The Prostate 36:256–263 (1998)

Differential Transactivation by the Androgen Receptor in Prostate Cancer Cells

Robert Snoek,[1] Nicholas Bruchovsky,[1] Susan Kasper,[2] Robert J. Matusik,[2] Martin Gleave,[1] Naohide Sato,[1] Nasrin R. Mawji,[1] and Paul S. Rennie[1]*

[1]*Department of Cancer Endocrinology, British Columbia Cancer Agency, Vancouver, British Columbia, Canada*

[2]*Department of Cell Biology, Vanderbilt University Medical Center, Nashville, Tennessee*

BACKGROUND. The purpose of this study was to determine the contribution of different transactivating regions of the androgen receptor (AR) to the induction of androgen-regulated promoters in poorly (PC3 cells) and well-differentiated (LNCaP cells) prostate cancer cell lines.

METHODS. PC3 and LNCaP cells were co-transfected with plasmids expressing full-length AR or deletion mutants together with luciferase reporters linked to the probasin (PB) and PSA promoters; as well as to ARR_3tk, a PB-derived recombinant promoter.

RESULTS. Androgen induction of the ARR_3tk promoter in the presence of AR was 8- to 10-fold higher than that seen with the PB promoter. Activation of ARR_3tk was greatest with an androgen-independent construct in which the first 231 amino acids and the ligand binding domain had been removed, indicating that this promoter is more responsive to activating functions in the N-terminal domain than in the ligand binding domain. By comparison, induction of the PB promoter was greatest with the full-length AR, which suggests that the ligand binding domain also makes a major contribution to the activation of this promoter. In similar analyses with the PSA promoter, AR regions required for promoter induction was dependent on the host cell type. In PC3 cells, the predominant AR transactivation function was androgen-independent and resided in the N-terminal domain, whereas in LNCaP cells, the highest level of induction was androgen dependent and also required participation of the ligand binding domain.

CONCLUSIONS. Our results indicate that the relative utilization of transactivating functions in N-terminal and ligand binding domains of the AR is promoter and cell specific. *Prostate 36:256–263, 1998.*

KEY WORDS: **prostate-specific antigen; probasin; androgen regulation; androgen receptor; prostate cancer**

INTRODUCTION

The androgen receptor (AR) is a member of a large family of nuclear transcriptional factors that generally require binding of a specific ligand for activation and that possess a structure composed of similar functional regions or domains [1,2]. In general, each member possesses (1) an N-terminal region containing a ligand-independent transcriptional activating functions (AF-1); (2) a centrally located DNA-binding domain and hinge region; and (3) in the C-terminal portion of the protein, a ligand binding domain, which contains a conserved ligand dependent transcriptional activating function (AF-2) [3].

The N-terminal domain has the highest degree of variability between members of the family of nuclear receptors and may interact with other nuclear proteins

Contract grant sponsor: Medical Research Council of Canada; Contract grant sponsor: NIH George M. O'Brien Research Center; Contract grant number: P50 DK47656.

Naohide Sato is currrently at the Department of Urology, Teikyo University, Ichihara Hospita, Ichihara-shi, Japan.

*Correspondence to: Paul S. Rennie, Ph.D., Department of Cancer Endocrinology, British Columbia Cancer Agency, 600 West 10th Avenue, Vancouver, BC, Canada V5Z 4E6. E-mail: prennie@bccancer.bc.ca

Received 18 December 1997; Accepted 17 April 1998

in a receptor-specific manner [4]. In the AR, this region is extensive and comprises more than half of the primary amino acid sequence [5–7]. The AR is unique in that several homopolymeric stretches of amino acids occur in this domain, including 8–31 repeating glutamine residues [8], 9 proline residues, and a 10–31 polyglycine stretch [9]. Interestingly, both the polyglutamine and polyglycine tracts differ significantly among races [10]. Although the precise functions of these repeating tracts of amino acids are unknown, abnormal expansion of the polyglutamine tract is associated with X-linked spinal and bulbar muscular atrophy [11] and a decrease in the size of this homopolymer and polyglycine has been linked with an increased risk of developing prostate cancer [10,12–14]. In transfection experiments a complete loss of transactivation function with deletions of N-terminal amino acid residues 38–296 have been reported [15–17]. Jenster et al. [18] found two variable transactivation regions in the AR N-terminal domain whose activity was dependent on the promoter context as well as the presence or absence of the ligand binding domain. Recently, Chamberlain et al. [19] have mapped the AF-1 function into two regions: AF-1a and AF-1b.

