Prostate cancer can be detected using assays for blood-borne prostate-specific antigen (PSA), which is the clinically most useful diagnostic marker of malignant disease. This paper characterizes the 5′-flanking prostate-specific enhancer which controls expression of the human PSA gene. This enhancer, located between −5824 and −3738, is androgen-responsive and requires a promoter for activity. Inductions of 12-100-fold activity occur at 1 nM concentrations of the testosterone analog R1881. The enhancer demonstrated tissue specificity as judged by transfections of several human cell lines. Electrophoretic mobility shift assays comparing nuclear extracts from breast cancer cells MCF-7, and prostate cancer cells LNCaP, showed three regions of prostate-specific binding. These three regions are −4168 to −4797 (region I), −4710 to −4479 (region II), and −4168 to −3801 (region III). Region III contained a putative androgen response element at −4136 that markedly affected activity if mutated. These data suggest that prostate-specific gene expression may involve interaction of prostate-specific proteins or protein complexes with the enhancer in addition to binding of the androgen receptor to androgen response elements.

The human kallikrein gene family consists of three members: prostate-specific antigen (PSA),1 glandular kallikrein (hGK-1), and pancreatic/renal kallikrein (hPRK) (1–3). PSA is a Mr = 34,000 chymotrypsin-like protein that is synthesized exclusively by normal, hyperplastic, and malignant prostatic epithelia (4, 5). This property of PSA has permitted its use clinically as a biomarker for benign prostatic hyperplasia and prostate carcinoma (CaP). Rising levels of PSA above 10 ng/ml in the blood are indicative of benign prostatic hyperplasia or CaP (6, 7). In end-stage metastatic CaP, serum PSA levels can exceed 200 ng/ml.

The clinical utility of PSA rests on its highly tissue-specific expression pattern, of which a portion is contributed by androgen regulation of transcription via the androgen receptor (AR) (5, 8, 9). The AR modulates transcription through its interaction with its consensus DNA binding site, GGTACAnnnTGTT/CCT, termed the androgen response element (ARE). Androgen ablation therapy of CaP leads to reduction in the level of circulating PSA (7). In LNCaP cells, a cell line derived from a lymph node metastasis of CaP and which synthesizes PSA (10), treatment with antiandrogens induces PSA transcription and treatment with antiandrogens results in suppression (11, 12). A close match to the androgen-response element (ARE) consensus sequence was identified in the 5′-flanking promoter region of the PSA gene (13). While this potential ARE was shown to be active by cotransfection of CV-1 cells with AR, the promoter fragment itself is inactive in LNCaP cells. It appears that additional sequences are required for the prostate-specific regulation of the PSA gene.

Other androgen-responsive genes such as the prostatic binding protein C3 (1) and mouse vas deferens protein gene are regulated by enhancers separated from the promoter region (14, 15). Given the medical importance of the PSA gene, regions farther upstream of the coding region were examined for regulatory sequences. In this paper, we describe the identification and characterization of an androgen-responsive enhancer located between −5327 and −3737 relative to the start of transcription of the PSA gene. This enhancer is selectively active in LNCaP cells in vitro and stimulated 10- to 100-fold increases in transcription when linked to the PSA gene promoter. Within the sequence of this enhancer is a potential ARE; alterations in the sequence of this element can either increase or decrease transactivation of linked reporter genes. When analyzed by electrophoretic mobility shift assay (EMSA), proteins from nuclear extracts were found to complex with extensive regions of the enhancer. Protein-DNA complexes specific to LNCaP extracts were formed with three regions of the enhancer, including the region around the putative ARE.

MATERIALS AND METHODS

Cells and Culture Methods—LNCaP cells (10) were obtained from the American Type Culture Collection (Rockville, MD) at passage 9. LNCaP cells were maintained in RPMI medium supplemented with 10% fetal bovine serum (Intergen Corp.), 100 units/ml penicillin, and 100 μg/ml streptomycin. The prostate-specific antigen (PSA) production of the cells was tested periodically and was consistently above 20 ng/ml per day.

The HBL100 (17), MCF-7 (18), Ovar-3 (19), Panc-1 (20), and DU-145 (21) cell lines were also obtained from the American Type Culture Collection. The 293 cell line (22) was obtained from Microbix, Inc. (Ontario, Canada). These cell lines were maintained in Dulbecco’s modified Eagle’s medium supplemented with 6 g/liter glucose, as well as fetal bovine serum and antibiotics as above.

