

# PART-1: A Novel Human Prostate-specific, Androgen-regulated Gene that Maps to Chromosome 5q12<sup>1</sup>

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## Abstract

Genes regulated by androgenic hormones are of critical importance for the normal physiological function of the human prostate gland, and they contribute to the development and progression of prostate carcinoma. We used cDNA microarrays containing 1500 prostate-derived cDNAs to profile transcripts regulated by androgens in prostate cancer cells. This study identified a novel gene that we have designated *PART-1* (prostate androgen-regulated transcript 1), which exhibited increased expression upon exposure to androgens in the LNCaP prostate cancer cell line. Northern analysis demonstrated that *PART-1* is highly expressed in the prostate gland relative to other normal human tissues and is expressed as different transcripts using at least three different polyadenylation signals. The *PART-1* cDNA and putative protein are not significantly homologous to any sequences in the nonredundant public sequence databases. Cloning and analysis of the putative *PART-1* promoter region identified a potential binding site for the homeobox gene PBX-1a, but no consensus androgen response element or sterol-regulatory element binding sites were identified. We used a radiation hybrid panel and fluorescence *in situ* hybridization to map the *PART-1* gene to chromosome 5q12, a region that has been suggested to harbor a prostate tumor suppressor gene. These results identify a new gene involved in the androgen receptor-regulated gene network of the human prostate that may play a role in the etiology of prostate carcinogenesis.

## Introduction

The AR<sup>3</sup> pathway is a critical mediator of normal and neoplastic prostate development. During human embryogenesis, the AR is expressed in mesenchymal cells of the urogenital sinus with subsequent temporal expression in epithelial cells, leading to a differentiated epithelial phenotype and the production of prostate-specific proteins (1). Testosterone and 5 $\alpha$ -dihydrotestosterone are the primary ligands activating the AR pathway. The majority of prostate cancers initially require androgens for growth, and the elimination of AR ligands by surgical or chemical castration leads to marked tumor regression through a mechanism of programmed cell death (2). However, surviving cancer cells lose their dependency on androgens over time and are capable of proliferation in the absence of serum androgens. The molecular events leading to androgen independence have not been defined, but potential mechanisms include overexpression of the AR,

mutations in the AR gene leading to promiscuous ligand binding, and activation of the AR or downstream regulatory molecules by other endocrine or paracrine growth factors (3, 4).

Despite the importance of androgens for the proliferation and differentiation of normal and neoplastic prostate epithelia, relatively few downstream targets of the AR pathway have been identified. Of the prostate genes shown to be regulated by androgens, the serine proteases PSA and hK2 have proven to be extremely interesting from a biological standpoint, due in part to their prostate-specific expression profile and their potential role in the proliferation and progression of prostate cancers (5).

Our objective in this study was to identify genes expressed in human prostate cells exhibiting transcriptional regulation by androgens. We hypothesize that such genes could be direct mediators of the AR pathway or could be involved in prostate-specific functions that could be exploited for understanding normal and neoplastic prostate growth. We used a cDNA microarray hybridization approach to simultaneously analyze the levels of transcripts corresponding to 1500 different cDNAs derived from human prostate tissues. Transcripts expressed in the androgen-sensitive LNCaP prostate tumor cell line were quantitated for comparison under conditions of androgen depletion and androgen stimulation. Here we report the cloning, chromosomal mapping, and partial characterization of a novel gene, *PART-1* (prostate androgen-regulated transcript 1), which exhibits no significant homology to functional domains of known genes or proteins. *PART-1* is predominantly expressed in prostate, with a low level of expression in the salivary gland, and is regulated by androgens in human prostate cancer cells. These data provide evidence for another member of the prostate androgen response program that may provide further insight into normal and neoplastic prostate development.

## Materials and Methods

**Cell Culture and General Methods.** DNA manipulations including transformation, plasmid preparation, gel electrophoresis, and probe labeling were performed according to standard procedures (6). The prostate carcinoma cell lines LNCaP, DU145, and PC3 were cultured in RPMI 1640 supplemented with 10% FCS (Life Technologies, Inc., Rockville, MD). LNCaP cells were transferred into RPMI 1640 with 10% CS-FCS (Life Technologies, Inc.) 24 h before androgen regulation experiments. This medium was replaced with fresh CS-FCS media or CS-FCS supplemented with 1 nM of the synthetic androgen R1881 (New England Nuclear Life Science Products, Inc.). Cells were harvested for RNA isolation at the 0- and 72-h time points.

**Microarray Fabrication.** A nonredundant set of 1500 prostate-derived cDNA clones was identified from the Prostate Expression Database, a public sequence repository of EST data derived from human prostate cDNA libraries (7). Five hundred of the selected cDNAs were derived from LNCaP cDNA libraries. Individual clone inserts were amplified by the PCR using 2  $\mu$ l of bacterial transformant culture as template with primers BL\_m13F (5'-GTA-AAACGACGGCCAGTGAATTG-3') and BL\_m13R (5'-ACACAGGAAA-CAGCTATGACCATG-3') as described previously (8). PCR products were purified through Sephadryl S500 (Pharmacia), mixed 1:1 with DMSO (Amer-

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<sup>3</sup> The abbreviations used are: AR, androgen receptor; PSA, prostate-specific antigen; hK, human glandular kallikrein; ARE, androgen response element; EST, expressed sequence tag; CS-FCS, charcoal-stripped FCS; RACE, rapid amplification of cDNA ends; FISH, fluorescence *in situ* hybridization; poly(A), poly-adenylated; nt, nucleotide; BAC, bacterial artificial chromosome.

sham), and spotted in duplicate onto coated Type IV glass microscope slides (Amersham) using a Molecular Dynamics GenII robotic spotting tool. After spotting, the glass slides were air-dried and UV-cross-linked with 500 mJ of energy and then baked at 95°C for 30 min.

