AlbZIP, a Novel bZIP Gene Located on Chromosome 1q21.3 That Is Highly Expressed in Prostate Tumors and of Which the Expression Is Up-Regulated by Androgens in LNCaP Human Prostate Cancer Cells

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ABSTRACT

Androgens play an important role in the development and physiology of the normal prostate as well as in prostate cancer cell proliferation. Comparison of the mRNA expression profiles of control and R1881-treated cultures of LNCaP human prostate cancer cells using cDNA subtraction led to the identification of a novel transcription factor that we named Androgen-Induced bZIP (AlbZIP) protein. AlbZIP is a 395 aa protein with homology to cyclic AMP-responsive element binding protein/activating transcription factor transcription factors. It contains an NH2-terminal activation domain, a central bZIP domain, and a COOH-terminal transmembrane domain. The AlbZIP gene is localized on chromosome 1q21.3 and consists of 10 exons. A major 1.7-kb transcript was detected exclusively in the prostate as well as in breast and prostate cancer cell lines. Androgens up-regulate AlbZIP mRNA and protein levels in a dose-dependent manner. The kinetics of AlbZIP mRNA up-regulation and the results of experiments with cycloheximide suggest that AlbZIP may be a delayed response gene. Immunoreactive AlbZIP protein was primarily detected in the cytoplasm of prostatic luminal epithelial cells. Similarly, full-length AlbZIP-green fluorescent protein fusion proteins were localized in the cytoplasm of LNCaP cells, whereas a truncated form of AlbZIP lacking the putative transmembrane domain was exclusively nuclear. Examination of AlbZIP protein and mRNA expression in a series of transurethral resection of the prostate and needle biopsy specimens indicated that AlbZIP is expressed at higher levels in cancerous prostate cells compared with noncancerous prostate cells. The highly tissue-specific expression profile, androgen regulation, chromosomal localization, and expression profile of AlbZIP in prostate tumors suggest that AlbZIP may play an important role in prostate cancer and in androgen receptor signaling in prostate cells. Future studies will confirm a possible relationship between AlbZIP and prostate cancer.

INTRODUCTION

The AR3 belongs to the superfamily of nuclear steroid hormone receptors, a family of transcription factors that modulate hormone-regulated gene expression (1). The AR gene is localized on the X chromosome (2). The AR gene encodes two functionally equivalent proteins that are translated from different initiation codons (3). Like other nuclear receptors, AR contains distinct functional domains consisting of an NH2-terminal domain, a central DNA-binding domain, and a COOH-terminal ligand-binding domain.

Although a number of cytokines and growth factors have been shown to modulate AR activity, the primary regulators of AR activity are the androgens testosterone and its active metabolite 5α-dihydrotestosterone. These steroid hormones interact with the ligand-binding domain of AR and induce conformational changes that allow AR to interact with transcriptional coregulators of which the specific roles in AR-mediated gene regulation remain to be determined (4). AR can modulate gene expression directly by interacting with specific elements in the regulatory regions of target genes (5) or indirectly by activating various growth factor signaling pathways (6).

Androgens play a determining role in prostate differentiation and development (7). Androgens have been shown to stimulate the growth of prostate cancer cells both in vitro and in vivo, and they are recognized to play an important role in prostate tumor growth (8). Since the original discovery of Charles Huggins in 1941, blockade of androgen formation and action has evolved as the cornerstone of the treatment of prostate cancer (9). Genetic alterations of AR consisting of AR gene amplification (10) and mutations in the AR coding sequence (11) are frequently observed in prostate cancer and are believed to contribute in some manner to cancer progression. In fact, AR mutations are found in several commonly studied human prostate cancer cell lines including LNCaP, CWR22, MDA PCa 2a, and MDA PCa 2b (12–15). Given the importance of androgens and the AR in prostate development and cancer, it is important to identify genes that are regulated by androgens in the human prostate.

Transcription factors of which the expression is regulated by androgen in prostate cells are a particularly interesting class of androgen-responsive genes because they control the expression of a specific subset of androgen-responsive genes. Relatively few androgen-responsive transcription factors have been identified to date. One of these, NKX3.1, is a homeobox gene located on chromosome 8p21 that is believed to play an important role in prostate differentiation and the control of prostate cell proliferation (16, 17). Disruption of NKX3.1 in mice results in dysplasia of the prostatic epithelium (18), and loss of NKX3.1 expression is observed in a high proportion of hormone-refractory and metastatic prostate tumors (19). Androgens also down-regulate the expression of the zinc finger transcription factor early growth response-α (20) and induce the orphan nuclear receptor TR3 (21), c-myc, and nm23 (22) in LNCaP cells. The orphan nuclear receptor TR3 is particularly interesting, because TR3 translocates from the nucleus to mitochondria in response to proapoptotic signals and induces cytochrome c release and apoptosis (23). Down-regulation of EGR-α by androgens in LNCaP cells is consistent with the growth-stimulatory effect of androgens in prostate cells, because the EGR-α protein is nearly identical to transforming growth factor-β-inducible early gene that has been shown to inhibit cell proliferation (24). In fact, EGR-α and transforming growth factor-β-inducible early gene are expressed from alternative promoters of the same gene through the use of alternative first exons (25).
More recently, Xu et al. (26) identified a number of putative androgen-responsive genes using serial analysis of gene expression to compare the expression profiles of control and R1881-treated LNCaP cells. Seven of these genes were transcription factors that were not known previously to be androgen-regulated, including KRAB-associated protein 1, prostate-derived Ets factor, mitochondrial transcription factor 1, a sox-like transcription factor, and the orphan nuclear receptor ear-2, all of which were up-regulated by androgens. Two transcription factors, the homeobox protein Hox B13 and the histone deacetyase CHD4/Mi-2β, were down-regulated by R1881. Whereas the androgen regulation of these genes remains to be confirmed, it is of interest to note that the prostate-specific transcription factor PDEF interacts with AR and enhances androgen-induced activation of the PSA promoter (27).

Our laboratory is interested in identifying androgen-regulated genes in human prostate cancer cells. We hypothesize that these genes may play an important role in androgen-induced prostate development and androgen-dependent prostatic diseases such as prostate cancer. We report here the cDNA cloning, gene structure, expression profile, androgen regulation, and partial characterization of a novel androgen-regulated transcription factor that we have named AlbZIP. AlbZIP is a novel member of the CREB/ATF family of transcription factors that is highly expressed in prostate tumors and of which the expression is up-regulated by androgen in LNCaP cells.