Plasmid Constructs—The plasmid CN0 contains the 5.8-kilobase (kb) HindIII fragment of the human PSA gene covering the region −5834 to +12 relative to the transcription start site. This fragment was subcloned into pUC18 and was a kind gift of Ake Lundwall (23). The plasmid pCATbasic and pGL2 promoter vector was obtained from Promega Corp. pCMVβ plasmid was purchased from Clontech, Inc. (Palo Alto, CA). The BlueScript KS II (+) (BskII+) vector was obtained from Stratagene.

DNA manipulations of various plasmids derived in this study were...
performed by conventional molecular biology techniques (24). Restriction enzymes and other modifying enzymes were purchased from various sources including Pharmacia Biotech Inc., Life Technologies, Inc., and New England Biolabs. CN33 was constructed by inserting the 5.8-kb HindIII fragment from CN0 in pCAT basic in the correct orientation. CN22 is the 5.8-kb HindIII fragment from CN0 in correct orientation. In the plasmid pGL2, linearized pGL2 was digested with BsKSII and KpnI, ligated into similarly cut CN33, and contains –5322 to –3738 relative to the PSA gene upstream region and the promoter from –541 to +12. CN68 was constructed from CN23 cut with Clal and Xhol, ligated into similarly cut CN33. It contains –5322 to –3783 of the PSE and –541 to +12 of the PSA promoter. CN71 was constructed from CN0 PCR-amplified with primers 15.59A and 10.150.1 (5'-AGGGTACCTTCGGGATCCTGAG) and KpnI, end-filled with Klenow, and ligated into similarly cut CN33. It contains –5322 to –4023 of the PSE and –541 to +12 of the PSA upstream region. CN71 was constructed from CN0 PCR amplified with primers 15.59A and 10.150.1 (5'-AGGCCTGAGAAGCAGGCATC-) and KpnI, end-filled with Klenow and ligated into similarly cut CN33. It contains –5322 to –4023 of the PSE and –541 to +12 of the PSA promoter. CN34 was constructed by excising the KpnI fragment from CN22 cut with XhoI, ligated into similarly cut CN33, and contains –5322 to –4023 of the PSE and –541 to +12 of the PSA promoter. CN71 was constructed as described for CN27 with the upstream region ligated in the wild type orientation. CN75 was constructed the same way as for CN71, except with the enhancer PCR fragment ligated to KpnI cut CN34; CN75 is in the wild type orientation relative to the promoter, and CN74 is in the opposite orientation. To construct a reporter plasmid with the PSA enhancer upstream of the SV40 early promoter in the correct orientation, DNA Sequencing—The SB24-bp HindIII 5'-flanking fragment of the PSA gene was sequenced by the dideoxy chain termination method using Sequenase T4 DNA polymerase (U. S. Biochemical Corp.). Sequence numbering is according to Lundwall (25). A series of 3'-5' nested PCR fragments were synthesized on the T7 promoter and cloned into the pUC19 vector. The cloned inserts were sequenced. The sequence has been deposited in GenBank under accession number U37672.

Selected constructs generated by PCR were sequenced to ensure sequence fidelity. The constructs were sequenced on one strand as described above. The constructs which were sequenced were: CN65, CN70 through CN75, CN109, and CN110.

Transfection Procedure—For transfection, LNCaP cells were plated at 5 x 10³ cells per 6-cm culture dish in complete medium. DNAs were introduced into the cells by complexing with N-[1-(2,3-dioleoyloxy)propyl]-N,N,trimethylammonium chloride (DOTMA)/dioleoylphosphatidylethanolamine (DOPE) (1:1) Lipofectin, Life Technologies, Inc., Briefly, 8 µg (24.2 mmol) of DNA was diluted into 200 µl of RPMI 1640 medium containing 10% fetal bovine serum (stripped serum) and supplemented with 10⁻⁴ M methylthiouracilone (R1881, DuPont NEN), followed by incubating for an additional 48 h at 37 °C before harvest.