The moderately conserved steroid/ligand binding domain is located in the C-terminal region of nuclear receptors and interacts with other domains to block transcriptional activation in the absence of the appropriate steroid hormone. In addition, there are conserved sequences within many nuclear receptors, which are associated with ligand-dependent transcriptional activation function-2 (AF-2) [3]. Conclusive evidence for a transcriptional activation function in the AR ligand binding domain was recently demonstrated in a cell-free, in vitro transcription system [20]. Point mutations in the ligand binding domain of the AR may result in transcriptionally inactive receptor forms [21], whereas extensive deletions often lead to molecules with the capacity for varying degrees of constitutive transcriptional activity [15,22]. Interaction between the ligand binding domain and the N-terminal domain of the AR may also occur [23–25].

It is likely that cellular and promoter specificity of AR transactivation is determined to a large extent by the occurrence and relative abundance of other transcription factors and receptor cofactors in different tissues. Co-effector proteins can either enhance (co-activators) or repress (co-repressors) nuclear receptors [26,27]. For instance, co-effectors ARA_{70} [28], TIF-2 [29], CBP, F-SRC-1, and RIP140 [25] enhance, whereas SRC-1 [25] represses AR-mediated transactivation. Thus, the presence or absence of certain co-effectors would greatly effect AR function. Yet virtually every study that has attempted to characterize the AR has used nonprostate cell lines (usually COS, CV-1, Chinese hamster ovary [CHO], or HeLa cells) [15–19,25,29] and thus has not taken into account the potential contribution of unique combinations of co-effectors and other prostate-specific components. Similarly, the promoters used have generally not been derived from naturally occurring genes that are normally androgen regulated in vivo. Therefore, the effect that a prostate cellular environment, with its particular complement of co-effectors, will have on AR transactivation of an androgen-regulated promoter remains unclear. To address these problems, we have compared the ability of AR deletion constructs with transactivate reporters containing androgen-regulated promoters (i.e., the prostate-specific antigen [PSA] and probasin [PB] genes) in transfection experiments, using well-differentiated and poorly differentiated prostate cancer cell lines (i.e., LNCaP and PC3 cells, respectively). Our results indicate that there is differential promoter sensitivity to regions of the AR and that there may be dissimilar co-effector concentrations in these prostate lines that modulate transcriptional activity.

MATERIALS AND METHODS

Plasmid Construction

ARR_3tk-luc was constructed by using −81tk-luc and excising −244 to −96 of the 5′-flanking region of the rat PB gene from PB-CAT plasmid [30] with *Hin*dIII and *Xba*I, followed by blunt ending and ligation to *Hin*dIII linkers. Three fragments were ligated in tandem into the *Hin*dIII site of pT81 luciferase vector (American Type Culture Collection [ATCC], Rockville, MD), which contains a thymidine kinase (tk) minimal reporter. PB-luc was made as follows. Using the PB 5′-flanking deletion starting with the *Hin*dIII site at −286 bp, the clone was digested with *Hin*dIII and *Fok*I, and the −286 to −53-bp PB fragment was isolated. Similarly, with the PB −426- to +28-bp clone (pBH500), the plasmid was digested with *Fok*I and *Bam*HI and the −53 to +28 fragment was isolated. The two PB fragments were subcloned in pBluescript II SK(+) (Stratagene, La Jolla, CA) to create the −286 to +28 bp PB promoter with a *Hin*dIII/*Bam*HI site. This PB fragment was then ligated into the *Hin*dIII and *Bgl*II site of pXP2 luciferase vector (ATCC) to form the PB-luc reporter. The PSA-luc plasmid was prepared by PCR amplification of nucleotides −630 to +12 using primers 5′CATTGTTTGCTGCACGTTGGAT and 5′TCCGGGTGCAGGTGGTAAGCTTGG obtained from the Nucleic Acid-Protein Service Unit (University of British Columbia, Vancouver, BC, Canada). The amplified DNA fragment was purified, blunt-end ligated into the *Eco*RV site of pBluescript SK I (–) excised with

*Hin*dIII and inserted into the *Hin*dIII site of pGL2 basic luciferase reporter plasmid (Promega, Madison, WI).