**MATERIALS AND METHODS**

Cloning of AlbZIP. A 299-bp cDNA fragment corresponding to nucleotides 314–612 of the full-length AlbZIP cDNA was isolated from androgen-treated LNCaP human prostate cancer cells using the CLONTECH PCR-Select cDNA Subtraction kit (Clontech Laboratories, Palo Alto, CA) as described previously (28). This fragment, designated A25, was excised as a 354-bp insert from the pCRII plasmid by EcoRI digestion, radiolabeled with [α-32P]dCTP using the DecaPrimer II DNA labeling kit (Ambion, Austin, TX), and used to screen 4 × 107 recombinant plaques of a LNCaP cDNA library. After three rounds of screening, positive clones were excised in vivo directly into the phagemid pBK-CMV and sequenced using the T7 sequencing kit (USB Corporation, Cleveland, OH) and Deaza G/A T7 sequencing mixes (Amersham Pharmacia Biotech, Aylesbury, United Kingdom) and X-ray film. A portion of the GAPDH cDNA (nucleotides 1203–1439) was cloned into pBluescript II KS (+) and radiolabeled with [α-32P]dCTP using T7 RNA polymerase. A probe designated as E4-7 was designed for the purpose of distinguishing the full-length AlbZIP transcript from alternative transcripts that were isolated as cDNA clones. To confirm the required probe, a cDNA fragment encompassing nucleotides 610–890 of the full-length AlbZIP cDNA was cloned into pbilueScript II KS (+) by PCR using oligonucleotides 5'-GATGAGGAGGAAGCCAAGA-3' and 5'-CTTCTGCACAGAACCA-3'. The probe was then biotinylated by nick translation and hybridized in situ to metaphase chromosomes from normal human lymphocytes (29). Chromosomes were counterstained with propidium iodide and 4',6-diamidino-2-phenylindole. After hybridization, the biotinylated probe was detected with avidin-FITC. Images of metaphase preparations were captured digitally using a cooled CCD camera (Biomedical Photometrics, Inc., Waterloo, ON). FISH was performed in Dr. S. Scherer's laboratory in the Department of Genetics at The Hospital for Sick Children, Toronto, Ontario, Canada.

Cell Lines and Culture Conditions. All of the cell lines used in these experiments were obtained from American Type Culture Collection (Manassas, VA). The human breast and prostate cancer cell lines and corresponding culture conditions were described previously (28). Human kidney 293 cells were cultured in MEM containing 10% FCS, 1% nonessential amino acids, 100 µg/ml streptomycin, and 100 IU/ml penicillin.

Experiments designed to characterize the regulation of AlbZIP expression by androgens in LNCaP cells were performed as described (28). In brief, the LNCaP cells used in the time course, R1881 dose-response and cycloheximide/actinomycin D experiments (Fig. 7) were cultured in RPMI 1640 supplemented with hormone-depleted 0.25% FCS for 6 days before each experiment. Under these conditions, LNCaP cells remain in G1 for 24 to 48 h after addition of 0.1 nM of R1881 (data not shown). The LNCaP cells used in the experiments designed to assess the effects of androgens on AlbZIP expression (Fig. 8) were cultured for 3 days in hormone-depleted 2% FCS before each experiment. The medium was changed at 2–3 day intervals. At the start of each experiment, the medium was replaced with identical medium containing the indicated concentrations of compounds. Each experiment was performed at least three times, and all of the experiments were performed in LNCaP cells between passage 27 and 37.

The synthetic androgen R1881 was purchased from NEN Life Science Products Inc. (Boston, MA). The antiandrogen hydroxyflutamide was kindly provided by Schering-Plough Research Institute (Kenilworth, NJ). Actinomycin D and cycloheximide were from ICN Biomedicals, Inc. (Aurora, OH).

Northern Blotting. The human multiple tissue blots (2 µg poly(A) RNA/lane) were obtained from Clontech Laboratories, whereas total RNA was isolated from cell lines using Tri-Reagent (Molecular Research Center, Inc., Cincinnati, OH). Electrophoresis of cancer cell line RNA samples, Northern blotting, hybridization, and autoradiography were performed as described (28). The human tissue and cell line blots were probed with the A25 cDNA probe that was used to screen the LNCaP cDNA library. The human tissue blots were also probed with the full-length AlbZIP cDNA. Radiolabeled cDNA probes for human β-actin (Clontech Laboratories) and human GAPDH were used as loading controls for the human multiple tissue blots and cell line blots, respectively. A portion of the GAPDH cDNA (nucleotides 172–717 of GenBank accession no. M33197) was amplified by PCR using oligonucleotides 5'-ATTGACCTCAACATCAGTGT-3' and 5'-CTTGCCCAACAGCTTG-GCAG-3' and radiolabeled with [α-32P]dCTP.

RNAse Protection Assays. To confirm that clone A25 corresponded to an androgen-responsive transcript, the plasmid containing the A25 cDNA fragment (pCRII-SH2-A25/AS) was used to generate a cRNA probe. The plasmid was linearized with EcoRV, and a 434-bp probe (including 135 bp of vector/ linker sequences) was synthesized using Sp6 RNA polymerase.

To confirm that AlbZIP corresponded to an androgen-responsive gene, we constructed a plasmid (pBSKs-A25-3'UTR/AS) to generate a probe that would hybridize to a region of the AlbZIP transcript that is not recognized by the A25 probe. A cDNA fragment containing the last 64 bp of the open reading frame and the first 173 bp of the 3' UTR of AlbZIP (nucleotides 1203–1439) was cloned into pbilueScript II KS (+) by PCR using oligonucleotides 5'-GATGAGGAGGAAGCCAAGA-3' and 5'-CATAATGCGACTAGGG-CTC-3'. The plasmid was linearized with BamHI, and the 318 bp probe (including 81 bp of vector sequences), designated 3' UTR, was produced using T3 RNA polymerase. The 3' UTR probe was used for most of the experiments presented in this report.

A probe designated as E4-7 was designed for the purpose of distinguishing the full-length AlbZIP transcript from putative alternative transcripts that were isolated as cDNA clones. To generate the required probe, a cDNA fragment encompassing nucleotides 610–890 of the full-length AlbZIP cDNA was cloned into pbilueScript II KS (+) by PCR. The 362 bp E4-7 probe (including 81 bp of vector sequences) was produced using T3 RNA polymerase after linearizing the plasmid (pBSKs-A25-E4-7/AS) with BamHI.

A cRNA probe for β-actin was used as an internal control in all of the RNase protection experiments. A fragment of the β-actin cDNA (nucleotides 704–947 of GenBank accession no. X00351) was amplified by PCR and cloned into the XbaI and KpnI sites of pbilueScript SK. The plasmid was linearized with Ddel, and a 145-bp cRNA probe that protects a 130-bp fragment of the human β-actin mRNA (nucleotides 818–947 of the cDNA) was synthesized using T7 RNA polymerase.