For CAT assays, the cells were harvested by removing the medium, washing the cells once with phosphate-buffered saline, and incubating in 1 ml of TEN buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA) for 5 min. Cells were scraped off the dishes, and extracts were made by pelleting the cells, resuspending the cell pellet in 100 µl of 0.25 M Tris, pH 7.8, and then sonicating the cell suspension to three freeze-thaw cycles. The supernatant was transferred to fresh tubes and stored at –80 °C. Protein concentration of extracts was measured using the Bradford assay.

Quantitative CAT assays were performed as described previously (24). Briefly, cell extracts were normalized for protein content, followed by diluting equal quantities of protein to 90 µl with 0.25 M Tris, pH 7.8, 500 mM NaCl, 10 mM KCl, 10 mM EDTA, 50 mM 2-mercaptoethanol, and 1 µM (32P)ATP. For each reaction, 5 µl of 0.1 mM substrate mixture (20 µCi/ml [3H]chloramphenicol (DuPont NEN), 0.63 x 10⁻³ M chloramphenicol, 2.5 x 10⁻⁴ M butyryl coenzyme A in water) was added to each sample and vortexed briefly. Samples were incubated at 37 °C for 2 h followed by a single extraction with 100 µl of a 1:1 mixture of xylene and tetramethylpentadecane. The organic phase was transferred to scintillation vials containing Biosafe NA scintillation fluid (Research Products International) and counted. A standard curve was constructed by assaying purified CAT enzyme assays. Assays for β-galactosidase were performed on cell lysates as described previously (26).

Electrophoretic Mobility Shift Assays (EMSA)—Nuclear extracts containing DNA-binding proteins were prepared from LNCaP cells by a modification of the method of Dignam et al. (27, 28). The crude extract was dialyzed against binding buffer (20 mM HEPES pH 7.9, 20% glycerol, 100 mM KCl, 0.2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride) and stored at –80 °C.

DNA probes for EMSA were constructed by synthesizing a series of 16 overlapping polymerase chain reaction (PCR) products spanning –5443 to –3738 relative to the start of the PSA gene transcription. The locations of the segments defined by the PCR primer pairs are diagrammed under “Results”; primer sequences are omitted here but will be provided upon request. PCR samples were composed of 1 unit of Taq DNA polymerase (Stratagene), 1 ng of CN0 PCR buffer supplied by the manufacturer, 200 µM nucleotides, and 50 pmol of each primer. Following an initial denaturing step at 94 °C for 2 min, for each of 25 cycles the template was denatured at 94 °C for 1 min as for CN0, followed by an extension at 72 °C for 2 min. The samples were then analyzed by 12% polyacrylamide slab gel electrophoresis, autoradiography, and quantitation by scintillation counting.

RESULTS

Androgen Responsiveness of the PSA Gene 5'-Flanking Region—In determining the contribution of regions upstream of the PSA coding region to androgen responsiveness, the 5322-bp XbaI/HindIII fragment residing at the 5' end of the PSA gene was used to drive the synthesis of reporter genes in PSA-secreting LNCaP cells. This upstream segment of the PSA gene, as well as truncations at –4136 (Clal) and –541 (BglII) were cloned upstream of the CAT gene to create plasmids CN23, CN25, and CN33. Fig. 1 demonstrates that CN23 was highly inducible by the synthetic testosterone analog R1881. Peak level of induction at 100 nM R1881 was 45-fold over the background value of 500 cpm.

In contrast, neither CN25...
the PSA gene in CN33 which was previously reported to be

Typically, the levels of induction of CAT synthesis by the ex-

Cla fragments somewhere upstream of the

HindIII site at

2

4136 and the

Cla site at

2

541 to

12) was inducible at all

1

2

12) nor CN33

541 to

12) was inducible at all

with R1881. The loss of inducibility with truncation beyond the

Xba site at

2

5322 reveals the presence of an upstream tran-

sitional regulatory region whose

5

Xba site and extending to variable distances

downstream of the CAT gene in CN75 also resulted in a high

downstream of the CAT gene in CN75 also resulted in a high

level of induction of 27-fold for CN23 while CN33 was not inducible. Removal of the sequences between

