M., and Pfahl, M. (1992) *Mol. Cell. Biol.* 12, 4666–4676.
33. Zhao, X.-Y., and Hung, M.-C. (1992) *Mol. Cell. Biol.* 12, 2739–2748.
34. Jonat, C., Rahmsdorf, H.J., Park, K.-K., Cato, A.C.B., Gebel, S., Ponta, H., and Herrlich, P. (1990) *Cell* 62, 1189–1204.
35. Schule, R., Rangarajan, P., Kliewer, S., Ransone, L.J., Bolado, J., Yang, N., Verma, I.M., and Evans, R.M. (1990) *Cell* 62, 1217–1226.
36. Yang-Yen, H.-F., Chambard, J.-C., Sun, Y.-L., Smeal, T., Schmidt, T.J., Drouin, J., and Karin, M. (1990) *Cell* 62, 1205–1215.
37. Akerblom, I.E., Slater, E.P., Beato, M., Baxter, J.D., and Mellon, P.L. (1988) *Science* 241, 350–353.
38. Biggin, M.D., and Tjian, R. (1989) *Cell* 58, 433–440.
39. Jaynes, J.B., and O'Farrell, P.H. (1991) *EMBO. J.* 10, 1427–1433.
40. Jones, N. (1990) *Seminars Cancer Biol.* 1, 5–17.
41. Angel, P., Allegretto, E., Ikino, S.T., Hattori, K., Boyle, W.J., Hunter, H., and Karin, M. (1988) *Nature* 332, 166–171.
42. Montminy, M.R., Gonzalez, G.A., and Yamamoto, K.K. (1990) *Trends Neurosci.* 13, 184–188.
43. Carson-Jurica, M.A., Schrader, W.T., and O'Malley, B.W. (1990) *Endocrine Rev.* 11, 201–220.