All rat AR plasmid constructs were prepared using PCR as described previously [30]. All primers included *BamH*I sites as well as a methionine start site in the 5′-end primers. The AR fragments synthesized were blunt-end ligated into the *Eco*RV site of pBluescript II SK(+). The inserts were digested at one end with *Cla*I, filled in with Klenow fragment of DNA polymerase (BRL) and excised using *Xba*I. They were then ligated into blunt-ended *Hin*dIII and *Xba*I sites generated in the pRc/CMV vector (Invitrogen, San Diego, CA). In all cases, the orientation and sequences of the inserts were confirmed by DNA sequence analysis using the dsDNA Cycle Sequencing System kit (GIBCO-BRL, Burlington, Ontario, Canada).

Transient Transfection Experiments

PC3 cells were plated at a density of 7×10^5 cells/60-mm dish in Dulbecco's Modified Eagle's Medium (DMEM)(Stem Cell Technologies, Vancouver, BC, Canada) supplemented with 5% fetal bovine serum (FBS). Transient transfections were performed using calcium phosphate/DNA precipitation method previously described [30] with 0.005–1.5 μg of wild-type AR or AR mutant expression vectors, or both. The pRC/CMV vector was used to make the final DNA concentration to 1.5 μg, which was co-transfected with 1 μg of ARR_3tk-luc or PB-luc reporter plasmid, 0.25 μg of β-galactosidase expression vector pCH110 (Pharmacia, Baie d'Ure, Quebec, Canada) and 0.75 μg pXP2 carrier plasmid (ATCC) per plate. The cells were harvested after 24 hr, and activity for luciferase (Promega luciferase assay kit; Promega) and β-galactosidase (Tropix galacto-light kit; Tropix Inc., Bedford, MA) was determined using an Optocomp I luminometer (Tropix) as specified by the manufacturers. For lipofection experiments, DNA was mixed with 10 μg of lipofectin reagent (BRL) in serum-free media, incubated at room temperature for 15 min, and then added to PC3 or LNCaP cells which had been initially plated at 3×10^5 cells/6-well plate in RPMI 1640 plus 5% FBS. The media was changed 6 h later to DMEM (PC3) or RPMI 1640 (LNCaP) plus 5% dextran-coated charcoal-stripped serum with or without 10 nM R1881. The cells were harvested 24 hr (PC3) or 48 hr (LNCaP) after transfections and analyzed as described above.

Western Analyses

PC3 and LNCaP cells were transfected with plasmids (2 μg) expressing the full-length AR and deletion mutant ARs using lipofectin as described above. Nuclear extracts were isolated [31] and 200 μg of nuclear protein run on a sodium dodecyl sulfate (SDS) polyacrylamide gel [30]. Western blots were done as previously described [30] using the anti-AR DNA binding domain antibody 15071A (Pharmingen, San Diego, CA) and detected by ECL (Amersham, Arlington Heights, IL). The corresponding AR peptide bands were quantitated by densitometry scanning. For comparative purposes, each set of experiments were run on the same gel to ensure identical sample treatment and the relative AR peptide expression calculated.

RESULTS

Construction of a Reporter System for Increased Sensitivity of Detection of AR Transactivation

The androgen response elements located between nucleotides −244 and −96 in the 5′-upstream DNA of the rat PB gene manifest a higher induction by androgens than do MMTV or tyrosine aminotransferase hormone response elements [30,32]. We sought to further increase the sensitivity of detection of transactivation by creating a luciferase-based reporter system with multiple PB androgen response elements linked to a thymidine kinase (tk) minimal promoter. This reporter construct, designated ARR_3tk-luc, was co-transfected with various concentrations of a rat AR expression vector into PC3 cells. In parallel experiments, a PB (nucleotides −286 to +28)-luciferase (PB-luc) reporter, containing just one hormone response region adjacent to its native promoter, was co-transfected with AR. The results shown in Figure 1 demonstrate that in the ARR_3tk-luc transfectants, the net fold-induction of luciferase activity after treatment with 10 nM R1881 increases linearly with AR plasmid concentrations of $\leq$0.7 μg and then reaching a plateau with the net induction of approximately 200-fold. By comparison, the induction of PB-luc by androgens levels off at about 20-fold after transfection with 0.2–0.4 μg of AR plasmid. The difference in AR plasmid concentrations required for maximal induction in each system probably reflects the three times greater amount of receptor protein required to occupy all the AR binding sites in ARR_3tk-luc reporter. Nevertheless, it is evident that ARR_3tk-luc is a much more sensitive (8–10 times more at AR plasmid concentrations >0.7 μg) reporter for detecting AR transactivation than is PB-luc.