The cRNA probes were labeled with [α-32P]UTP (800 Ci/mmol) using the Riboprobe in vitro Transcription System (Promega Corp., Madison, WI). RNase protection assays were performed using 10 µg of total RNA using the RPA III kit (Ambion, Inc.) according to the manufacturer’s instructions. Protected fragments were separated by electrophoresis in 6% acrylamide 7 M urea gels. The gels were exposed to Hyperfilm MP (Amersham Pharmacia Biotech, Aylesbury, United Kingdom) and X-ray films were quantitated by scanning densitometry using the Bioimage 110 S (Millipore Corp., Bedford, MA). AlbZIP mRNA levels were corrected for β-actin mRNA levels, and the AlbZIP/β-actin ratios in the control cells of each experiment were assigned a value of 1. AlbZIP mRNA levels in treated cells are expressed as AlbZIP/β-actin ratios relative to those in the corresponding control cells.

Expression Vectors. To express AlbZIP as a fusion to the NH2 terminus of EGFP, the coding sequence of AlbZIP was amplified from the full-length
cDNA by PCR and cloned into the BglII and KpnI sites of pEGFP-N3 (Clontech Laboratories). The fusion protein encoded by plasmid pEGFP-N3-AlbZIP contains aa 1–395 of AlbZIP, 10 amino acids (GATGPGSIAT) encoded by vector polylinker DNA, and EGFP. Plasmid AlbZIP (1–290)-EGFP was made by amplifying the first 290 codons of AlbZIP by PCR and subcloning the DNA fragment in-frame with the EGFP open reading frame in plasmid pEGFP-N3.

To express AlbZIP as a fusion to the COOH-terminus of the DNA-binding domain of GAL4, the coding sequence of AlbZIP was amplified from the full-length cDNA by PCR and cloned into the EcoRI and XhoI sites of pM (Clontech Laboratories). The fusion protein encoded by pM-AlbZIP contains aa 1–147 of GAL4, three amino acids (PEF) encoded by vector polylinker DNA, aa 1–395 of AlbZIP, and two residues (SR) that are encoded by the XhoI site that precedes the termination codon. Plasmids expressing GAL4 fused to various deletion mutants of AlbZIP were constructed using conveniently placed restriction sites or PCR. The sequence of each AlbZIP expression plasmid used in our experiments was verified.

**AlbZIP Localization Experiments.** For transient expression of EGFP and EGFP fusion proteins, I × 10⁶ LNCaP cells were seeded in four-well chamber slides (9 × 18 mm; Nalge Nunc International, Naperville, IL) in RPMI 1640. The cells were transfected with pEGFP-N3, pEGFP-N3-AlbZIP, or pEGFP-N3-AlbZIP (1–290) (1 μg DNA/well) 24 h after plating using Lipofectin (Life Technologies, Inc., Rockville, MD). After transfection (24 h), the cells were fixed with Formalin solution (Sigma Chemical Co, Diagnostics, St. Louis, MO). Crystal/Mount (Biomeda Corp., Foster City, CA) was used to mount the coverslips to the slides. Fluorescent proteins were detected by fluorescence microscopy, and photographs were taken using a digital camera (SPOT RT Slider; Diagnostic Instruments, Inc., Sterling Heights, MI) mounted directly on a BX-60 microscope (Olympus, Melville, NY). The stability of AlbZIP-EGFP fusion proteins was verified in human kidney 293 cells. The cells were cultured in MEM, and the corresponding expression plasmids were transfected using ExGen 500 (MBI Fermentas, Amherst, NY). The cells were harvested 24 h after transfection.

**GAL4-AlbZIP Experiments.** Human kidney 293 cells were seeded in MEM containing 10% FCS at 1.5 × 10⁵ cells/well in 12-well plates (21 mm/well; Becton Dickinson Labware, Bedford, MA) 24 h before transfection. Each well was transfected with 1.5 μg of plasmid DNA comprised of 1.125 μg pM plasmid expressing either the GAL4 DNA-binding domain alone, GAL4 fused to AlbZIP, or GAL4 fused to VP16 (pM3-VP16), and 0.25 μg of a GAL4-responsive luciferase reporter plasmid (pGL3-G5E1BTATA). pRLnull (0.125 μg; Promega) was used as an internal control for transfection efficiency. Transfections were performed using Exgen 500 (MBI Fermentas). After 48 h, the cells were lysed in Passive Lysis Buffer (Promega), and Firefly and Renilla luciferase activities were determined using a Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer’s instructions. Firefly luciferase activity values were normalized using Renilla luciferase activity values. The stability of GAL4-AlbZIP fusion proteins was verified in T-47D cells. The cells were transfected with the corresponding plasmids using FuGENE (Roche, Indianapolis, IN) and harvested 48 h after transfection.

**AlbZIP Antibody Preparation.** A synthetic peptide corresponding to aa 379–392 of AlbZIP (KRPSGRIRSVLHA) was synthesized on an ABI 433A peptide synthesizer using FastMoc chemistry. The peptide was purified on a Vydate 22 × 250 mm C18 reverse-phase high-performance liquid chromatography column using a 0.1% trifluoroacetic/acetonitrile gradient. Rabbits were immunized with a total of 0.5 mg of peptide per rabbit.

**Immunoblotting.** For immunoblotting, cells were harvested in lysis buffer [6 mM urea, 20 mM Tris (pH 6.8), 1% SDS, Roche Complete Protease Inhibitor mixture, peptatin, and DTT] and then sonicated. Protein extracts (30 or 40 μg) were separated by SDS-PAGE on 12% or 13.5% acrylamide gels and transferred to nitrocellulose. EGFP fusion proteins, GAL4 fusion proteins, and α-tubulin were detected using the following commercially available primary antibodies: Living Colors full-length A.v. polyclonal antibody (Clontech Laboratories), mouse monoclonal antibody RK5C1 raised against the DNA-binding domain of GAL4 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and mouse IgM antibinulin antibody (Santa Cruz Biotechnology). The following peroxidase-conjugated AffiniPure secondary antibodies were obtained from Jackson ImmunoResearch Laboratories Inc. (West Grove, PA): goat antirabbit IgG, goat antimaus IgM, and goat antimaus IgG. Immune complexes were revealed using the Supersignal Ultra Chemiluminescent detection system (Pierce, Rockford, IL).

**AlbZIP Immunoblot.** To assess AlbZIP protein levels in R1881-treated LNCaP cells, equal amounts of protein extracts (30 μg/lane) were electrophoresed on 12% SDS-PAGE gels and transferred to nitrocellulose filters. The blots were incubated overnight at 4°C with rabbit polyclonal antibody against AlbZIP (diluted 1:1000 in 5% milk and 1 × Tris-buffered saline; 0.9% NaCl and Tris-HCl 10 mM) followed by a 3-h incubation at 37°C with peroxidase-conjugated AffiniPure goat antimaus IgG (Jackson ImmunoResearch Laboratories Inc.). AlbZIP protein levels were quantified using scanning densitometry. AlbZIP protein levels in R1881-treated cells are expressed relative to the levels determined in control LNCaP cells, which were assigned a value of 1. The AlbZIP levels (relative to control) achieved with 0.01, 0.1, and 10 nM R1881 in two independent experiments were within 4% of the calculated average values.

