Androgen-independent Induction of Prostate-specific Antigen Gene Expression via Cross-talk between the Androgen Receptor and Protein Kinase A Signal Transduction Pathways*

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Transcription of the prostate-specific antigen (PSA) gene escapes regulation by androgens in advanced prostate cancer. To determine the molecular mechanism(s) of androgen-independent regulation of the PSA gene, the possibility that the androgen receptor (AR) is activated in the absence of androgen by stimulation of protein kinase A (PKA) was investigated. Activation of PKA by forskolin resulted in elevated expression of the PSA gene in androgen-depleted LNCaP cells, an effect that was blocked by the antiandrogen, bicalutamide. Further evidence that induction of PSA gene expression was dependent on AR was obtained from experiments using PC3 cells devoid of AR. Neither PSA, PB, nor ARR3, androgen-responsive reporters could be induced by activation of PKA in the absence of transfected AR. In addition, when nuclear AR from forskolin-treated LNCaP cells was incubated with oligonucleotides encoding an androgen response element of the PSA promoter and examined by electromobility shift assay, an increase in AR-androgen response element complex formation was observed. Lastly, cotransfection of an expression vector for a chimeric protein encoding the amino-terminal domain of the human AR linked to Gal4 and a 5xGal4UAS reporter gene construct resulted in activation of the amino-terminal domain of the AR by stimulation of PKA activity. These results demonstrate androgen-independent induction of PSA gene expression in prostate cancer cells by an AR-dependent pathway.

The androgen receptor (AR)† belongs to the superfamily of nuclear receptors that mediates the actions of lipophilic ligands, including steroids, retinoids, vitamin D3, and thyroid hormones (1). These receptors have distinct functional domains that include a carboxyl-terminal ligand binding domain, a highly conserved DNA binding domain (DBD) comprising two zinc finger motifs, and a poorly conserved amino-terminal domain that may contain one or more transcriptional activation domains. Binding of ligand to the receptor results in activation or transformation such that the receptor can effectively bind to its specific DNA element. The mechanism of ligand-induced transformation of the AR is not clear, although it is known that the conformation of the AR becomes more compact upon ligand binding, heat shock proteins are dissociated, and dimerization and phosphorylation occur before DNA binding (2). Thus, the ligand-activated AR may stimulate or repress androgen-regulated genes. However, it has been suggested that the AR can also be transformed in the absence of androgen by elevation of cAMP levels and by growth factors (3–5). The mechanism of such ligand-independent activation of AR has not been clarified but may involve the bypassing of one of the above-mentioned processes associated with ligand-dependent transformation. Of these, phosphorylation has been implicated in the ligand-independent activation of the progesterone, estrogen, and retinoic acid receptors. On the other hand, although there are three identified phosphorylation sites on the AR, its phosphorylation does not appear to be essential for the induction of androgen-regulated genes (6).

Prostate-specific antigen (PSA) is a clinically important androgen-stimulated gene that is used to monitor treatment responses, prognosis, and progression in patients with prostate cancer. The transcriptional regulation of PSA is initially androgen-regulated and undergoes a sharp decline after medical or surgical castration (7). When the tumor becomes androgen-independent, PSA mRNA is constitutively up-regulated through an unknown mechanism that presumably involves the promoter and enhancer regions of the PSA gene. These regions have been sequenced as far as –5824 from the start site of transcription (8, 9), and the following DNA response elements have been characterized: 1) TATA box, −28 to −23 (10); 2) androgen response elements (AREs), −170 to −156 (10) and −4148 to −4134 (9); and 3) androgen response region (ARR), −395 to −376 (11). The fact that PSA production ultimately increases in an androgen-deprived environment suggests that other factors not directly related to androgens but possibly acting via the AR become paramount, leading to androgen-independent induction of PSA gene expression.

In the present study, the possibility that androgen-independent induction of PSA gene expression by cross-talk between the AR and PKA signal transduction pathways was investigated in prostate cancer cell lines. The experiments confirmed that PSA gene expression was induced by activation of PKA and demonstrated, for the first time, activation of the amino terminus of the AR by stimulation of PKA activity.