Androgen-Independent Transactivation by an AR Deletion Mutant With a Truncated N-Terminus and Lacking a Ligand Binding Domain

We next tested different plasmids expressing AR-constructs that lacked the ligand binding domain. Deletion mutant $AR_{1–649}$, coding for AR amino acids 1–

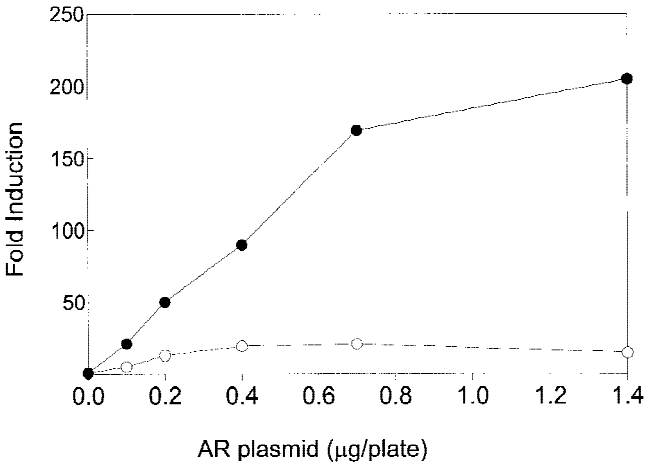

Fig. 1. Relative induction by AR of ARR_3tk-luc and PB-luc reporters. PC3 cells were co-transfected by the calcium phosphate method with 1 μg of ARR_3tk-luc (●) or PB-luc (○) reporter plasmid, 0.25 μg of β-galactosidase expression vector pCH110, 0.75 μg XP2 carrier plasmid, and 0–1.4 μg of AR (together with sufficient pRC/CMV vector for a final AR + pRC/CMV DNA concentration of 1.5 μg) per plate. The fold induction (mean, $n \geq 3$) is the ratio luciferase activity (relative light units min^{-1} mg $protein^{-1}$ corrected for β-galactosidase activity) in cells grown in the presence of 10 nM R1881 over luciferase activity in cells grown in the absence of androgen.

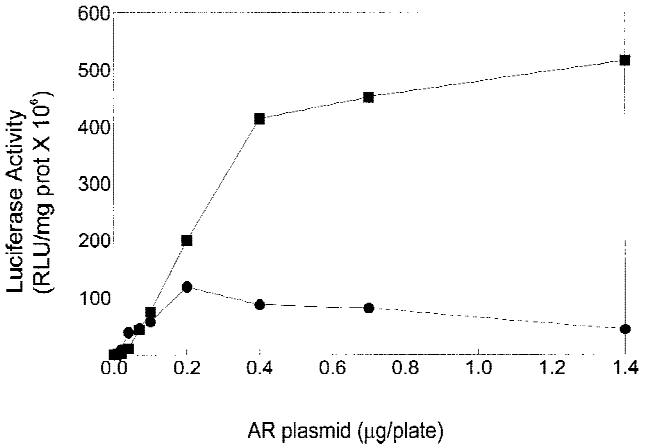

Fig. 2. Transactivation of ARR_3tk-luc in PC3 cells by an AR construct ($AR_{232–649}$) lacking the ligand binding domain and a portion of the N-terminal. After co-transfections with ARR_3tk-luc and 0–1.4 μg of AR or $AR_{232–649}$, with lipofection, PC3 cells were grown in the presence or absence of 10 nM R1881. The results, corrected for β-galactosidase activity, are expressed as mean ($n \geqslant 3$) relative luciferase units min^{-1} mg $protein^{-1}$. AR +R1881 (●), $AR_{232–649}$ +R1881 (○).

649 (numbering system used by Tan et al. [33]) encompasses the N-terminal domain, the DNA-binding domain, and the hinge region (for its nuclear localization signal) but lacks the ligand binding domain. When co-transfected with ARR_3tk-luc into PC3 cells, the amount of transactivation was always <1% of that seen with full-length AR (data not shown). This result differs from other reports in which removal of the ligand binding domain was found in one case to have constitutive activity equivalent to wild-type AR in COS-1 cells with pG29Gtk-CAT reporter [22], and on the other to have 10–13% of wild type in CV-1 cells using MMTV-CAT reporter [15]. These differences may reflect the cells and/or promoters used in the experiments.