3738 and

541 (CN65) resulted in a 38-fold induction. When the deletion was ex-

3738 and

541 (CN65) resulted in a 38-fold induction. When the deletion was ex-

extended further upstream to

3872 (CN71), the level of induc-

3872 (CN71), the level of induc-

3738 is not necessary for enhancer activity but contains ele-

3738 is not necessary for enhancer activity but contains ele-

One of the defining features of enhancer elements is their

ability to function in stimulating transcription despite their

location or orientation relative to the promoter upon which they act (32). To determine if the element defined above has

these properties, the segment of the upstream region from the

Xba site to

3738 was inserted upstream of the PSA promoter/ CAT gene transcription unit in both orientations; the same gene segment was also inserted downstream of the CAT gene in both orientations (Fig. 5). CN73 is essentially the same as CN65 (Fig. 4B) and yielded a 44-fold induction in LNCaP cells treated with R1881. The same segment in the opposite orienta-

tion resulted in a 36-fold induction (CN72). This level of induction remained unchanged when the upstream element was moved downstream of the CAT gene with an orientation opposite that in CN73 (CN74). Reversal of this orientation downstream of the CAT gene in CN75 also resulted in a high

level of induction of 25-fold. These levels of induction compared to a 1-fold induction using the promoter construct CN33. These results confirm that the element characterized above possesses the properties of an enhancer.

Despite the presence of ARE sequences within the upstream enhancer, it is conceivable that the androgen regulation of PSA expression is contributed by the ARE at

170. To determine if the PSA enhancer contributes to androgen responsiveness, the PSA enhancer was placed upstream of the SV40 early promoter (CN110, Fig. 6). Cultures of LNCaP cells transfected with CN110, CN109 (containing the SV40 early promoter alone),
FIG. 2. Sequence of the 5.8-kb HindIII fragment 5' to the PSA gene. Sequencing was performed as described under "Materials and Methods," and sequence segments were assembled using AssemblyLign software (IBI/Kodak). Overlined sequences indicate homonucleotide sequences, underlined sequences indicate consensus recognition sites for transcription factors as described in the text. The TATA box is indicated by both over- and underlines. Nucleotide position 11 corresponds to the 5'-most cap site as defined by Lundwall (13).
CN65 (containing the PSA promoter plus enhancer), or CN33 (containing the PSA promoter alone) were treated with increasing concentrations of R1881, then assayed for CAT activity. As shown in Fig. 6, activity of CN110 ranged from 76-fold induction over background in the absence of R1881 to 206-fold induction at 10^{-28} M R1881 while activity of CN65 ranged from 37- to 137-fold at these same R1881 concentrations. CN109 stimulated approximately 16-fold induction of transcription at all R1881 concentrations, while CN33 activity did not exceed 2-fold activation. CN110 and CN65 displayed similar patterns of response to increasing R1881 concentrations with peak levels of transcriptional stimulation observed at 10^{-28} M R1881. These data show both that the PSA enhancer is androgen-responsive and that its activity is independent of the promoter used.

To determine if the potential ARE at -24148 functions in androgen inducibility of the upstream element, alterations were made within the ClaI site in CN23 to construct CN68 and CN69. The plasmid CN23 was cut with ClaI, then either end-filled with Klenow (CN68) or treated with S1 nuclease (CN69), then religated. The former treatment resulted in addition of a CG dinucleotide at -24136, while the latter treatment removed bases at -24137 to -24134. The wildtype sequence in CN23 yielded a 27-fold induction in LNCaP cells, while addition of two bases within the ClaI site resulted in half the level of induction (data not shown). Interestingly, removal of the 5 bases within the ClaI site resulted in a 3-fold increase in the level of CAT synthesis relative to the wild type sequence (data not shown). The bases removed reside in the rightmost portion of the potential ARE. These results suggest that this ARE may be functional and that its activity may be influenced by neighboring sequences. These sequences may bind transcription factors which might be required for formation of prostate-specific DNA-protein complexes and for prostate-specific PSA expression (see below).

Tissue Specificity of the PSA Gene Enhancer—The PSA protein is recognized to be the most useful biomarker of malignant disease. Very little, or no, PSA protein is synthesized by tissues outside of the prostate. It was therefore important to determine if the enhancer described above retained not only androgen responsiveness, but also the high level of tissue specificity characteristic of the PSA gene.