**Probe Construction and Microarray Hybridization.** Total RNA was isolated from LNCaP cells after 72 h of androgen depletion or supplementation using TRIzol (Life Technologies, Inc.) according to the manufacturer's directions. Polyadenylated RNA was purified using oligodeoxythymidylic acid magnetic beads (Dynal). Fluorescence-labeled probes were made from 1 µg of polyadenylated RNA or 30 µg of total RNA in a reaction volume of 20 µl containing 1 µl of anchored oligodeoxythymidylic acid primer (Amersham); 0.05 mM Cy3-dCTP (Amersham); 0.05 mM dCTP; 0.1 mM each dGTP, dATP, and dTTP; and 200 units of Superscript II reverse transcriptase (Life Technologies, Inc.). Reactants were incubated at 42°C for 120 min, followed by heating to 94°C for 3 min. Unlabeled RNA was hydrolyzed by the addition of 1 µl of 5 N NaOH and heating to 37°C for 10 min. One µl of 5 M HCl and 5 µl of 1 M Tris-HCl (pH 7.5) were added to neutralize the base. Unincorporated nucleotides and salts were removed by chromatography (Qiagen), and the cDNA was eluted in 30 µl of distilled water. One µg of dA/dT 12–18 (Pharmacia) and 1 µg of human Cot1 DNA (Life Technologies, Inc.) were added to the probe, heat-denatured at 94°C for 5 min, combined with an equal volume of 2× microarray hybridization solution (Amersham), and prehybridized at 50°C for 1 h. The mixture was then placed onto a microarray slide with a coverslip and hybridized in a humid chamber at 52°C for 16 h. The slides were washed once with 1× SSC, 0.2% SDS at room temperature for 5 min and then washed twice with 0.1× SSC, 0.2% SDS at room temperature for 10 min. After washing, the slide was rinsed in distilled water to remove trace salts and dried.

**Image Acquisition and Data Analyses.** Fluorescence intensities of the immobilized targets were measured using a laser confocal microscope (Molecular Dynamics). Intensity data were integrated at a pixel resolution of 10 µm using approximately 20 pixels/spot and recorded at 16 bits. Quantitative data were obtained with the SpotFinder V 2.4 program written at the University of Washington. Local background hybridization signals were subtracted before comparing spot intensities and determining expression ratios. For each experiment, each cDNA was represented twice on each slide, and the experiments were performed in duplicate producing 4 data points/cDNA clone/hybridization probe. Intensity ratios for each cDNA clone hybridized with probes derived from androgen-stimulated LNCaP and androgen-starved LNCaP cells were calculated (stimulated intensity:starved intensity). Gene expression levels were considered significantly different between the two conditions if (a) all four replicate spots for a given cDNA demonstrated a ratio of >2 or <0.5, and (b) the signal intensity was greater than 2 SDs above the image background. We have previously determined that expression ratios less than 1.5 are not reproducible in our system (data not shown).

**Northern Analysis.** Ten µg of total RNA were fractionated on 1.2% agarose denaturing gels and transferred to nylon membranes by using the capillary method (6). The human multiple tissue and master blots were obtained from CLONTECH. Blots were hybridized with DNA probes labeled with [ $\alpha$ -<sup>32</sup>P]dCTP by random priming using the Random Primers DNA labeling kit (Life Technologies, Inc.) according to the manufacturer's protocol. Filters were imaged and quantitated by using a phosphor-capture screen and Imagequant software (Molecular Dynamics).

**RACE.** RACE reactions were performed using the human prostate Marathon-ready cDNA cloning kit (CLONTECH) following the manufacturer's instructions. Templates for RACE reactions were prostate Marathon-ready cDNA (CLONTECH) and androgen-stimulated LNCaP cDNA prepared using the Marathon cDNA amplification kit (CLONTECH). Nested 5'-RACE reactions were performed with primers 14D7-196 L (5'-GTGACGGTCTTGGA-CAGTAAGGG-3') and 14D7-85 L (5'-AGAGTATTGTTGGCTTGTCT-GTC-3'), followed by a second reaction using the first reaction product as a template and primers 14D7RC3 (5'-CTTCCCTCCGACAAGGAAGCTG-3') and 14D7RC4 (5'-CTCATCTGTGTTGTTCCAGTGCAGCC-3'). The RACE products were subcloned into PCR2.1-TOPO vectors with the TOPO TA cloning kit (Invitrogen) and sequenced.

**Promoter Cloning by Genomic Walking.** Genomic DNA sequence upstream of the putative *PART-1* transcriptional start site was obtained using the GenomeWalker kit (CLONTECH). Briefly, libraries of adapter-ligated genomic DNA fragments were used as a template for PCR reactions with the *PART-1* gene-specific primer 14D7RC3 and a primer corresponding to the

library adapter sequence AP1 5'-GTAATACGACTCACTATAGGGC-3' according to the manufacturer's instructions. PCR products were cloned into the pCR2.1-TOPO vector and sequenced using M13 forward and M13 reverse primers. Sequences were examined for promoter and potential transcriptional start sites using a neural network promoter prediction program<sup>4</sup> and for transcription factor binding sites using the Transcription Element Search Software program.<sup>5</sup>