We next tested a plasmid that expressed $AR_{232–649}$, having the N-terminus truncated to amino acid 232. In order to compare PC3 cell transfection results with those using LNCaP cells, we used lipofection as a means of transfection, as we were unable to transfect LNCaP cells using calcium phosphate/DNA precipitation methods. Co-transfection of PC3 cells with a range of $AR_{232–649}$ plasmid concentrations and with the ARR_3tk-luc reporter gave the transactivation profile shown in Figure 2. The level of AR required for maximal induction is lower in Figure 2 (0.2 μg) than in Figure 1 (0.7–1.4 μg) because of the higher transfection efficiencies of lipofection over calcium phosphate. The profile was the same in the presence or absence of androgens for $AR_{232–649}$, whereas the full-length AR is inactive in the absence of androgens (data not shown). In these experiments, activation by $AR_{232–649}$ at a plasmid concentration of 0.2 μg is slightly higher than the maximum induction observed with AR. Activation of the reporter by $AR_{232–649}$ occurs androgen independently and is consistently higher than the highest level seen with AR in the presence of hormone.

Western blots of the AR deletion mutants show that the $AR_{232–649}$ and the full-length AR protein were expressed in PC3 cells (Fig. 3; $AR_{232–649}$ protein lane 4; AR protein lane 6). A small amount of immunoreactive material corresponding to the band position of full-length AR is observed in the control sample (lane 5) and in the $AR_{232–649}$ transfected sample (lane 4). This may be a nonrelated protein that cross-reacts with the antibody or represent the low level of nonfunctional, endogenous AR seen in PC3 cells by others [34,35]. Immunoprecipitation experiments confirmed the synthesis of $AR_{232–649}$ and AR (data not shown). The AR protein was expressed at approximately twice the molar amount (2.4 ± 1.2 (3); mean ± S.D., n) of $AR_{232–649}$ protein. This implies that the higher transactivational competence of $AR_{232–649}$ was the result of its superior transcriptional activation in this system.

Transcriptional Activity of $AR_{232–649}$ in Well-Differentiated LNCaP Prostate Cancer Cells

LNCaP cells are more differentiated than PC3 cells in that they contain an endogenous AR, are growth

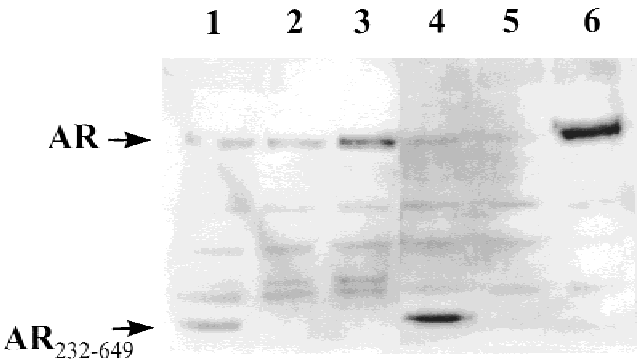

Fig. 3. Expression of AR proteins. Nuclear proteins were extracted from LNCaP **(lanes 1–3)** and PC3 **(lanes 4–6)** cells, which had been transfected by lipofection with 2.0 μg of plasmid expressing $AR_{232–649}$ (lanes 1 and 4), AR (lanes 3 and 6), or the empty vector (pRC/CMV) (lanes 2 and 5), and were separated on 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), blotted, probed with anti-androgen receptor antibody, and visualized with the ECL Western blotting detection kit.

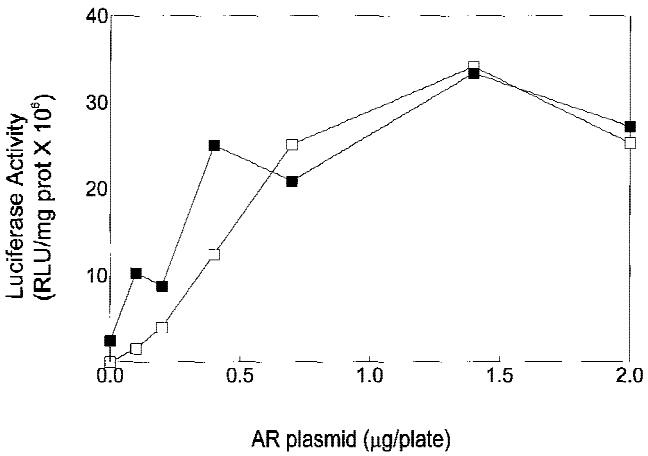

Fig. 4. Transactivation of ARR_3tk-luc in LNCaP cells by $AR_{232–649}$. After co-transfection with ARR_3tk-luc and 0–2 μg of $AR_{232–649}$, LNCaP cells were grown in the presence (■) or absence (□) of 10 nM R1881. The results, corrected for β-galactosidase activity, are expressed as mean ($n \geq 3$) relative luciferase units min^{-1} mg $protein^{-1}$.