To this end, a variety of cell lines were transfected with three reporter constructs: CN13 containing the 5836-bp 5' region, CN65 containing the minimal enhancer/promoter, and CN33 containing the promoter alone. The cell lines used represent several hormone-responsive tissues including human breast epithelia (HBL100), human breast carcinoma (MCF-7), pancreatic cancer (PANC-1), ovarian carcinoma (OVCA-3), and prostate carcinoma (LNCaP, DU145). The 293 cell line was derived from human embryonic kidney cells transformed by adenovirus DNA. The cell lines were transfected as described under "Materials and Methods" with reporter DNAs admixed with an internal control plasmid, pCMVβ.
The results of such an analysis are shown in Fig. 7. In LNCaP cells, both CN13 and CN65 stimulate CAT synthesis approximately 9-fold above background, while CN33 stimulated a 2-fold accumulation of CAT. In no other cell line did CN13 or CN65 lead to more than a 2-fold induction of CAT synthesis. The highest levels of activity outside of LNCaP cells were observed in Panc-1 and Ovcar-3, where CN13 reached approximately 1.5-fold and 2.5-fold, respectively; CN65 exhibited less than a 2-fold induction in both of these cell lines. Not surprisingly, all three PSA reporter constructs were inactive in the DU145 prostatic carcinoma cell line since the PSA gene is inactive (33). In contrast to the enhancer-containing reporters, the CN33 construct stimulated CAT synthesis to approximately 2-fold in each of the cell lines tested except Panc-1 where a 5-fold induction was observed. In each case, CN33 was more active than the enhancer-containing constructs.

Preliminary results using LNCaP tumor xenografts in the nude mouse model support the retention of tissue specificity in vivo. When CN23 complexed with a lipid delivery vehicle (eg., DOTMA/DOPE, 1:1) was introduced into LNCaP tumors as well as other mouse tissues either by direct injection or systemically, CAT activity was detected only in tumor samples (data not shown). These results also indicate that the PSA enhancer/promoter combination has retained tissue-specific properties to a large degree both in vitro and in vivo.

DNA-Protein Complexes on the PSA Gene Enhancer—The above data demonstrate that an enhancer resides upstream of the PSA gene promoter. Furthermore, it suggests that this enhancer is responsible, at least in part, for the narrow tissue distribution of PSA expression. Protein-DNA complexes which form on this enhancer are likely to control this tissue-specific expression. In characterizing these protein-DNA complexes, segments of the enhancer were assayed for their ability to form such complexes by EMSA. Sixteen subsegments of the enhancer were created by PCR with end-labeled primers for use as probes in LNCaP cell and MCF-7 cell extracts by EMSA. The locations of these subsegments and a summary of the results of this survey are presented in Fig. 9.

The PCR-amplified segments which bound proteins only in LNCaP extracts span −4980 to −4797 (segment 5), −4710 to −4479 (segments 7 and 8), and −4168 to −3801 (segments 13 through 15). Autoradiographs of EMSA with these probes are shown in Fig. 8. In each example, multiple complexes are formed with the probes; often, the faster-migrating complexes are formed with both cell extracts and appear to be nonspecific. Complexes specific to LNCaP extracts are indicated by arrows in each panel (LNCaP extract + mock competitor). The absence or reduction of these complexes in lanes 3 (LNCaP extract + specific competitor) demonstrated the specific nature of these complexes. Four specific complexes were formed with segment 8, while three specific complexes were formed with probe 13. Two each were formed with probes 5, 13, and 15. One complex each was formed with probes 7 and 14. The relatively large size of the probes and the slow migration of the complexes observed suggests a higher order arrangement of multiple proteins on the DNA segments.

The segments are numbered starting with the 5′-most region and range in size from 87 bp (segment 16) to 184 bp (segment 5). The ability of the DNA segments to form protein-DNA complexes was scored by two criteria: specific complex formation as judged by competition with unlabeled homologous PCR product and the formation of the complexes by one or both of the cell extracts. Each of the segments formed specific complexes (indicated by a bold bracket) except for segments 4 and 6. Of the segments which formed complexes, six (segments 5, 7,
8, 13, 14, and 15) formed specific complexes only with LNCaP extracts (indicated by *). Two findings are apparent from this analysis: 1) protein-DNA complexes are formed at various locations across the entire enhancer, and 2) complexes specific to LNCaP extracts are formed at several locations on the enhancer.