**Prostate Specimens.** The prostate specimens used in our studies were kindly provided by Dr. Jean Emond (Hôtel-Dieu de Lévis, Lévis, Québec, Canada), Dr. Bernard Tétu (Hôtel-Dieu de Québec, Québec City, Québec, Canada), and by Drs. Martin Lemay and Michel Tremblay (Centre Hospitalier de l’Université Laval, Québec City, Québec, Canada). Thirty-seven different prostate specimens were used to evaluate AlbZIP expression by immunohistochemistry and/or in situ hybridization. These tissues consisted of 20 specimens from men undergoing TURP for symptomatic benign prostatic hyperplasia and 17 paraffin blocks of formalin-fixed archival prostatic needle core biopsies displaying infiltrating adenocarcinoma. The TURP specimens were fixed by immersion in 2% glutaraldehyde, 4% formaldehyde, and 3% dextran in 0.05 M phosphate buffer (pH 7.4) for in situ hybridization or in 10% neutral buffered formalin for immunohistochemistry. They were then processed and embedded in paraffin blocks. BT-474 and MDA-MB-231 breast cancer cells were used as controls for the immunohistochemical detection of AlbZIP. The cells were harvested, fixed in 10% neutral buffered formalin, centrifuged in 2% agaro, postfixed in 10% neutral buffered formalin, and embedded in paraffin.

**In Situ Hybridization.** Paraffin sections (4 μm) were collected on Superfrost Plus (Fisher) glass slides. The sections were deparaffinized in toluene, rehydrated through graded alcohol, and then treated with proteinase K [1 μg/ml in 100 mM Tris-HCl, 50 mM EDTA, (pH 8.0)] for 30 min at 37°C. Sections were then immersed in prehybridization buffer [50% formamide, 0.8 M NaCl, 120 mM Tris base (pH 8.0), 8 Mm EDTA, 200 μg/ml salmon sperm DNA, 200 μg/ml RNA, and 4% dextran sulfate] for 2 h at room temperature. The [35S]UTP-labeled 318-bp antisense AlbZIP riboprobe was generated by in vitro transcription using T3 RNA polymerase from pBSKS-A25–3′/UTR/AS linearized with BamHI. The [35S]UTP-labeled 303-bp sense (control) riboprobe was generated by in vitro transcription using T7 RNA polymerase from pBSKS-A25–3′UTR/AS linearized with EcoRI. Sections were incubated overnight at 53°C in hybridization buffer (prehybridization buffer plus 10 mM DTT and 2 × 10⁴ Cpm probe). To remove the excess probe, the sections were washed twice in 50% formamide, 2 × SSC at 58°C for 90 min. The slides were dehydrated in graded alcohol, air-dried, and then dipped in NBT-2 emulsion (Eastman Kodak, Rochester, NY; diluted 1:1 in distilled water) and exposed for 14 days. Slides were developed and counterstained with H&E. The silver grains resulting from hybridization of sense and antisense cRNA probes were counted manually by overlaying photomicrographs with acetate sheets.

**Immunohistochemistry.** Immunostaining was performed using Zymed SP kits (San Francisco, CA). Paraffin sections (4 μm) were deparaffinized in toluene and rehydrated through ethanol. Endogenous peroxidase activity was eliminated by preincubation with 3% H₂O₂ in methanol for 20 min. A microwave retrieval technique using citrate buffer was applied (30). After cooling the slides, nonspecific binding sites were blocked using 10% goat serum for 30 min. Sections were incubated with the rabbit polyclonal AlbZIP antibody diluted 1:400 for 2 h at room temperature, washed in PBS buffer, and incubated with biotinylated antirabbit secondary antibody for 12 min and then with streptavidin-peroxidase for another 12 min. Under microscope monitoring, diaminobenzidine was used as the chromogen to visualize the biotin/streptavidin-peroxidase complexes. Counterstaining was performed using #2 Gill’s hematoxylin for 45 s. For the negative control, the preimmune serum was used at the same dilution as the primary antibody.
The GenBank accession no. of the AIbZIP clone sequence reported here is AF394167.

RESULTS

Cloning of AIbZIP. We used PCR-select cDNA subtraction to identify new androgen-regulated genes in LNCaP human prostate cancer cells that were exposed to the synthetic androgen R1881 for 24 h. The cDNA fragments that were isolated were sequenced to determine their identity to known genes, and novel cDNAs were then used as probes in RNase protection assays to confirm that the corresponding mRNAs were truly androgen-regulated. One of the validated androgen-responsive transcripts identified in LNCaP cells was detected using a probe derived from clone A25, a 299-bp cDNA that did not match any known human gene in the GenBank database.

To obtain a full-length A25 cDNA we screened a LNCaP cDNA library using radiolabeled clone A25 as the probe. Twenty-five cDNA clones were isolated and characterized. The longest cDNA, number A25-15, was 1564 bp in length and contained a 1188-bp open reading frame preceded by a 78-bp 5'-UTR (Fig. 1). A termination codon (TGA) is located 30 bp upstream of and in-frame with the first putative methionine is double underlined. The leucine and cysteine residues that constitute the leucine zipper are shown as white letters in black boxes. The termination codon is indicated by *, and the polyadenylation signal is underlined. The cDNA fragment isolated by cDNA subtraction (clone A25) contained nucleotides 314–612. The cRNA probe used for RNase protection assays contained nucleotides 1203–1439.

Functional Domains of AIbZIP. The putative initiating methionine of cDNA A25-15 is located in a purine-rich region with similarity to a Kozak consensus sequence (5'-AAGCATG-3') that would be predicted to favor translation initiation, whereas the second methionine lies in a less favorable context 110 codons further downstream (31). Assuming that translation initiates at the first ATG codon, the A25-15 open reading frame encodes a 395 amino acid protein. A search of the Conserved Domain Database using RPS-BLAST4 with the amino acid sequence of cDNA A25-15 revealed that it contained a region with extensive similarity to the bZIP domain of CREB/ATF transcription factors (Fig. 2A). For this reason, the protein encoded by cDNA A25-15 was designated AIbZIP.