MATERIALS AND METHODS

Cell Culture—All chemicals were purchased from Sigma, unless stated otherwise. PC3 cells between the 30th and 45th generation were maintained in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal bovine serum (Life Technologies, Inc.). LNCaP cells between

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† The abbreviations used are: AR, androgen receptor; DBD, DNA binding domain; PSA, prostate-specific antigen; ARE, androgen response element; ARR, androgen response region; tk, thymidine kinase; PB, probasin; PKA, protein kinase A; PKAc, catalytic subunit of protein kinase A; EMSA, electrophoretic mobility shift assay.
the 44th and 55th generation were maintained in RPMI 1640 supple-
mented with 5% fetal bovine serum. When the plates or wells were 60–70% confluent with cells, the culture medium was changed to se-
rum-free medium containing vehicle (MeSO), R1881, or forskolin.

Northern Blot Analysis—Total RNA was extracted from LNCaP cells with Trizol® (Life Technologies, Inc.) and fractionated by elec-
trophoresis before blotting onto Hybond-N+ filters (Amersham Pharmacia Bio-
tech). The 1.4-kilobase pair EcoRI fragments of the PSA cDNA and 18 S RNA were labeled with [α-32P]dCTP by Random Primers DNA label-
ing kit (Life Technologies, Inc.). Hybridization was performed according to the method described by Sato et al. (7). The mRNA bands were quantified with the STORM 860 PhosphorImager (Molecular Dynam,
ics, Sunnyvale, CA).

PSA Promoter Plasmid Constructs—PSA 5′-flanking DNA (−630/ +12) was obtained by polymerase chain reaction–mediated amplification of human genomic DNA using oligonucleotide primers corresponding to the PSA gene and ligated with EcoRV-digested pBluescript (pBS) (Stratagene, La Jolla, CA) as described previously (12).

Other Plasmids—The expression plasmid for full-length wild-type human AR was a gift from Dr. A. O. Brinkman (Erasmus University, Rotterdam, The Netherlands), and the expression plasmid for full-
length wild-type rat AR has been described (12). ARR3-tk-luciferase reporter construct consists of three congruent rat probasin AREs (−244 to −96) ligated in tandem into the HindIII site of the pBluescript vector (Stratagene, PathDetect CREB trans-Reporting System).

Transient Transfections and Luciferase Activity Assay—LNCaP cells (3 × 10⁴) were plated on 6-well plates and incubated in RPMI 1640 with 5% fetal bovine serum before transfection as described previously (12). The total amount of plasmid DNA used was normalized to 3 μg/well by the addition of empty plasmid. Medium was replaced after 24 h by serum-free RPMI 1640 containing MeSO, R1881, or forskolin. Cells were incubated after 48 h of incubation. Luciferase activity, measured in cell lysates was measured using the Dual Luciferase assay system (Promega, Madison, WI). The protein concentration of the cell lysates was determined by the method of Bradford (15). Luciferase activities were normalized by the Renilla activities and protein concentrations of the samples. The results are presented as the fold induction, which is the relative luciferase activity of the treated cells over that of the control cells. All transfection experiments were carried out in triplicate wells and repeated 2–8 times using at least 2 sets of plasmids prepared separately.

Immunoblots—LNCaP cells were incubated in RPMI 1640 (serum-
free) for 24 h before the addition of vehicle (0.01% MeSO), 10 nM R1881, or 1 μM forskolin. After incubation with compounds, whole cell lysates and nuclear extracts were prepared (16). Western blots were performed with 40 μg of total protein/lane. Immunoblots were blocked overnight in 5% milk (w/v) in 20 mM Tris-HCl, pH 7.4, containing 500 mM NaCl (TBS). Blots were incubated for 4 h with antibodies to the AR (2 μg/ml) (PAI–111A, Affinity Bioreagents Inc, Golden, CO). The blots were washed and incubated for 1 h with the secondary antibody (1: 5000). Antibodies were diluted in 5% milk, TBS solution. AR protein was detected using the ECL luminescence kit (Amersham) and quantified using the Personal Densitometer (Molecular Dynamics).