**Chromosomal Localization of *PART-1* by Radiation Hybrid Panel Mapping and by FISH.** The G3 Gene bridge radiation hybrid panel (Research Genetics, Huntsville, AL) was used to map the chromosomal localization of *PART-1* with primers 14D7mapR (5'-TGCTTTGTTAAGATGAG-GCAGGC-3') and 14D7mapF (5'-CATTCCAGGTGTCATGGATAAAG-AGC-3'), according to the manufacturer's instructions. After 35 cycles of amplification, the reaction products were separated on a 1.2% agarose gel, and the resulting product pattern was analyzed through the Stanford genome center web server<sup>6</sup> to determine the probable chromosomal location. In addition, a *PART-1* cDNA probe was used to screen an arrayed human BAC genomic library (Research Genetics). Positive clones were identified and confirmed by PCR using primers 14D7mapR and 14D7mapF. BAC DNA was biotinylated by nick translation, prehybridized in the presence of human Cot1 DNA, and hybridized to metaphase spreads of a normal male following procedures described in detail previously (9). After hybridization and washing, the hybridized sites were labeled with fluorescein-conjugated avidin, and the chromosomes were counterstained with 4',6-diamidino-2-phenylindole to produce a QFH-like banding pattern. Images were digitized as described elsewhere (10). Ten well-spread and well-banded metaphases were analyzed to localize the hybridization signals.

## Results

**Identification of a Novel Androgen-regulated cDNA *PART-1* by Microarray Hybridization.** Microarrays comprised of prostate-derived cDNAs were hybridized with total cDNA probes produced from androgen-stimulated and androgen-starved LNCaP prostate cancer cells. Four independent data points for each arrayed cDNA were generated because each cDNA was spotted twice per slide, and each hybridization was performed in duplicate. The hybridization ratios of 10 different cDNAs were consistently increased by >2-fold in androgen-stimulated cells relative to androgen-starved cells. We did not observe any cDNAs with consistent hybridization ratios of <0.5, a ratio that would indicate down-regulated expression. The genes induced by androgens included hK2 and hK3, also known as PSA (Fig. 1A). The regulation of hK2 and PSA expression has previously been shown to be mediated by androgens through a mechanism involving ARE binding sites in the promoter regions of these genes (11, 12). In addition to hK2 and PSA, other cDNAs transcriptionally up-regulated by androgens in the microarray analysis were the serine protease TMPRSS2 (13, 14), the lipid metabolic genes acyl-CoA synthetase 3 and 3-hydroxy-3-methylglutaryl-CoA synthase, and six anonymous ESTs. The expression level of the cDNA clone corresponding to one of these ESTs, 14D7, increased 6-fold in androgen-stimulated LNCaP cells relative to androgen-deprived cells as assayed by microarray hybridization (Fig. 1A). Sequence comparison against the nonredundant nt and protein subdivision of the GenBank database revealed homology only to an uncharacterized partial-length cDNA (GenBank accession number AL050198). We have named this cDNA *PART-1*.

**Androgen-regulated and Prostate-localized Expression of *PART-1*.** The androgen-regulated expression of *PART-1* was confirmed by Northern analysis using the same LNCaP RNA that was used for the microarray analysis. Phosphorimage quantitation of the Northern blot demonstrated a 3.5-fold induction of *PART-1* expression after 72 h of androgen exposure (Fig. 1B). PSA expression

<sup>4</sup> <http://www-hgc.lbl.gov/projects/promoter.html>.

<sup>5</sup> <http://www.cbil.upenn.edu/tess/index.html>.

<sup>6</sup> [www.shgc.stanford.edu](http://www.shgc.stanford.edu).