**DISCUSSION**

The studies described above have identified and characterized an enhancer located greater than 3700 bp upstream of the PSA gene and spanning approximately 1500 bp of DNA sequence. Previous studies of PSA transcriptional regulation had localized elements within 631 bp of the cap site which could alter transcription of reporter genes in an androgen-responsive manner (13). However, this activity was observed in non-prostate cells by co-transfection with cloned androgen receptor; the 631-bp 5’-flanking region of the PSA locus was inactive in LNCaP cells, the only prostate cell line which synthesizes PSA. The enhancer described in this work stimulated high levels of reporter genesynthesis in LNCaP cellsviathissamepromoterfragment. Therefore, it is likely that this element forms a major part of the transcriptional regulatory apparatus of the PSA gene.

The upstream enhancer of the PSA gene possesses the classic set of properties used to define enhancer elements (34, 35). The enhancer resides on a DNA segment distinct from the promoter and requires a promoter in order to stimulate transcription initiation. The enhancer can be moved as a unit relative to the promoter and coding sequences and its orientation reversed, both without substantially affecting activity. Typically, enhancers also are promiscuous with respect to the promoter they activate. Likewise, the PSA enhancer can stimulate high levels of transcription via its native promoter or a heterologous promoter.

In addition to their ability to stimulate transcription, enhancer elements often add specific qualities to the overall process of transcription initiation. While promoters are often quite active in multiple cell types, enhancers may either amplify or restrict their activity, depending on the cell type. Thus, the tissue-specific activity of this upstream element further cements its classification as an enhancer.

The PSA gene belongs to the class of androgen-responsive genes. In vivo, PSA expression correlates with and is induced by androgens (2). Treatment of LNCaP cells with dihydroxytestosterone or R1881 results in increased PSA mRNA synthesis.
The potential ARE half-site at −4148 and −4134 conforms to the previously determined ARE (GGTACAnnnTGTT/CCT (31)) at 13 of 15 positions. Insertion of two bases adjacent to the left half-site match diminished activity, while changing the first two bases of the left half-site match to AT from GA markedly increased activity of the enhancer even though the match to the consensus GRE is even lower. A similar effect on AR-mediated transcription was observed by Ham et al. (16) In their study, single base changes in the left half-site of an ARE from the C3(1) gene resulted in both increases and decreases in activity. These data suggest this putative ARE may be functional although further study is required to determine if AR interacts with this site.

The potential ARE half-site at −4079 also resides within the 140-bp region at the 3' end of the enhancer which was shown to be required for activity. Footprinting studies have revealed that this site is protected when the enhancer is bound by proteins from LNCaP extracts. These results indicate a possible functional role for this element.

Additional elements within the enhancer above and beyond the AREs must contribute to PSA expression restricted to the prostate. While genes are regulated by androgens in a variety of tissues, PSA expression is restricted to prostate. The results of this study (see Fig. 7) show that sequences within the 541 bp of the PSA gene will stimulate transcription at low levels in response to androgen; however, this segment is also active in other cell types such as HBL100 (breast carcinoma) and PANC-1 (pancreatic carcinoma). Induction of the enhancer with this promoter segment restricts its activity to PSA secreting LNCaP cells in vitro (Fig. 7) and to LNCaP tumor xenografts in nude mice in vivo (data not shown). This tissue-specific expression is probably mediated by prostate-specific DNA-protein complexes which require other regions of the enhancer in addition to ARE sequences.

Current hypotheses on how tissue-specific regulation is achieved invoke the following: 1) the combinatorial interaction of multiple transcription factors which are more widely distributed and/or 2) the interaction of key tissue-restricted transcription factors with the enhancer (32). In either case, one would expect to observe the interaction of transcription factors with the enhancer to produce a DNA-protein complex specific to LNCaP cells. This is in fact what was observed by EMSA (Figs. 8 and 9). While protein-DNA complexes common to both LNCaP cells and MCF-7 cells (breast cancer) were detected across most of the enhancer, LNCaP-specific complexes were detected in three distinct regions. The 3'-most of these segments encompasses the putative AREs between −4150 and −4000, suggesting DNA-protein complexes containing AR.

If the function of the PSA enhancer is to code for assembly of a multiprotein complex exclusively in prostate epithelial cells, then an important goal is to understand the three-dimensional structure of such a complex. As a key component guiding tissue-specific expression of genes, understanding the behavior of such complexes would provide important insights into the loss of differentiated gene expression in tumor cells during progression. In addition, a clear understanding of the molecular mechanisms of tissue-specific expression will facilitate usage of such DNA elements in applications such as gene therapy and development of novel pharmaceutical agents.

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