stimulated by androgens, and express PSA in response to androgens [36,37]. Figure 4 shows the results obtained after co-transfection of LNCaP cells with ARR_3tk-luc and plasmid concentrations of $AR_{232–649}$ of 0–2 μg. The contribution of the endogenous AR to transcriptional activation is manifested as the difference in activity both in the presence and absence of androgen at low concentrations (<0.5 μg) of $AR_{232–649}$ plasmid. As with the PC3 cells, $AR_{232–649}$ protein expressed in LNCaP cells, is a strong inducer of ARR_3tk-luc. The activity reaches a maximum at plasmid concentrations of 0.75–1.5 μg and then declines slightly. Overall, the pattern of $AR_{232–649}$ induction in LNCaP cells is similar to that in PC3 cells; implying that in both cell types, $AR_{232–649}$ is a very potent, androgen-independent activator of the ARR_3tk promoter.

The AR and $AR_{232–649}$ protein was readily detectable in LNCaP cells using Western blots (Fig. 3; lanes 1–3). As in the PC3 cells, the relative molar amounts of AR was twice (2.4 ± 0.9 (3)) that of $AR_{232–649}$.

Differential Induction of ARR_3tk, PB, and PSA Promoters

The reporter plasmids ARR_3tk-luc, PB-luc, and PSA-luc were co-transfected by lipofection with plasmids expressing AR, $AR_{232–649}$, or $AR_{232–902}$ into PC3 and LNCaP cells. $AR_{232–902}$ encodes the equivalent of $AR_{232–649}$ but also includes the C-terminal ligand binding domain. The results shown in Figure 5 indicate that the induction of transcription by the three AR proteins is cell and promoter specific.

In both PC3 and LNCaP cells (Fig. 5A and B, respectively), $AR_{232–649}$ is significantly more active in stimulating the ARR_3tk-luc reporter than is transfected AR (*t*-test, $P < 0.05$) or the endogenous AR (control in Fig. 5B). $AR_{232–902}$ is inactive with respect to induction of this promoter. The results suggest that ARR_3tk-luc is more responsive to activating functions in the N-terminal domain than in the ligand binding domain of the AR.

With the PB promoter (PB-luc), there is a markedly different profile of activities (Fig. 5C,D) in the two cell types. In PC3 cells (Fig. 5C), there is a high background level in the absence of hormone and the most potent transactivator tested is AR, which is three to four times more active than either $AR_{232–649}$ or $AR_{232–902}$. In LNCaP cells (Fig. 5D), the strongest induction is also seen with transfected AR, but $AR_{232–902}$ is more active than in PC3 cells, about 70% as potent as AR. The androgen-independent activity of $AR_{232–649}$ only contributes a slight increment (+20% to 25%) in activity above that ascribed to the endogenous AR (control). These results imply that PB-luc is more strongly activated by AR constructs containing the ligand binding domain.

In PC3 cells, the PSA promoter (PSA-luc) has a similar transactivation and background profile as seen with the PB promoter, except that $AR_{232–649}$ is as active as AR (Fig. 5E). In the LNCaP cells (Fig. 5F), the results with the PSA promoter are quite different; $AR_{232–902}$ is almost threefold more active than the transfected AR (*t*-test, $P < 0.001$). This may be attibutable to the presence of a promoter-specific co-repressor in LNCaP cells, which interacts with amino acids in the N-terminal portion of the AR. $AR_{232–649}$ displays very strong transcriptional activation of PSA-luc in LNCaP