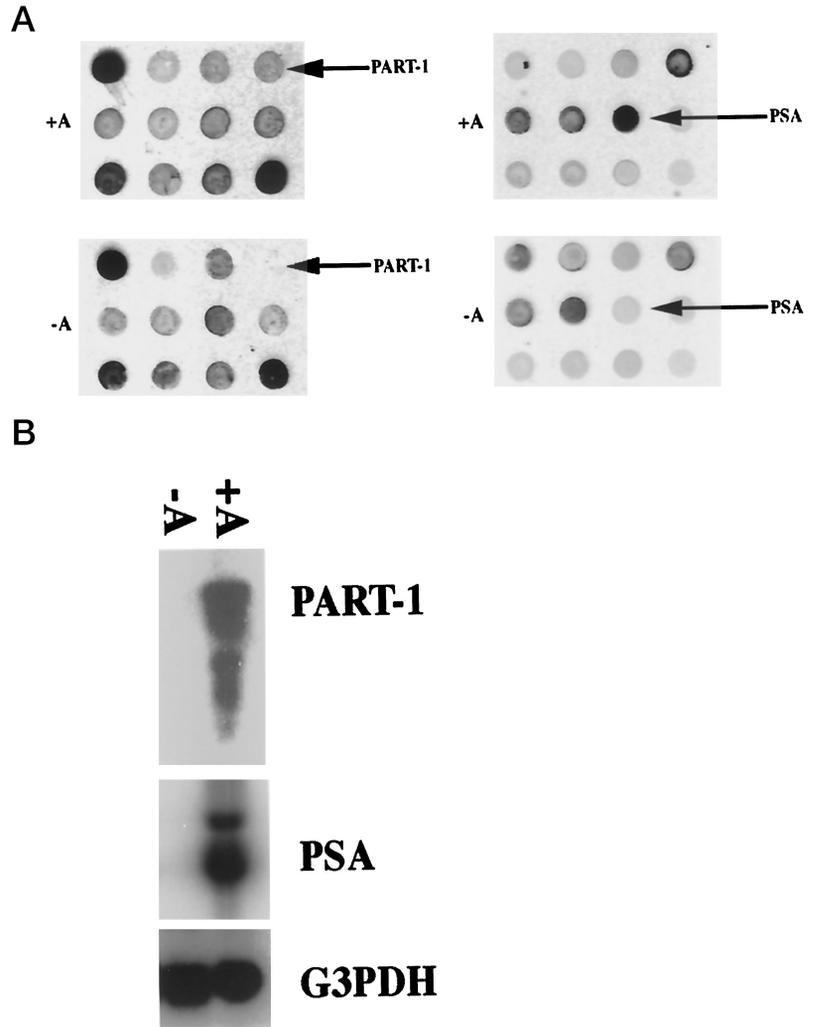


Fig. 1. *A*, cDNA microarray analysis of androgen induction of *PART-1* expression in LNCaP cells. Regions of the 1500 clone microarray are shown containing the *PART-1* and PSA cDNAs following hybridization with labeled total cDNA probes from the LNCaP cell line with (+) or without (-) exposure to androgen. *B*, Northern blot analyses of *PART-1* and PSA androgen-regulated expression. The androgen-stimulated and androgen-starved LNCaP RNA samples used for the microarray experiments were examined for *PART-1* and PSA expression by Northern hybridization. The blot was also hybridized with glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*) probe to verify equivalent RNA loading.

increased 25-fold, and the expression of the *G3PDH* loading control did not change significantly (Fig. 2).

The distribution of *PART-1* transcripts in normal human tissues was determined by Northern analysis. Of 16 adult tissues examined, a *PART-1* message of 2.1 kb was predominantly expressed in the prostate, with no expression detected in the colon, lung, liver, kidney,

pancreas, spleen, thymus, testes, ovary, peripheral leukocytes, heart, brain, placenta, or skeletal muscle (Fig. 2). Additional bands of smaller molecular weight were observed on the Northern blot and may represent cross-hybridization to genes homologous to *PART-1* or represent alternate *PART-1* transcripts as described below. The *PART-1* expression profile was confirmed using a RNA Master dot blot (CLONTECH) comprised of RNA from 50 different tissues (data not shown). *PART-1* expression was detected predominantly in the prostate with a low level of expression in salivary gland tissue, an expression pattern also described for PSA and the prostate-specific membrane antigen (15).

***PART-1* Cloning and Sequence Analysis.** The original *PART-1* cDNA clone contains a 1.8-kb insert with a poly(A) region located at the putative 3'-end of the clone. Two rounds of 5'-RACE from prostate and LNCaP cDNA using *PART-1*-specific primers produced a 300-bp fragment from both cDNA sources. Sequence analyses of eight individual RACE clones selected from both cDNA templates revealed that all overlapped with the original *PART-1* cDNA, and all originated with the same 5' base. A total of 2109 bp was obtained, which corresponds to the 2.1-kb band seen on the Northern blot (Fig. 2). The cDNA sequence was submitted to GenBank under the accession number AF163475.<sup>7</sup>

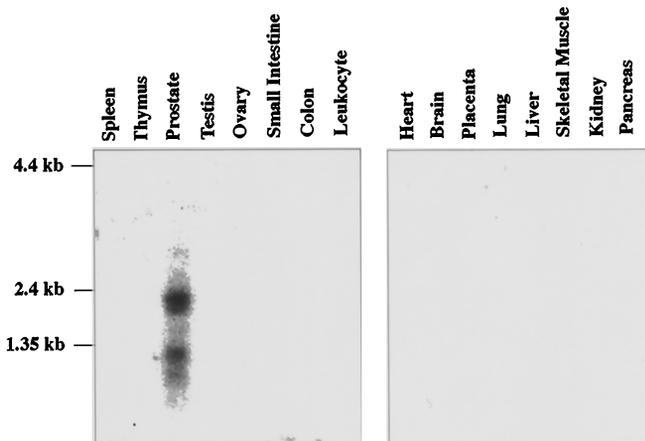


Fig. 2. Northern blot analysis of *PART-1* expression in 16 human tissues. The filters were obtained from CLONTECH and contain 2  $\mu$ g of polyadenylated RNA in each lane. A  $\beta$ -actin control probe was used to verify equivalent loading of RNA (data not shown).

<sup>7</sup> Nucleotide sequence data from this report have been deposited in GenBank under accession numbers AF163474 and AF163475.

The *PART-1* cDNA encodes a putative 60-amino acid protein (Fig. 3). The translational start site AagATGC conforms to the Kozak consensus motif, RNNatgY (where R is a purine and Y is a pyrimidine), in an adequate context (16). Polyadenylation signals, AAUAAA, were identified at nts 633 and 1558 3' of the TAG stop codon. A common natural variant of the polyadenylation signal, AUUAAA (17), was identified at nts 644 and 2054 3' of the TAG stop codon (Fig. 3). Alternate usage of the polyadenylation signals has previously been shown to produce mRNA of different sizes (18). Prolonged exposure of the *PART-1* multitissue Northern blot demonstrated three bands in the prostate lane, and these likely arise from alternate usage of the polyadenylation signals. A search of the database of ESTs identified several ESTs with homology to *PART-1*.

10	20	30	40	50
GATTACTCACACAGTCTTGAAGATGCAATGTCAGCTATTTAGGACAGAAA				
		M	Q	C
		Q	L	F
		R	T	E
60	70	80	90	100
CATCCAAGGCCGTGTCAGAACTCAATTACGACTACATATGCATTAAGGCA				
T	S	K	A	V
S	E	L	N	Y
D	Y	I	C	I
K	A			
110	120	130	140	150
GGAAGTGGCAGGCCTCAGGGTACGCCAACTATAGGACTCGTGTCTCTCGT				
G	T	G	R	P
Q	G	T	P	T
I	G	L	V	L
L	V			
160	170	180	190	200
ACGCTGGGCTATAATCTATGAAACTGAGCTCCAGAGCCCAATCACTT				
R	W	A	I	I
Y	E	T	E	L
Q	S	Q	P	I
T				
210	220	230	240	250
AGCTCCTCATAACAAGTCTAAC TGGCTCTGGAAGCTGAAAGGGCTGCAC				