The bZIP domain of AIbZIP is located between residues 219 and 280 (Fig. 2B). It is comprised of a lysine- and arginine-rich basic region separated from the leucine zipper by a 5-amino acid spacer. A bipartite nuclear localization signal (KKVRRKIRNKQSAQDSRRRRKKE) is contained within the bZIP domain (aa 223–240).
A BZIP domain of AIbZIP is most similar to that of human CREB-H (69% amino acid identity), human CREB3 (62% amino acid identity), and mouse OASIS (54% identity) proteins (32–34). In fact, 17 of 21 amino acids that constitute the basic domain as well as the 5 spacer amino acids present in AIbZIP are identical to those in CREB-H. The leucine zipper of AIbZIP is similar to that of CREB-H. It contains five repeats and consists of five leucine residues and one cysteine residue at the second heptad position (aa 258) of the leucine zipper. CREB3 and OASIS contain tyrosine substitutions at the same position. Twenty-two of the 36 amino acids present between the first and last leucines are identical in AIbZIP and CREB-H. The bZIP domain of AIbZIP is most similar to that of human CREB-H (69% identity) and mouse OASIS (54% identity) proteins (32–34). In fact, 17 of 21 amino acids that constitute the basic domain as well as the 5 spacer amino acids present in AIbZIP are identical to those in CREB-H. The leucine zipper of AIbZIP is similar to that of CREB-H. It contains five repeats and consists of five leucine residues and one cysteine residue at the second heptad position (aa 258) of the leucine zipper. CREB3 and OASIS contain tyrosine substitutions at the same position. Twenty-two of the 36 amino acids present between the first and last leucines are identical in AIbZIP and CREB-H. The bZIP domain of AIbZIP shows less homology (26–35% identity) to the bZIP domains of human ATF3, ATF4, and ATF6 proteins.

On the basis of the similarities between the bZIP domains of AIbZIP, CREB-H, and CREB3, we have assigned AIbZIP to the CREB/ATF subfamily of bZIP proteins (Fig. 2C).

Analysis of the protein sequence of AIbZIP (PSORTII server) also revealed the presence of two putative transmembrane domains (aa 169–191 and 294–314) flanking the bZIP domain. The COOH-terminal TMD of AIbZIP (TSTCVIIILLSFLALIPFS) bears significant homology to the TMD of CREB-H (CVAVLILLSFLALIPFS), aa 323–339). The putative TMD located in the NH2-terminal half of AIbZIP (RAGTVAPVPCCTLLPCQTLFLTD) shows little homology to either the COOH-terminal TMD of AIbZIP or that of CREB-H.

**Gene Structure and Chromosomal Location of AIbZIP.** To determine the genomic organization of the AIbZIP gene we searched the GenBank genomic sequence database and found that the complete sequence of the AIbZIP cDNA is contained in human chromosome 1 clone RP11-422P24 (GenBank accession no. AL358472). Comparison of the genomic and cDNA sequences allowed us to determine the exon-intron junctions of the AIbZIP gene (Table 1). The AIbZIP gene consists of 10 exons ranging from 74 to 493 bp in size. The first exon codes for the 5' UTR of the mRNA, whereas the AIbZIP open reading frame is encoded by exons 2–10. As shown in Fig. 3A, the AIbZIP gene lies in close proximity to the JTB/PAR gene (36, 37). The two genes are arranged in a head-to-head orientation, and the last 94 bp of AIbZIP exon 10 correspond to the last 94 bp of JTB/PAR exon 5. We used FISH to confirm the chromosomal location of the gene encoding AIbZIP. Hybridization of biotinylated BAC clone RP11-422P24 to metaphase chromosomes confirmed that this BAC maps to 1q21.3 (data not shown).

**Table 1 Exon-intron structure of AIbZIP gene**

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<td>10</td>
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<td>492</td>
<td>ctcccaag/TGACTTC</td>
<td>AAAATGT/poly A</td>
</tr>
</tbody>
</table>

* Internet address: http://psort.imperial.ac.uk

**Fig. 2. Features of AIbZIP.** A, a schematic representation of AIbZIP protein. The relative positions of the basic region, putative transmembrane domains (TMD), and leucine (L) and cysteine (C) residues of the leucine zipper are indicated. B, alignment of the bZIP domain of AIbZIP with those of human CREB-H (AB050902), human CREB3 (AF211848), mouse OASIS (AB017614), Drosophila melanogaster BFF-2 (A42140), human ATF-6 (XM_018271), human ATF-4 (NM_001675), and human ATF-3 (L19871). The basic and leucine zipper domains are indicated. Invariant residues are shown in white on a black background. C, a dendogram of representative CREB/ATF proteins, CREB-H, CREB3, and AIbZIP. The dendogram was derived from the complete open reading frames of AIbZIP, CREB-H, CREB3, ATF3, B-ATF (U15460), CRE-BP1 (NM_001880), CREB (M27691), ATF4, and ATF6 using the GCG computer program PILEUP.
Alternative Forms of AlbZIP. ATF/CREB genes frequently produce alternatively spliced transcripts that encode functional proteins (38). The full-length AlbZIP cDNA represents the most common form of AlbZIP transcript in LNCaP cells. Nineteen other cDNAs were characterized as identical in sequence to cDNA A25-15 except that their 5' UTRs varied in length from 32 to 78 bp (median = 44 bp). Two sequence variants of cDNA A25-15 were represented in five other cDNA clones. One variant, form 2 (found in two cDNA clones), had a deletion of nucleotides 764–1043, which corresponds to a complete deletion of exons 7 and 8, as well as partial deletions of exons 6 and 9 (Fig. 3B). The second variant, designated form 3 (found in three cDNA clones), contained intron 5 sequences. Both variants result in frameshifts that would produce truncated proteins lacking all (form 3) or most (form 2) of the bZIP domain. To determine whether these transcripts were expressed in appreciable levels in LNCaP cells, we designed a cRNA probe (E4–7) that would discriminate between the wild-type AlbZIP transcript and the two variants. Neither variant was detected in LNCaP cells (data not shown), and these two clones were not additionally studied.

In addition to these cDNAs, some ESTs contained in the GenBank database (B1087317, BG763732, BF347981, and AL528505) are identical to AlbZIP exon 2 except that they contain an additional 241–277 bp DNA at their 5' end, which suggests that alternative transcripts could be expressed from the AlbZIP gene. On the basis of the available genomic data, this alternative exon is predicted to lie 72 bp 5' to exon 1. We did not isolate a matching cDNA in LNCaP cells, but this alternative transcript would not be predicted to alter the open reading frame of AlbZIP unless other exons were also alternatively spliced.

AlbZIP Localization. To assay the subcellular localization of AlbZIP, the complete open reading frame of AlbZIP was cloned in-frame with sequences encoding EGFP to produce a plasmid expressing AlbZIP as a fusion protein to the NH2-terminus of EGFP (AlbZIP-EGFP). To evaluate the potential role of the COOH-terminal transmembrane domain of AlbZIP we constructed a plasmid that expresses the fusion protein AlbZIP (1–290)-EGFP, which lacks the last 105 amino acids of AlbZIP (Fig. 4A). Plasmids expressing EGFP alone, AlbZIP-EGFP, or AlbZIP (1–290)-EGFP were transiently transfected into LNCaP cells. EGFP alone was distributed evenly in the nuclei and cytoplasm of cells, whereas AlbZIP-EGFP was predominantly localized in the cytoplasm in structures that corresponded to the Golgi apparatus (Fig. 4B). Interestingly, deletion of the COOH-terminal portion of AlbZIP that includes the putative transmembrane domain produced a fusion protein that localized exclusively in the nuclei of transfected cells. These results indicate that a domain responsible for the cytoplasmic localization of AlbZIP resides within its COOH-terminal region. The putative NH2-terminal transmembrane domain does not appear to play an essential role in the cytoplasmic localization of AlbZIP. Immunoblots performed using an antibody against EGFP confirmed that the expression plasmids produced fusion proteins of the expected sizes (Fig. 4C).