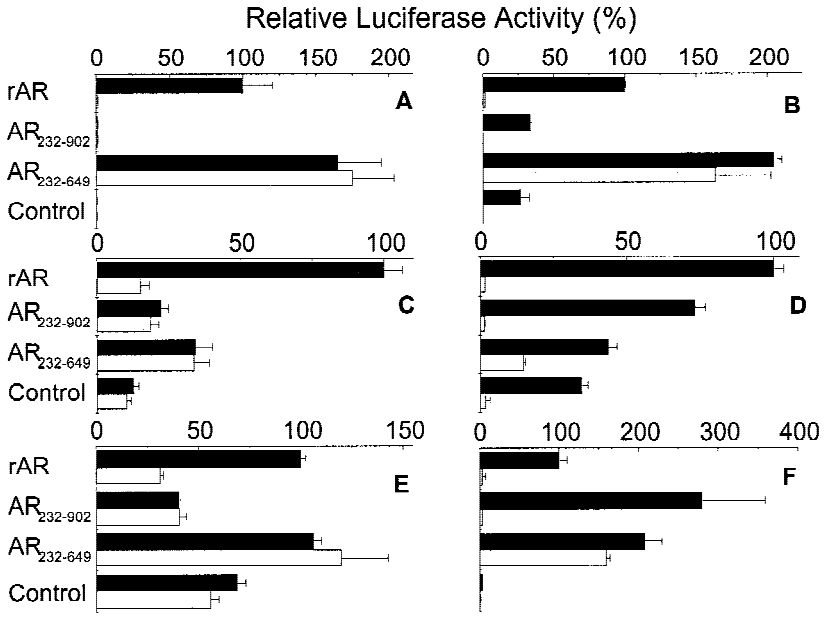

Fig. 5. Differential induction of ARR_3tk, PB, and PSA promoters. PC3 **(A,C,E)** and LNCaP **(B,D,F)** cells were transfected by lipofection with 0.5 μg of plasmid expressing AR, $AR_{232–649}$, $AR_{232–902}$, or empty vector control (pRC/CMV) together with 1 μg of ARR_3tk-luc (A,B), PB-luc (C,D), or PSA-luc (E,F) and 0.5 μg of gal reporter plasmid CH110. The cells were grown in the presence *(black bars)* or absence *(gray bars)* of 10 nM R1881. The results are expressed as the mean (±SD; n ≥ 3) relative % activity, with AR values in the presence of androgen set to 100% in each case. The actual 100% mean values in luciferase units min^{-} $mg\ protein^{-}$ are as follows: A: 16.7 $\times 10^6$; B: 14.5 $\times 10^6$; C: 2.9 $\times 10^6$; D: 3.4 $\times 10^6$; E: 1.8 $\times 10^6$; F: 1.0 $\times 10^6$.

cells, but overall has about 55–70% of the potency of $AR_{232–902}$. Collectively, these results suggest that in LNCaP cells activating functions in both the N- and C-terminus make a substantial contribution to induction of PSA-luc, whereas in the less differentiated PC3 cells, the N-terminal activity predominates.

DISCUSSION

The purpose of this investigation was to study the transactivation functions of the AR on the induction of two well-characterized androgen-regulated genes, PB and PSA. The in vivo expression of both of these genes is largely confined to the prostate [38–40] and in transgenic experiments, the PB promoter has been shown to confer androgen and prostate-specific expression [41]. We hypothesize that these naturally occurring androgen-responsive promoters uniquely support AR transactivation. In addition, we tested the pattern of induction of a reporter-construct (ARR_3tk-luc) composed of three cassettes of the PB androgen response region (ARR) linked in tandem to a tk-promoter [20,30,42]. The fold-induction of this reporter by androgens was about 10 times higher than that observed with the PB promoter (Fig. 1). We sought to increase the physiological relevance of our results by performing our transfection experiments in poorly differentiated (PC3) and well-differentiated (LNCaP) prostate cancer cells, where appropriate AR accessory factors might be expected to be found in varying abundance.