\*  
 TGGAAACAACAGATGAGATATCTACACATTAATCTACTTATCTGGAAT  
 CACTTTGCCTCTAAAGGCCAGAGAAAAATCACAGCTTCCCTGTGCGGAGGG  
 GAAAAGGACAGGTGATCTGGGAAAAACGACGTACACCTGGAGCAAGGTC  
 TCTCCGGCTTGGCAATCTCAGCTGTGCGCGCTACGGGACCGGACGCC  
 GTCCAGAAAACCAAGGGCAGGCAGCGCAGCAAACGCCTGAGTGTGCTG  
 CCTTCGGTACTATATGAGAATGGAACCTCTAAGGAAGCCAGGTGTGTA  
 GAATTGTTACCCCTTTACTCAGAGATAACATAGATTATCCAGGCTGAGA  
 TGGAAAACAAGCCCTTTATGAAATTTCAACACAGACTCCCTGCTCTCA  
 TCTCCTTAAATAAAATTTCAATTAATAATCCCTTGAACCTCCATGTTCAAA  
 TCTCCATTTGTTGACAGACAAAGCCAACAATACTCTAAACTGAGGCTGCA  
 AGTCATTTTCAATTTGATTTTTGTCCAGAAATTTCCCATAGGAAGACTTCA  
 CCTCTACAACCTCGAAGAAAACCTTACTGTCCAAGACCGTCACCAGCA  
 ACCATCCGCGAGTCAATTCAGTGGAAAGCTTTCACAGCTTTTGTACATTTCT  
 TGTGTCAATATACAACCTGAGTTACAGACTGTCCCTGGCTCCCTGACCC  
 TACAACACTAAAAGTTTTGTTGACTCAACTTCAAGCTGCTCATCTGTT  
 AGTAAAGTGTGTTCACTCCAGAACACATTCATGATGAGAACTTTTAAAA  
 GACCAGCACTGCTCTCCCTCCGCTATAATCATATAATAACTGATCAACTG  
 AAACATGTTACTGGGACTCGACATTTTTCTGGGGATTGAAATCTTTAGTC  
 CTTGGAGCTGTCACATAGCAGGGCAACCTCACACTGAAACAAAGGAAGT  
 GATGTCCCATTTATCCACCCTGAGCCACCATAATATGCTGTTTACATT  
 TATTTCTTCAGCCTGTGCAAAACAAGCAATGGAAAAGGAAACTAAAA  
 ATATACACTAGTACCATTTCTTTCTTTTTCCTTAAATTTACTAATGAC  
 CACGTCAGTCTGCTTCTTCAGGCATCATCTCAATTCATCAGGACTTGT  
 ATTAGCAGGTTCTGGCTAGAGAGACTATCTCCTGTCATCAGATCAATTA  
 ATGTTTTCTGGTATCACATCAGGCCCTATCTAAGAAGCTCATGGTATAC  
 AAGGTCACCCAAATAGCTGAGTGCAGTCCCTGCTCATATTTCTTCTCATC  
 TTAACCCCGCAAACAAGAATTAAGATGATCCCAATAAAAGAAAAATTGCT  
 CAGGAACTGAACCTTTTCTGAAACCAAGCACTGTGACGAAATCTCAGGT  
 ATTAGAGCAACTATGGTTGATTGAAAAGTGTCTCAAAATCTGGGCCAAGA  
 ATGATGTCTAGGTCATAGCTAATTTGCTGTGCTGCTGCTGCTGCTGCTGCTG  
 GCCAAAGAAAGTCACTCATGAGTAAACTATAGAAAAGCTTACAGCCCATC  
 CTGTTAGTATGTCAAATCAACTAAGACTGGCAGGGTATTAACCTCATTCC  
 AGGTGTCATGGATAAAGAGCCCCATTTTTCACAGTGCAGCCCTTACC  
 TAAGGAAACCCTAGACCTTGAACCAAGTTCCTGGTAGGGAAGTCTGAC  
 AGTTTCAATGCTGACAGTTGGAGCCAATGCCTCATAGTGTAAACTGAAAG  
 AAAAAATAGTTGCTTTTAAAAATGTCAGCAAGAAGGCTGCCTCATCTTAA  
 CAAAGCAAAAAAATGCTTTAATTCAAAATTAATAATCATGATACAAAA  
 AAAAA

Fig. 3. *PART-1* cDNA sequence and putative protein translation. The predicted translational start site ATG codon is shown in **bold**. Putative polyadenylation signals ATAAAA and AATAAA are underlined. The 3' polyadenylation signal present in the original *PART-1* cDNA is double underlined.

Several of these sequences, accession numbers AA410580, AA411616, and AA419011, have a poly(A) stretch that would arise from the usage of the AAUAAA signal located 633 nts from the TAG stop codon. EST AL245259 has a poly(A) stretch that would use the AUUAAA signal located 644 nts from the stop codon. EST AL050198 has a poly(A) stretch that would require the usage of the AAUAAA signal 1558 nts from the stop codon. Our *PART-1* cDNA has the longest 3'-untranslated region and would derive from the usage of the AUUAAA signal located 2054 nts from the stop codon.

The putative protein has two protein kinase C phosphorylation sites and one tyrosine kinase site. We used the full-length *PART-1* nt and the putative amino acid sequences to search the National Center for Biotechnology Information nonredundant sequence database using BLAST and BEAUTY algorithms. No significant homology to any database sequence was identified. Queries against the BLOCKS protein motif database<sup>8</sup> (19) revealed only weak homology to the XPG\_1 BLOCK, a motif found in the DNA damage inducible gene Din7 from yeast (20) and in the XPG DNA repair endonuclease (Ref. 21; data not shown).