Transcriptional Activity of AlbZIP. To determine whether AlbZIP had transcriptional activity we constructed a plasmid expressing full-length AlbZIP as a fusion protein to the COOH terminus of the DNA-binding domain of GAL4. The GAL4-AlbZIP-expressing plasmid was cotransfected with a luciferase reporter plasmid containing GAL4 DNA-response elements into human kidney 293 cells. Preliminary experiments confirmed that GAL4-AlbZIP could indeed activate a GAL4-responsive promoter in a dose-dependent manner (data not shown). To map the activation domain of AlbZIP we then constructed plasmids expressing GAL4-AlbZIP fusion proteins containing various NH2- or COOH-terminal deletions within AlbZIP and tested their ability to activate the GAL4-responsive promoter (Fig. 5A). Deletion of the first 48 or 98 amino acids of AlbZIP completely abrogated GAL4-AlbZIP-induced transactivation of the GAL4-responsive luciferase reporter. These results suggested that the activation domain of AlbZIP resides within its NH2-terminal portion. In fact, experiments performed using fusion proteins that express the first 71 or 100 amino acids of AlbZIP confirmed that the NH2-terminal region of AlbZIP is sufficient for activation. It is interesting to note that these fusion proteins were 30 times more active than the full-length GAL4-AlbZIP fusion protein. Moreover, fusion proteins containing the first 150–280 amino acids of AlbZIP were less active than the 71- and 100-residue fusion proteins. Immunoblots performed using a GAL4-specific antibody confirmed that the proteins that displayed less transcriptional activity were not expressed at lower levels (Fig. 5B). Therefore, we conclude that the transcriptional activity of the AlbZIP activation domain is negatively regulated by the COOH-terminal portion of AlbZIP.

Tissue Expression Profile of AlbZIP. We performed Northern blots to confirm the size of the AlbZIP mRNA and to determine whether other AlbZIP-related transcripts are expressed in LNCaP cells. Total RNA was isolated from LNCaP cells after exposure to 0.1 nM of R1881 for 24 h, and AlbZIP mRNA was detected using radiolabeled A25 cDNA as the probe. As shown in Fig. 6A, the AlbZIP probe detected a major 1.7-kb band in control LNCaP cells. The size of this transcript is in agreement with the length of the longest AlbZIP cDNA we isolated (1564 bp). A less abundant transcript that migrated slightly above the major band and which could correspond to an alternatively spliced AlbZIP transcript was also detected. Exposure of LNCaP cells to R1881 caused a marked increase in the amount of 1.7-kb mRNA as well as in that of the less abundant longer transcript.

To determine the tissue expression profile of AlbZIP we performed Northern blot analysis using polyadenylated RNA purified from 16 human tissues. CLONTECH Human and Human IV multiple tissue
batches were hybridized to the AlbZIP A25 cDNA probe. A 1.7-kb band was detected only in human prostate, whereas a shorter 700-bp band was detected in the colon (Fig. 6B). AlbZIP or AlbZIP-related transcripts were not detected in any other human tissue even after prolonged exposure (6 days) of the blots to autoradiography film. An identical tissue distribution pattern was observed using the full-length AlbZIP cDNA as the probe (data not shown). Although some ESTs containing portions of the AlbZIP cDNA have been isolated in non-prostate cDNA libraries, the absence of detectable full-length AlbZIP transcript in tissues other than the prostate suggested that AlbZIP might be a prostate-specific gene.

To additionally test this hypothesis we performed RNase protection assays using the 3' UTR cRNA probe to determine the expression profile of AlbZIP mRNA in human breast and prostate cancer cell lines. In addition to LNCaP cells, AlbZIP mRNA was detected in the androgen-insensitive prostate cancer cell lines DU145 and PC-3 (Fig. 6C). This finding indicates that AlbZIP expression is not exclusively regulated by androgens, because DU 145 and PC-3 cells do not express AR. Interestingly, AlbZIP mRNA was also detected in all of the human breast cancer cell lines tested except for MDA-MB-231 cells. As in prostate cancer cells, AlbZIP expression in breast cancer cells was not strictly dependent on AR expression, because some of the cell lines that express AlbZIP do not express AR. The full-length 1.7-kb AlbZIP transcript was also detected by Northern blot analysis in those cell lines in which AlbZIP mRNA was most abundant (data not shown). We did not verify whether AlbZIP is expressed in normal mammary cells. However, based on these data, it is possible that AlbZIP expression is restricted to hormone-sensitive tissues, i.e., the prostate and mammary gland.

Androgen Regulation of AlbZIP Expression in LNCaP Cells.

To additionally characterize the regulation of AlbZIP expression by androgens we assessed the effect of R1881 on AlbZIP mRNA levels as a function of time and dose. AlbZIP mRNA levels were determined in LNCaP cell cultures between 1 and 72 h after the addition of 0.1 nM of R1881. This dose of R1881 was selected because it is the same dose that was used to treat LNCaP cultures marked up to 12 h after the addition of 0.1 nM of R1881. This dose of R1881 was selected because it is the same dose that was used to treat LNCaP cultures that served for the cDNA subtraction. As shown in Fig. 7A, AlbZIP mRNA levels (expressed as AlbZIP:β-actin ratios) did not change markedly up to 12 h after the addition of 0.1 nM of R1881. However, 2–3-fold changes in AlbZIP mRNA levels were observed starting 24 h after the addition of R1881. Similar results were observed in two other experiments.

The induction of AlbZIP expression by R1881 was less rapid than that of other androgen-responsive genes such as NKX3.1 (17) and PSA (28), which suggests that AlbZIP is not an early response gene. Therefore, we examined the effect of the protein synthesis inhibitor cycloheximide and the RNA synthesis inhibitor actinomycin D on R1881-induced AlbZIP expression in LNCaP cells. The effects of actinomycin D and cycloheximide were compared...
with that of Casodex, an AR antagonist. The cell culture conditions and the dose of R1881 used in this experiment were identical to those used for the cDNA subtraction experiment and the time course experiment. In the experiment shown in Fig. 7B, R1881 caused a 2-fold increase in AIbZIP mRNA levels at 24 h. This effect was completely blocked by actinomycin D (1 \( \mu \)g/ml), cycloheximide (10 \( \mu \) g/ml), and Casodex (3 \( \times \) 10\(^{-6}\) M), which suggests that androgens regulate AIbZIP expression indirectly. The dose of R1881 that we used to isolate androgen-responsive transcripts by cDNA subtraction (0.1 nM) reproducibly caused a 2–3-fold increase in AIbZIP mRNA levels in several independent experiments (Figs. 6A, and 7, A and B). To determine whether higher doses of R1881 could produce a correspondingly larger increase in AIbZIP mRNA levels, LNCaP cells were cultured for 24 h in the presence of 0.001–10 nM R1881. As shown in Fig. 7C, 1 nM of R1881 and 10 nM of R1881 caused ~5- and 6-fold increases in AIbZIP mRNA levels, respectively, compared with a 2.3-fold increase in cells exposed to 0.1 nM of R1881.