Electrophoretic Mobility Shift Assay (EMSA)—Nuclear extracts from LNCaP cells were used for EMSA studies. Nuclear extracts were prepared from cells (17) that had been treated with 10 nM R1881 or 1 μM forskolin for 4 h. DNA binding reactions were carried out with 10 μg of total nuclear extracts in a total volume of 20 μl containing DNA binding buffer (10 μM HEPES, pH 7.9, 10% (v/v) glycerol, 100 μM KC1, 1 mM EDTA, 5 mM MgCl₂, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 2 μg of poly(dI-dC) (Amersham Pharmacia Biotech) with approximately 1.5 fmol of double-stranded ³²P-labeled PSA-ARE oligonucleotide (5′-TTGACAGAAGCAGAATGCTGACTGTC-3′), or PSA mutant ARE (5′-TTGACAAAAAGGAGAATGCTGACTGTC-3′). Protein-DNA complexes were separated under nondenaturing conditions in a 4% polyacrylamide gel (29:1) containing 2.5% glycerol and run in 0.5 × TBE (1× = 89 mM Tris-borate, 89 mM boric acid, and 2 mM EDTA, pH 8.3) at 200 V. Protein-DNA complexes were quantified with the STORM 860 PhosphorImager (Molecular Dynamics).

RESULTS

Effect of Forskolin on the Levels of PSA mRNA in LNCaP Cells—Forskolin activates adenyl cyclase to synthesize cAMP, which in turn stimulates PKA activity. Taking advantage of this effect, experiments were undertaken to determine whether PSA gene expression could be elevated in the absence of androgen by forskolin-induced activation of the PKA signal transduction pathway. To this end, LNCaP cells were employed because these cells express endogenous AR and PSA. As shown in Fig. 1A, when LNCaP cells were exposed for 16 h to various concentrations of forskolin (0.1 to 50 μM), the maximum induc-
tion of PSA mRNA was achieved using a concentration of 1 μM. Concentrations of forskolin in excess of 10 μM had little to no effect on PSA mRNA levels when compared with those in controls, which is in agreement with other reports (18, 19). As shown in Fig. 1B, the optimal induction of PSA mRNA by forskolin (1 μM) was comparable with that achieved with the synthetic androgen, R1881. A mixture of R1881 and 1 μM forskolin resulted in an additive increase in the mean level of PSA mRNA; however, this change was not statistically significant (Student’s t test, p > 0.05). A mixture of R1881 with higher concentrations of forskolin (>10 μM) reduced PSA mRNA to levels below those of R1881 alone (data not shown), which is consistent with a previous report (19).

The results of a time-course study of PSA mRNA in LNCaP cells (Fig. 1C) demonstrated that the optimal time for maximum induc-
tion was between 8 and 16 h after the addition of forskolin; after that, the levels of PSA mRNA decreased. In contrast, the induction of PSA mRNA by R1881 continued to increase for the duration of the experiment (48 h). The tran-
sient increase in PSA mRNA induced by forskolin, as compared with the increase obtained with R1881, may reflect the half-
lives of these compounds. R1881 is considered to be a poorly metabolized compound, whereas forskolin is relatively labile.

Induction of Reporter Constructs Containing AREs—To check whether the induction of PSA mRNA may involve changes in the activity of the PSA promoter as opposed to changes in post-transcriptional regulation, LNCaP cells were transfected with the PSA (−630/+12) promoter-luciferase re-
porter plasmid. This region of the PSA promoter contains both the ARE and ARR regions required for androgen induction (10, 11). After transfection, LNCaP cells were incubated in the presence of R1881 or forskolin. At the optimal concentration of R1881 (10 nM) (12), PSA luciferase activity was increased 5-fold (Fig. 2A). In comparison, the optimal concentration of forskolin (50 μM) increased PSA luciferase activity by 70-fold. Elevation of PKA activity by transfection of cells with an expression vector encoding the catalytic subunit of PKA (pPKAc) resulted in a greater than 200-fold induction of PSA luciferase activity. These results show that the induction of PSA by activation of PKA is mediated, at least in part, through the −630 to +12 region of the PSA promoter.

To determine whether other reporter constructs that contain AREs could be induced by forskolin, two additional reporters were tested in LNCaP cells. The first of these was the probasin promoter (PB-luciferase), a naturally occurring androgen-reg-
egulated promoter from the rat. As shown in Fig. 2B, the PB-
luciferase reporter construct was induced 65-fold by R1881, 35-fold by forskolin, and greater than 80-fold by overexpression of pPKAc. The second of these reporters was the ARR3-tk-luciferase, which is an artificial reporter construct that contains three repeats of the rat probasin ARE1 and ARE2 region.
Androgen-independent Induction of PSA

The induction of PSA by forskolin appears to be dependent on the presence of the AR, as implied by the increased levels of AR protein in extracts prepared from cells exposed to forskolin, whole cell levels of AR remained relatively constant for the 50-h duration of the experiment (Fig. 4A). In androgen-treated cells, there was a transient increase in whole cell levels of AR, which returned to control levels after 24 h. The increased levels of AR protein in extracts prepared from cells exposed to R1881 may reflect stabilization of the protein in the presence of ligand (20, 21).