**Cloning of the Promoter Region of *PART-1* by Genomic Walking.** Genomic walking with primers 14D7RC3 and AP1 produced a 1.3-, a 2.3-, and a 0.8-kb band from the *DraI*, *PvuII*, and *SspI* human GenomeWalker libraries, respectively (data not shown). The 2.3-kb band from the *PvuII* library was cloned and sequenced. A *DraI* site and a *SspI* site were found in the sequences, and these sites would generate the respective 1.3- and 0.8-kb genome walking PCR bands from the *DraI* and *SspI* libraries.

The 2.3-kb sequence overlaps with the 5'-end of the *PART-1* cDNA and extends 1969 bp 5' of the *PART-1* translational start site (GenBank accession number AF163474). A TATA box (TATAAAA) was identified at nt -562 (where +1 is the A of the ATG start codon). A putative transcriptional start site (TGTCTTCAAT) is located 30 nts 3' of the TATA box. We were unable to identify an ARE consensus sequence in the cloned genomic DNA fragment. However, a binding site (TTGATTGAT) for the homeodomain containing protein Pbx-1a (22) is located at nt -978, and a binding site (AAGAGGAAAA) for nuclear factor of activated T cells (23) is located at nt -588 relative to the putative transcriptional initiation site.

**Chromosomal Localization of 14D7.** The Stanford G3 radiation hybrid panel was used to map the chromosome location of *PART-1* using primers 14D7mapR and 14D7mapF. Analysis of the typing results indicated that *PART-1* is located closest to SHGC-14390 on chromosome 5 with a lod score of 8.60 and a cR 10,000 distance of 18 cR. SHGC-14390 is mapped between markers D5S2376 and D52604. The locations of D5S2376, D5S427, and D5S2370 have not been refined beyond 5pter-5qter.<sup>9</sup> The only finely mapped marker in the region, D5S624, was inconclusively mapped either to 5q12-13 or 5pter-5qter.<sup>9</sup>

To more precisely determine the chromosomal location of *PART-1*, we mapped a *PART-1* containing BAC by FISH. Three BACs (370E12, 493B12, and 508J22) were identified by screening a human BAC library (Research Genetics) using a *PART-1* cDNA probe. BAC 493B12 was biotinylated and hybridized to metaphase spreads of a normal male donor. The *PART-1* BAC mapped to proximal chromosome 5q12.1 (Fig. 4). This result should resolve the map location of D5S624 and help to localize other DNA markers in the region.

## Discussion

Androgens influence vital aspects of prostate cellular growth and function including proliferation (24), apoptosis (2), differentiation (1),

<sup>8</sup> <http://www.blocks.fhcr.org>.

<sup>9</sup> <http://www.gdb.org>.

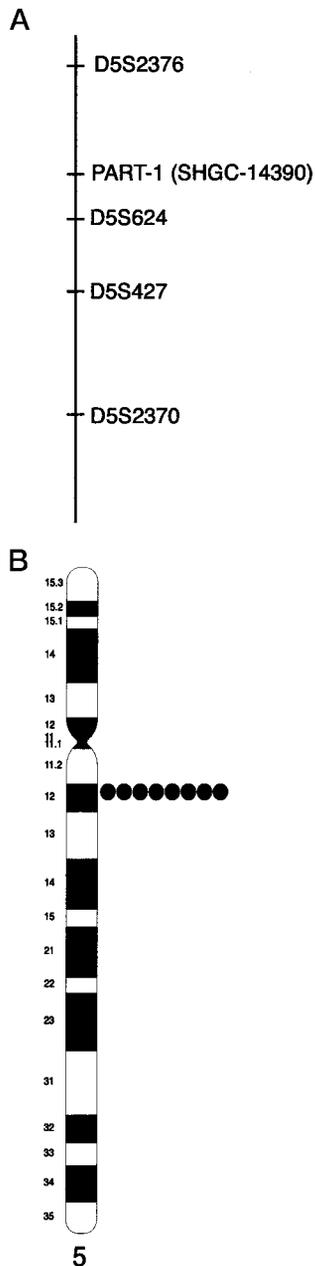


Fig. 4. Localization of *PART-1* to chromosome 5q12.1. *A*, mapping of *PART-1* by PCR typing of the Stanford G3 radiation hybrid panel. *PART-1* is mapped closest to SHGC-14390 on chromosome 5 with a lod score of 8.60. *B*, idiogram of human chromosome 5 and the localization of 14D7 by FISH, summarized from FISH signals observed among 10 mitotic cells hybridized with BAC 493B12 DNA. Each ● represents a double fluorescent signal on chromosome 5q12.1.

lipid metabolism (25), and secretory action (12). Although these processes involve a complex series of metabolic steps, the direct androgen response gene expression program, as assessed by the transcriptional regulation of downstream genes, is limited. Studies designed to identify genes regulated by androgens in the rat prostate estimated that androgens increase the transcription of about 56 genes and decrease the transcription of less than 10 genes (26). This estimate does not include transient alterations in transcript levels, posttranscriptional regulation, or the potential cascade of molecular interactions set in motion by the initial transcriptional alterations.

Using a cDNA microarray-based approach we identified a novel gene, *PART-1*, as an androgen-induced gene in prostate adenocarcinoma cells. We have also shown that *PART-1* exhibits a pattern of

expression essentially restricted to the prostate and salivary glands. The identification of genes with selective expression in specific organs or cell types provides an entry point for understanding biological processes that occur uniquely within a particular tissue. In addition, the identification of genes and their cognate proteins whose expression is specific for the prostate has greatly aided the diagnosis and treatment of prostate carcinoma. The most useful of these proteins to date is PSA, a serine protease expressed by the luminal epithelium of normal prostate tissue and also produced by malignant prostate cells. If the tissue expression profile of the *PART-1* protein corresponds to the transcript expression profile, then *PART-1* may represent an additional target for prostate cancer diagnostic and therapeutic interventions.