We performed two additional experiments to examine the effect of R1881 on AIbZIP protein levels in LNCaP cells. The cells were grown under the same culture conditions that were used for the RNA experiments presented in Fig. 7C. Protein extracts were prepared from cells that were exposed to increasing doses of R1881 for 24 h, and AIbZIP protein levels were determined using a rabbit polyclonal antibody raised against a COOH-terminal peptide of AIbZIP (aa 379–392, KPRPSGRIRSVLHA). This polyclonal antibody is specific for AIbZIP, because it recognizes a protein of the expected size (Mr \( \approx \) 43,000) in BT-474 cells, which express high levels of AIbZIP mRNA, whereas no protein was detected in MDA-MB-231 cells that express low levels of AIbZIP mRNA (see Fig. 6C). Preimmune serum did not detect AIbZIP (data not shown). R1881 elicited nearly identical dose-related increases in AIbZIP protein levels in two independent experiments (Fig. 7D). A 2.9-fold (average of two experiments) increase in AIbZIP protein levels was observed at the dose of 1 nM of R1881.
Casodex did not completely block the effect of 10 nM of R1881. These results provide additional confirmation that R1881 regulation of AlbZIP is mediated by the AR. It is of interest to note that the AR expressed in LNCaP cells contains a mutation in its hormone-binding domain that alters the ligand-binding specificity of the AR and which imparts “androgen-like” activity to antiandrogens such as hydroxyflutamide (12). Because such aberrant behavior can result from specific ligand-induced alterations in receptor conformation, it is of interest to identify genes that may be differentially regulated by androgens and antiandrogens to gain some insight into AR structure-function relationships, AR-coactivator interactions, and target genes. Therefore, LNCaP cells were cultured in the presence of increasing concentrations of either R1881 (0.001–10 nM), hydroxyflutamide (0.001–500 nM), or Casodex (0.001–500 nM) for 48 h. The cells were harvested after 48 h, and RNA was isolated and processed for determination of AlbZIP mRNA levels by RNase protection assay. As expected, R1881 up-regulated AlbZIP mRNA levels, whereas Casodex did not (Fig. 8B). Surprisingly, hydroxyflutamide, which up-regulated PSA mRNA levels under these conditions (data not shown), did not markedly increase AlbZIP mRNA levels.

AlbZIP mRNA Expression in Human Prostate. To evaluate AlbZIP mRNA localization and expression in the prostate we performed in situ hybridization. AlbZIP mRNA was not detected in noncancerous human prostate (Fig. 9, A and B). In fact, in normal prostate, the hybridization pattern obtained with the AlbZIP antisense probe was similar to that obtained with the control sense probe. In both cases, scattered silver grains were equally distributed throughout the epithelium, stroma, and lumen. The number of silver grains overlying epithelial cells that were generated by the antisense probe was similar to that obtained with the control sense probe (75 versus 80 in the specimen presented in Fig. 9). In contrast, hybridization of the antisense probe to sections of prostate adenocarcinoma revealed a more intense and epithelial-specific labeling (Fig. 9C). The number of silver grains overlying epithelial cells obtained with the antisense probe was approximately twice that obtained with the control sense probe (574 versus 236 in the specimen presented in Fig. 9). The results presented in Fig. 9 are representative of experiments performed on 10 sections of normal prostate and 11 sections of cancerous prostate. These results suggested that AlbZIP could be expressed at higher levels in prostate cancer cells compared with normal prostate.

Modulation of AlbZIP Expression by Antiandrogens. As shown in Fig. 8A, 3 × 10⁻⁶ M Casodex completely reversed up-regulation of AlbZIP mRNA levels induced by doses of R1881 ranging from 0.001 to 0.1 nM. On the other hand, the same dose of Casodex did not completely block the effect of 10 nM of R1881. These results provide additional confirmation that R1881 regulation of AlbZIP is mediated by the AR. It is of interest to note that the AR expressed in LNCaP cells contains a mutation in its hormone-binding domain that alters the ligand-binding specificity of the AR and which imparts “androgen-like” activity to antiandrogens such as hydroxyflutamide (12). Because such aberrant behavior can result from specific ligand-induced alterations in receptor conformation, it is of interest to identify genes that may be differentially regulated by androgens and antiandrogens to gain some insight into AR structure-function relationships, AR-coactivator interactions, and target genes. Therefore, LNCaP cells were cultured in the presence of increasing concentrations of either R1881 (0.001–10 nM), hydroxyflutamide (0.001–500 nM), or Casodex (0.001–500 nM) for 48 h. The cells were harvested after 48 h, and RNA was isolated and processed for determination of AlbZIP mRNA levels by RNase protection assay. As expected, R1881 up-regulated AlbZIP mRNA levels, whereas Casodex did not (Fig. 8B). Surprisingly, hydroxyflutamide, which up-regulated PSA mRNA levels under these conditions (data not shown), did not markedly increase AlbZIP mRNA levels.

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AlbZIP Protein Expression in Prostate Carcinoma. We used a rabbit polyclonal antibody raised against aa 379–392 of AlbZIP to assess AlbZIP protein expression by immunostaining. As shown in Fig. 10, this polyclonal antibody is specific for AlbZIP, because it produced a strong reaction in BT-474 cells, which express high levels of AlbZIP protein, whereas no reaction was detected in MDA-MB-231 cells that do not express AlbZIP protein (see Fig. 7D). As expected, the preimmune serum did not produce a detectable reaction (Fig. 10B).

We then assessed AlbZIP protein expression in prostate needle biopsy specimens that contained both noncancerous and cancerous prostate cells. Immunoreactive AlbZIP expression was low (Fig. 11A) or undetectable (Fig. 11C) in noncancerous prostate tissue. In contrast, AlbZIP protein levels were appreciably higher in prostatic carcinoma cells compared with the adjacent noncancerous tissue (Fig. 11A, and compare Fig. 11C to Fig. 11D). The immunostaining reaction was primarily localized in the cytoplasm of luminal epithelial cells. In noncancerous cells, the reaction was detected in structures that could correspond to the Golgi apparatus (see inset in Fig. 11A). In cancerous cells, a more intense reaction was detected in the cytoplasm, especially in the apical portions of these cells. A total of 20 noncancerous TURP specimens and 15 adenocarcinoma-containing needle biopsy specimens were examined. The noncancerous cells present in these specimens exhibited low or undetectable expression of AlbZIP similar to that presented in Fig. 11, A and C. All 15 of the tumor-containing specimens exhibited an expression pattern similar to that presented in Fig. 11, A and D.