Nuclear extracts prepared from cells exposed to forskolin demonstrated a transient increase in nuclear levels of AR protein (Fig. 4B). After 3 h, forskolin-treated cells were characterized by a 5-fold increase in nuclear AR protein compared with control levels. R1881 increased nuclear levels of AR protein within 90 min after its addition to the cells. The nuclear level of AR protein at 3 h was 38-fold higher in R1881-treated cells than in control cells analyzed at the same time point. In the continuing presence of R1881, nuclear levels of AR remained elevated in LNCaP cells for at least 48 h.

Inhibitory Effect of Bicalutamide on the Induction of PSA mRNA by Forskolin.—The induction of PSA by forskolin appears to be dependent on the presence of the AR, as implied by the increased levels of AR protein in extracts prepared from cells exposed to forskolin, whole cell levels of AR remained relatively constant for the 50-h duration of the experiment (Fig. 4A). In androgen-treated cells, there was a transient increase in whole cell levels of AR, which returned to control levels after 24 h. The increased levels of AR protein in extracts prepared from cells exposed to R1881 may reflect stabilization of the protein in the presence of ligand (20, 21).

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the above experiments. To further test the role of the AR in this mechanism, the antiandrogen, bicalutamide, was employed. As shown in the Northern blot in Fig. 5, preincubation of LNCaP cells with bicalutamide blocked the induction of PSA mRNA by forskolin (compare lane 5 to lane 6). Induction of PSA mRNA by R1881 was also prevented by bicalutamide, as expected (compare lane 2 to lane 4). These results demonstrate that the induction of PSA mRNA by forskolin requires a functional AR.

**Inhibitory Effect of Bicalutamide on the Forskolin Induction of Reporter Constructs Containing AREs**—To clarify whether the induction of transfected androgen-responsive reporter constructs (PSA, PB, and ARR3) by forskolin was dependent upon the presence of a functional AR, LNCaP cells were preincubated with bicalutamide. Because lengthy exposure of LNCaP cells to bicalutamide causes cell death, a relatively short 24-h incubation period was employed with no evidence of cytotoxicity (data not shown). As shown in Fig. 6A, PSA-luciferase activity was induced approximately 70-fold by forskolin, and this induction was blocked (approximately 80%) by preincubation of cells with bicalutamide. Results with R1881 are not shown because no induction was observed with this construct at 24 h. The induction of PB- (Fig. 6B) and ARR3-luciferase (Fig. 6C) reporters by R1881 was completely blocked (100%) by bicalutamide, whereas forskolin induction of these reporters was only blocked by 80% with bicalutamide. Thus, bicalutamide blocks the forskolin-induced luciferase activities of ARE-containing reporters, consistent with a role for the AR in this mechanism.

**Forskolin Increases AR ARE Complex Formation**—To determine whether forskolin increases DNA binding activity of AR protein to the PSA ARE, EMSAs were employed using radiolabeled oligonucleotides of the PSA-ARE promoter with nuclear extracts from LNCaP cells. Nuclear extracts from cells treated with guest on September 7, 2017 http://www.jbc.org/ Downloaded from
PKA Activates the Amino-terminal Domain of the Human AR—There are two potential PKA phosphorylation sites on the human AR, and both of these reside within the amino-terminal domain (amino acids 16 and 213). Therefore, the amino-terminal domain of the AR may be the target of PKA for the induction of ligand-independent activation. To test this hypothesis, amino-terminal fragments of the human AR were cloned into the carboxyl terminus of Gal4 DBD. Expression vectors for these chimeric proteins were cotransfected into LNCaP cells with PSA (1.0 μg), PB (1.0 μg) (B), or ARRtk-luciferase (1.0 μg) (C) reporter constructs for 24 h and then preincubated with bicalutamide (BIC, 50 μM) for 2 h before the addition of forskolin (FSK, 50 μM), R1881 (10 nM), or vehicle (Me2SO, 0.5%) and then incubated for an additional 24 h under serum-free conditions. The normalized luciferase activities were divided by the normalized activity of cells transfected with reporter plasmid and exposed to Me2SO (control) to give the fold-induction.