The mechanism of androgen-mediated *PART-1* expression remains to be characterized. The lack of a consensus ARE in the 5'-upstream regulatory region suggests that either the AR is interacting with nonconsensus DNA binding sites or an ARE(s) is located further upstream than the proximal 1.9-kb promoter-containing sequence we have analyzed. The AR NH<sub>2</sub> terminus (amino acids 142–485) has been shown to selectively bind to the basal transcription factors TFIIF and the TATA box-binding protein (27). *PART-1* contains a TATA box sequence, and it is possible that the AR can bind to TFIIF and TATA box-binding protein and potentially recruit other transcriptional factors that regulate the expression of TATA box-containing genes, such as *PART-1*. A more likely hypothesis is that *PART-1* may be regulated indirectly by androgens through the expression of an intermediary transcription factor. Such a mechanism has been shown for the regulation of several lipid-metabolizing enzymes that are regulated by sterol regulatory element-binding proteins that in turn are regulated by the activated AR.

The identification of a binding site for the homeodomain protein PBX-1a (22) in the *PART-1* sequence suggests additional regulatory mechanisms. Homeobox genes are known to control the expression of many genes involved in development and organogenesis. PBX-1a may contribute to *PART-1* regulation. A human homeobox gene, NKX3.1, which encodes a protein related to the *Drosophila* NK gene family, has been cloned and is restricted in expression to the prostate and testis (28). Of further interest, NKX3.1 expression is androgen-regulated in LNCaP cells (29). Functional studies will be required to determine whether PBX-1a or NKX3.1 activates *PART-1* transcription. A DNA-binding site sequence for a protein hypothesized to direct prostate-specific gene expression has recently been described (30). However, the proposed prostate-specific binding site sequence, 5'-GAAATATGATA-3', is not present in our cloned *PART-1* sequence upstream of the transcriptional start site. Thus, factors dictating the prostate-specific expression of *PART-1* remain to be defined.

We have mapped *PART-1* to chromosome 5q by radiation hybrid panel mapping and, more specifically, to chromosome 5q12.1 by FISH. Cytogenetic studies have identified deletions in chromosome 5q in prostate cancer cell lines (31) and in 39% of prostate cancer metastasis (32). The introduction of chromosome 5 into metastatic PC3 prostate cancer cells by microcell-mediated transfer decreases the cellular growth rate and suppresses their tumorigenic potential (33). These findings suggest that 5q might contain one or more tumor suppressor genes for human prostate cancer development, and *PART-1* should be investigated as a potential candidate.