**DISCUSSION**

**AlbZIP** is a new gene that we discovered using cDNA subtraction to identify androgen-induced transcripts in LNCaP human prostate cancer cells. The full-length human AlbZIP cDNA encodes a 395-amino acid protein of which the outstanding feature is the presence of a bZIP domain, hence the name AlbZIP. BZIP domains are comprised of a region of basic amino acids (bZIP) that is located immediately NH₂-terminal to a “leucine zipper” (bZIP), i.e., repeats of leucine residues that are separated by six amino acids (39). These domains, which mediate both DNA binding and protein-protein interactions, are present in proteins of the bZIP superfamily, which includes the ATF/CREB family of transcription factors.

Several characteristics of AlbZIP are unique. First, the structure of AlbZIP differs from that of most known ATF/CREB proteins. The sequence of the bZIP domain of AlbZIP is most closely related to those of CREB-H (32) and CREB3 (33), two recently identified and relatively understudied members of the ATF/CREB family. The sim-
ABZIP EXPRESSION AND REGULATION IN PROSTATE CANCER

A paraffin section of MDA-MB-231 cells. No immunostaining reaction was detected. A, staining reaction can be detected in the cytoplasm of most cells. B, paraffin section of BT-474 cells incubated with the preimmune serum. No staining reaction can be detected. C, the antibody used in A was used to detect ABZIP in a paraffin section of MDA-MB-231 cells. No immunostaining reaction was detected.

Immunohistochemistry and GFP fusion protein localization experiments revealed that ABZIP is primarily a cytoplasmic protein. The protein appears to be localized in structures related to the secretory pathway such as the Golgi apparatus. Whereas this finding will need to be confirmed using more precise localization techniques such as electron microscopy, this characteristic of ABZIP is particularly interesting considering that the closely related CREB-H and CREB3 proteins, as well as ATF6, are associated with the endoplasmic reticulum. Like ABZIP, all three of the proteins contain COOH-terminal transmembrane domains, which, when deleted, cause the recombinant proteins to localize to the nucleus (32, 40, 41). Despite their highly homologous bZIP and transmembrane domains, a few structural features distinguish ABZIP from CREB-H. First, CREB-H contains a second COOH-terminal leucine zipper (aa 411–432) anchored by four leucine residues that is not present in ABZIP. Second, CREB-H contains a COOH-terminal KDEL-like sequence (GDEL; aa 458–461) that can function as an endoplasmic reticulum retrieval sequence (42). A similar motif is not present in ABZIP.

Given the fact that ABZIP possesses the functional domains and activity of a transcription factor, it is highly possible that ABZIP translocates to the nucleus to regulate gene expression in response to specific though as yet undetermined stimuli. In support of this hypothesis, during the endoplasmic reticulum stress response (unfolded protein response), ATF6 translocates from the endoplasmic reticulum to the nucleus where it activates genes that encode molecular chaperones (43–45). Haze et al. (40) found that this process involves cleavage of ATF6 from a mature M90,000 polypeptide to a nuclear-localized M50,000 form of ATF6 that includes the bZIP and activation domains. Thus, it is conceivable that ABZIP could exist in a transcriptionally inactive form in cytoplasmic compartments and that removal of the COOH-terminal “anchor” would activate ABZIP.

One unique feature of ABZIP is that androgens induce its expression in LNCaP cells. To our knowledge, ABZIP is the first example of a human CREB/ATF protein of which the expression is regulated by androgen. Most CREB/ATF proteins are regulated post-translationally, primarily through phosphorylation (38) or, as demonstrated recently for ATF6, by proteolysis (40). Contrary to CREB, ABZIP does not contain protein kinase A phosphorylation sites nor does it contain a kinase-inducible domain (38). CREB/ATF proteins may also mediate the effects of androgens in other androgen-sensitive tissues. Kim et al. (46) reported recently that CREB mRNA levels increase in the submandibular glands of rats treated with testosterone. Induction of bZIP proteins by steroid hormones has also been observed in mammary epithelial cells, hXBP-1, a bZIP transcription factor more closely related to jun proteins, was found to be up-regulated by estrogen in MCF-7 human breast cancer cells (47). Thus, bZIP transcription factors may play an important role in steroid hormone-dependent cellular events in hormone-sensitive tissues.

One particularly interesting characteristic of ABZIP is its highly restricted expression profile. On the basis of Northern blot analyses, full-length ABZIP mRNA is only expressed in the human prostate as well as in breast and prostate cancer cell lines. Indeed, the shorter transcript (0.7 kb) expressed in the colon is unlikely to encode full-length ABZIP. This highly tissue-specific expression profile additionally supports the hypothesis that ABZIP may mediate steroid-responsive events in these tissues. However, ABZIP expression is not exclusively responsive to androgen in LNCaP cells, because ABZIP mRNA was detected in the absence of androgenic stimulation. In preliminary experiments conducted to date, ABZIP expression was not modulated by steroid hormones in human breast cancer cell lines.
JTB/PAR gene. The fact that these genes are arranged head-to-head, possibly involve common regulatory elements or, alternatively, some regulation of JTB/PAR and AIbZIP expression by androgens could possibly result in the loss of expression but down-regulate JTB/PAR expression, suggesting that regulation of this cluster of androgen-responsive genes.

In summary, we have identified a new androgen-induced transcription factor that is a novel member of the CREB/ATF family of bZIP transcription factors. Additional study of the role of androgen-responsive transcription factors such as AIbZIP should contribute significantly to our understanding of androgen action in the prostate and prostate cancer.

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REFERENCES


Fig. 11. AIbZIP protein expression in two prostate needle biopsy specimens displaying infiltrating adenocarcinoma (×600 magnification). A and B are two consecutive serial sections from one specimen, whereas C and D are two different areas from a single section of a second specimen. Noncancerous tissue is labeled “N,” whereas cancerous tissue is labeled “C.” A, the cytoplasm of noncancerous epithelial cells is weakly labeled, whereas a strong immunostaining reaction is detected in all cancerous cells. The two noncancerous cells identified by arrows are additionally magnified in the inset (top left hand corner of A). The staining reaction is detected in the Golgi apparatus. B, no reaction was detected when the preimmune serum was used. C, AIbZIP protein was not detected in the noncancerous cells of this specimen. D, a strong immunostaining reaction was detected in the cancerous cells present on an area adjacent to that shown in C.


AlbZIP, a Novel bZIP Gene Located on Chromosome 1q21.3 That Is Highly Expressed in Prostate Tumors and of Which the Expression Is Up-Regulated by Androgens in LNCaP Human Prostate Cancer Cells

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