for 3 h with forskolin (maximum nuclear levels of AR protein) showed an increase in AR-ARE complex formation (Fig. 7, lane 2) compared with control (lane 4). In comparison, cells treated with R1881 (lane 3) also had an increase in AR-ARE complex formation compared with control (lane 4). As expected, substitution with a mutated PSA-ARE showed an 85–90% decrease in complex formation with the nuclear extracts prepared from forskolin-treated (lane 6), R1881-treated (lane 7), and control cells (lane 8). These results show that forskolin increases the DNA binding activity of the AR.

DISCUSSION

Steroid hormone receptors are considered ligand-activated transcription factors. However, recent evidence shows that the human estrogen and the chicken, rat, and rabbit progesterone receptors can mediate extracellular signals in the absence of cognate ligand by dopamine, epidermal growth factor, heregulin, gonadotropin-releasing hormone, tumor growth factor α, insulin and insulin-like growth factor I, keratinocyte growth factor, epidermal growth factor, and compounds that elevate cAMP (4, 5). However, induction of PSA gene expression by androgen-independent activation of the AR in human prostate cancer LNCaP cells has not been previously reported. Therefore, the present studies investigated androgen-independent induction of PSA gene expression via cross-talk between the AR and PKA signal transduction pathways, and these studies revealed the following: 1) PSA gene expression is induced by activation of PKA; 2) PKA induction of androgen-responsive reporter genes is promoter-specific; 3) induction of PSA gene expression by PKA requires a functional AR; and 4) PKA appears to target the amino terminus region of the AR.

Forskolin induction of PSA gene expression in LNCaP cells was shown by both Northern blot and the PSA reporter gene construct. The transient induction of PSA mRNA by activation of PKA using forskolin was shown to be dose-dependent, with...
the optimal concentration at 1 μM. At higher concentrations of forskolin, the levels of PSA mRNA did not plateau, but rather these concentrations resulted in a decrease in the induction of PSA mRNA. Forskolin concentrations of 10 μM or higher did not induce PSA mRNA above control levels. High concentrations of forskolin also decreased induction of PSA mRNA by R1881. This lack of induction of PSA mRNA at high concentrations of forskolin could not be explained by cytotoxic effects. However, these results are consistent with the report of Blok et al. (19), showing that a high concentration of forskolin decreases the phosphorylation of AR, thereby attenuating its DNA binding activity.

In agreement with the results showing the induction of PSA mRNA by forskolin, PSA reporter activity was also induced by forskolin and overexpression of PKAc in LNCaP cells. The PB-luciferase reporter was also induced by forskolin in LNCaP and PC3 cells that were transfected with wild-type human AR. Curiously, the powerful androgen-responsive ARΔ7-tk-luciferase reporter construct, which contains three repeats of the probasin ARE1 and ARE2, was poorly induced by forskolin and PKAc relative to R1881. One explanation for this weak induction may be a requirement of different factors for the thymidine kinase minimal promoter, as compared with the PSA and PB natural promoters.

Evidence that forskolin activates the AR to induce PSA gene expression is based on the following: 1) the induction of PSA gene expression by forskolin in LNCaP cells was blocked by bicalutamide; 2) the induction of PSA and other ARE reporter gene constructs by forskolin or PKAc in PC3 cells did not occur in the absence of transfected human AR; and 3) an increase in AR-ARE complex formation was observed using nuclear extracts from cells treated with forskolin.

Bicalutamide is an antiandrogen that prevents dissociation of the heat shock protein complex from the AR, thereby preventing DNA binding activity and possibly AR nuclear localization (29–31). It has been employed in numerous studies to determine the role of the AR in the activation of androgen-responsive reporter gene constructs (4, 5). In this study, application of bicalutamide blocked forskolin induction of PSA mRNA and androgen responsive reporter activities. These results show that the induction of PSA gene expression by forskolin is dependent upon a functional AR and imply cross-talk between the AR and PKA signal transduction pathways.