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## References

1. Bonkhoff, H., and Remberger, K. Differentiation pathways and histogenetic aspects of normal and abnormal prostatic growth: a stem cell model. *Prostate*, 28: 98–106, 1996.
2. Denmeade, S. R., Lin, X. S., and Isaacs, J. T. Role of programmed (apoptotic) cell death during the progression and therapy for prostate cancer. *Prostate*, 28: 251–265, 1996.
3. Isaacs, J. T., Wake, N., Coffey, D. S., and Sandberg, A. A. Genetic instability coupled to clonal selection as a mechanism for tumor progression in the Dunning R-3327 rat prostatic adenocarcinoma system. *Cancer Res.*, 42: 2353–2371, 1982.
4. Bruchofsky, N., Brown, E. M., Coppin, C. M., Goldenberg, S. L., Le Riche, J. C., Murray, N. C., and Rennie, P. S. The endocrinology and treatment of prostate tumor progression. *Prog. Clin. Biol. Res.*, 239: 347–387, 1987.
5. Rittenhouse, H. G., Finlay, J. A., Mikolajczyk, S. D., and Partin, A. W. Human Kallikrein 2 (hK2) and prostate-specific antigen (PSA): two closely related, but distinct, kallikreins in the prostate. *Crit. Rev. Clin. Lab. Sci.*, 35: 275–368, 1998.
6. Sambrook, J., Fritsch, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.
7. Hawkins, V., Doll, D., Bumgarner, R., Smith, T., Abajian, C., Hood, L., and Nelson, P. S. PEDB: The Prostate Expression Database. *Nucleic Acids Res.*, 27: 204–208, 1999.
8. Nelson, P. S., Ng, W. L., Schummer, M., True, L. D., Liu, A. Y., Bumgarner, R. E., Ferguson, C., Dimak, A., and Hood, L. An expressed-sequence-tag database of the human prostate: sequence analysis of 1168 cDNA clones. *Genomics*, 47: 12–25, 1998.
9. Trask, B. J. *Fluorescence in Situ Hybridization*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1997.
10. Wise, C. A., Clines, G. A., Massa, H., Trask, B. J., and Lovett, M. Identification and localization of the gene for EXTL, a third member of the multiple exostoses gene family. *Genome Res.*, 7: 10–16, 1997.
11. Riegman, P. H., Vlietstra, R. J., van der Korput, J. A., Brinkmann, A. O., and Trapman, J. The promoter of the prostate-specific antigen gene contains a functional androgen responsive element. *Mol. Endocrinol.*, 5: 1921–1930, 1991.
12. Murtha, P., Tindall, D. J., and Young, C. Y. Androgen induction of a human prostate-specific kallikrein, hK2: characterization of an androgen response element in the 5' promoter region of the gene. *Biochemistry*, 32: 6459–6464, 1993.
13. Paoloni-Giacobino, A., Chen, H., Peitsch, M. C., Rossier, C., and Antonarakis, S. E. Cloning of the TMRSS2 gene, which encodes a novel serine protease with transmembrane, LDLRA, and SRCR domains and maps to 21q22.3. *Genomics*, 44: 309–320, 1997.
14. Lin, B., Ferguson, C., White, J. T., Wang, S., Vessella, R., True, L. D., Hood, L., and Nelson, P. S. Prostate-localized and androgen-regulated expression of the membrane-bound serine protease TMRSS2. *Cancer Res.*, 59: 4180–4184, 1999.
15. Troyer, J. K., Beckett, M. L., and Wright, G. L., Jr. Detection and characterization of the prostate-specific membrane antigen (PSMA) in tissue extracts and body fluids. *Int. J. Cancer*, 62: 552–558, 1995.
16. Kozak, M. An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.*, 15: 8125–8148, 1987.
17. Wilusz, J., Pettine, S. M., and Shenk, T. Functional analysis of point mutations in the AAUAAA motif of the SV40 late polyadenylation signal. *Nucleic Acids Res.*, 17: 3899–3908, 1989.
18. Lin, B., Rommens, J. M., Graham, R. K., Kalchman, M., MacDonald, H., Nasir, J., Delaney, A., Goldberg, Y. P., and Hayden, M. R. Differential 3' polyadenylation of the Huntington disease gene results in two mRNA species with variable tissue expression. *Hum. Mol. Genet.*, 2: 1541–1545, 1993.
19. Henikoff, J. G., Henikoff, S., and Pietrokovski, S. New features of the Blocks Database servers. *Nucleic Acids Res.*, 27: 226–228, 1999.
20. Mieczkowski, P. A., Fikus, M. U., and Ciesla, Z. Characterization of a novel DNA damage-inducible gene of *Saccharomyces cerevisiae*, DIN7, which is a structural homolog of the RAD2 and RAD27 DNA repair genes. *Mol. Gen. Genet.*, 253: 655–665, 1997.
21. O'Donovan, A., Scherly, D., Clarkson, S. G., and Wood, R. D. Isolation of active recombinant XPG protein, a human DNA repair endonuclease. *J. Biol. Chem.*, 269: 15965–15968, 1994.
22. Van Dijk, M. A., Voorhoeve, P. M., and Murre, C. Pbx1 is converted into a transcriptional activator upon acquiring the N-terminal region of E2A in pre-B-cell acute lymphoblastoid leukemia. *Proc. Natl. Acad. Sci. USA*, 90: 6061–6065, 1993.
23. Rao, A. NF-ATp: a transcription factor required for the co-ordinate induction of several cytokine genes. *Immunol. Today*, 15: 274–281, 1994.
24. Schuurmans, A. L., Bolt, J., Voorhorst, M. M., Blankenstein, R. A., and Mulder, E. Regulation of growth and epidermal growth factor receptor levels of LNCaP prostate tumor cells by different steroids. *Int. J. Cancer*, 42: 917–922, 1988.
25. Swinnen, J. V., and Verhoeven, G. Androgens and the control of lipid metabolism in human prostate cancer cells. *J. Steroid Biochem. Mol. Biol.*, 65: 191–198, 1998.
26. Wang, Z., Tufts, R., Haleem, R., and Cai, X. Genes regulated by androgen in the rat ventral prostate. *Proc. Natl. Acad. Sci. USA*, 94: 12999–13004, 1997.
27. McEwan, I. J., and Gustafsson, J. Interaction of the human androgen receptor transactivation function with the general transcription factor TFIIF. *Proc. Natl. Acad. Sci. USA*, 94: 8485–8490, 1997.
28. He, W. W., Scivolino, P. J., Wing, J., Augustus, M., Hudson, P., Meissner, P. S., Curtis, R. T., Shell, B. K., Bostwick, D. G., Tindall, D. J., Gelmann, E. P., Abate-Shen, C., and Carter, K. C. A novel human prostate-specific, androgen-regulated homeobox gene (NKX3.1) that maps to 8p21, a region frequently deleted in prostate cancer. *Genomics*, 43: 69–77, 1997.
29. Prescott, J. L., Blok, L., and Tindall, D. J. Isolation and androgen regulation of the human homeobox cDNA, NKX3.1. *Prostate*, 35: 71–80, 1998.
30. Patrikainen, L., Shan, J., Porvari, K., and Vihko, P. Identification of the deoxyribonucleic acid-binding site of a regulatory protein involved in prostate-specific and androgen receptor-dependent gene expression. *Endocrinology*, 140: 2063–2070, 1999.
31. Ozen, M., Navone, N. M., Multani, A. S., Troncoso, P., Logothetis, C. J., Chung, L. W., von Eschenbach, A. C., and Pathak, S. Structural alterations of chromosome 5 in twelve human prostate cancer cell lines. *Cancer Genet. Cytogenet.*, 106: 105–109, 1998.
32. Cher, M. L., Bova, G. S., Moore, D. H., Small, E. J., Carroll, P. R., Pin, S. S., Epstein, J. I., Isaacs, W. B., and Jensen, R. H. Genetic alterations in untreated metastases and androgen-independent prostate cancer detected by comparative genomic hybridization and allelotyping. *Cancer Res.*, 56: 3091–3102, 1996.
33. Ewing, C. M., Ru, N., Morton, R. A., Robinson, J. C., Wheelock, M. J., Johnson, K. R., Barrett, J. C., and Isaacs, W. B. Chromosome 5 suppresses tumorigenicity of PC3 prostate cancer cells: correlation with re-expression of  $\alpha$ -catenin and restoration of E-cadherin function. *Cancer Res.*, 55: 4813–4817, 1995.

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## ***PART-1: A Novel Human Prostate-specific, Androgen-regulated Gene that Maps to Chromosome 5q12***

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