Further evidence that the AR is required for forskolin induction of reporter gene constructs containing AREs can be drawn from studies using the PC3 cell line. These cells are devoid of AR and therefore provide a good model for studying the requirement of AR in the induction of these reporters by forskolin. In the absence of transfected AR, forskolin induction of PSA-, PB-, and ARΔ7-luciferase activities did not occur. However, when the wild-type human AR was expressed in these cells, forskolin was able to induce these reporters. These data are consistent with the conclusion that forskolin activates the AR to induce androgen-responsive genes. In addition, these data demonstrate that the induction of PSA gene expression by forskolin in LNCaP cells is not unique to the mutated AR endogenously expressed in this cell line (32).

If the AR is activated by forskolin, an increase in AR-ARE complex formation should be observed. Application of EMSA confirmed that indeed an increase in AR-ARE complex formation did occur when nuclear extracts from LNCaP cells treated with forskolin were used together with PSA-ARE oligonucleotides. Unexpectedly, there was more AR-ARE complex formation when using nuclear extracts from forskolin-treated cells than from R1881-treated cells. This was not anticipated because the data in Fig. 4B shows a 40-fold increase in nuclear levels of AR protein in R1881-treated cells, as opposed to a 5-fold increase in nuclear AR levels in forskolin-treated cells. Although AR DNA binding activity is dependent on the amount of AR present in the assay, other factors are also crucial for DNA binding activity. Thus, it is conceivable that forskolin-transformed AR may have a greater affinity for the PSA-ARE than the AR activated by R1881. Such enhancement of affinity may result from differential interactions with other proteins or modulation of the receptor that may include a change in its phosphorylation state.

Upon establishing that forskolin elevates PSA gene expression by an AR-dependent pathway, the next step was to map what region of the AR was targeted. To do this, chimeric fragments of the AR receptor fused to the DBD of the Gal4 protein, together with the Gal4 reporter system, were employed (Fig. 8). These data provide the first demonstration of activation of the amino-terminal domain of the AR by stimulation of the PKA signal transduction pathway. In vivo, the transcriptional activation of the AR requires the AF-1 in the amino-terminal domain (28, 33–37), which has been mapped to two discrete overlapping regions between amino acids 110 to 379 and 369 to 494 (33, 36). Although distinct transactivation regions may be active for specific responsive promoters, ligand-dependent versus ligand-independent activation of the AR also may target different transactivation regions. This would result in recruitment of different co-activators or altered interactions with the basal transcriptional machinery. Studies examining ligand-independent activation of the estrogen receptor by growth factors and PKA have determined that epidermal growth factor (38) and insulin growth factor (39) act primarily by means of the transactivation domain AF-1 (in the amino terminus), whereas PKA acts through the transactivation domain AF-2 (in the carboxyl terminus) (38). Hence, the estrogen receptor can be activated through three signaling molecules, estradiol, cAMP, and growth factors, each acting through a different discrete domain. Although this study shows forskolin activation of the amino terminus of the AR in LNCaP cells, further experiments are in progress to map the precise site.

Whether forskolin/PKA directly alters the phosphorylation state of the amino-terminal domain of the AR is not known. There is reason to believe that a change in the phosphorylation state may be involved because this domain contains numerous potential serine phosphorylation sites for not only PKA but also for mitogen-activated protein kinase, DNA-dependent protein kinase, protein kinase C, casein kinase II, and serine-proline-directed kinase (40). Such a mechanism could potentially affect the ability of the receptor to dissociate from the heat shock proteins, shuttle to and from the nucleus, bind DNA, interact with other transcription factors, or activate particular responsive genes. However, early work examining AR phosphorylation strongly indicates that PKA does not directly alter the total phosphorylation state of the AR (41). In addition, although there are different phosphorylated isoforms of the AR (21, 42, 43) and phosphorylation increases with ligand (44, 45), AR transactivation does not appear to be affected by phosphorylation. Therefore, a more likely scenario may involve the changes in the phosphorylation state of a co-regulator or another protein factor that binds to the amino-terminal domain of the AR, thereby increasing AR transactivation activity. Such a hypothesis may also help to explain the promoter specificity of AR that is activated by forskolin seen in Fig. 2.

In summary, the data presented here provides evidence that the amino terminus of the AR can be activated by PKA or elevation of cAMP by forskolin in LNCaP cells to initiate transcription of some genes containing AREs, such as PSA. Identification of such a mechanism may be of critical importance in
the understanding of the molecular changes that are involved in the progression of prostate cancer to androgen independence. Through mapping of the site on the AR that is targeted by PKA, new approaches to averting androgen-independent prostate cancer might be developed.

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