In both types of prostate cell lines, the strong ARR_3 enhancer appeared to be very responsive to activating functions located in the N-terminal domain. The $AR_{232–649}$ plasmid exhibits strong androgen-independent transactivation on the ARR_3 enhancer (Fig. 2). Truncation of the first 231 amino acids of the N-terminus resulted in elimination of the polyglutamine tract, which has been reported to inhibit transactivation function [16,43]. Using the MMTV promoter in CV-1 cells, Chamberlain et al. [19] have delineated two AF-1 domains in the N-terminal of the AR, AF-1a (from amino acids 154–167) and AF-1b (amino acids 295–359). Removal of either one of these domains resulted in approximately a 50% decrease in AR activity [19]. Although the AF-1a has been deleted in $AR_{232–649}$, no drop in activity was seen here. Jenster et al. [18] found in HeLa cells, that removal of the ligand-binding domain resulted in a shift in the location of the AF-1 to amino acids 360–485, which could explain the high activity of $AR_{232–649}$. A shift in AF-1 location with the removal of the ligand-binding domain was also seen by Ikonen et al. [25] using CV-1 and CHO cells. These investigators also observed that AR transactivation was dependent upon the type of promoter and cell type used, supporting the concept that transcriptional proteins and co-effectors interact in a cell and promoter specific manor. For instance, in COS-1 cells, deletion of the AR-polyglutamine tract had no effect on the MMTV promoter but resulted in an increased transcriptional activity of the AR on the PSA promoter [44]; showing that different promoters give different results. Also, Gordon et al. [45] found that the MMTV-CAT was more active in stably expressed AR cells of prostate origin than of hepatoma origin, indicating that the type of cell line used is also important. In any event, the net induction of ARR_3tk-luc by $AR_{232–649}$ is greater than that observed with full-length AR in prostate cells.

In contrast to the ARR_3tk promoter, the PB promoter was more responsive to the full-length AR than to $AR_{232–649}$ in PC3 cells. Similarly, in LNCaP cells, induction of PB-luc with $AR_{232–649}$ was far less than that observed with AR and $AR_{232–902}$, implying that the PB promoter also requires the involvement of the ligand binding domain.

Experiments with the PSA promoter suggest that the predominant transactivator in PC3 cells resides in the N-terminal domain, while in LNCaP cells, the ligand binding domain also contributes to its induction. In both cell lines, $AR_{232–649}$ equals or slightly exceeds the full-length AR in stimulating the PSA-luc reporter. However, in LNCaP cells $AR_{232–902}$ is associated with the highest levels of luciferase activity. One interpretation of this observation is that LNCaP cells contain a co-repressor that normally interacts with residues within the first 231 amino acids of the AR N-terminal to modulate the expression of the PSA gene.

Overall, the results indicate that AR transactivation is cell and promoter dependent. This may be attributable in large part to the relative availability of accessory co-effectors proteins that may interact with specific promoters and/or different regions of the AR to modulate AR-induced transcription.

ACKNOWLEDGMENTS

We thank Dr. Colleen Nelson for many helpful discussions.

REFERENCES

1. Evans RM: The steroid and thyroid hormone receptor superfamily. Science 1988;240:889–895.
2. Truss M, Beato M: Steroid hormone receptors: Interaction with deoxyribonucleic acid and transcription factors. Endocr Rev 1993;14:459–479.
3. Danielian PS, White R, Lees JA, Parker MG: Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors. EMBO J 1992; 11:1025–1033.
4. Strahle U, Schmid W, Schutz G: Synergistic action of the glucocorticoid receptor with transcription factors. EMBO J 1988;7: 3389–3395.
5. Chang CS, Kokontis J, Liao ST: Molecular cloning of human and rat complementary DNA encoding androgen receptors. Science 1988;240:324–332.
6. Lubahn DB, Joseph DR, Sar M, Tan J, Higgs HN, Larson RE, French FS, Wilson EM: The human androgen receptor: Complementary deoxyribonucleic acid cloning, sequence analysis and gene expression in prostate. Mol Endocrinol 1988;2:1265–1275.
7. Trapman J, Klaassen P, Kuiper GG, van der Korput JA, Faber PW, van Rooij HC, Geurts van Kessel A, Voorhorst MM, Mulder E, Brinkmann AO: Cloning, structure and expression of a cDNA encoding the human androgen receptor. Biochem Biophys Res Commun 1988;153:241–248.
8. Edwards A, Hammond HA, Jin J, Caskey CT, Chakraborty R: Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 1992;12:241–253.
9. Lumbroso R, Beitel LK, Vasiliou DM, Trifiro MA, Pinsky L: Codon-usage variants in the polymorphic (GGN)n trinucleotide repeat of the human androgen receptor gene. Hum Genet 1997; 101:43–46.
10. Irvine RA, Yu MC, Ross RK, Coetzee GA: The CAG and GGC microsatellites of the androgen receptor gene are in linkage disequilibrium in men with prostate cancer. Cancer Res 1995;55: 1937–1940.
11. La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH: Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 1991;352:77–79.
12. Giovannucci E, Stampfer MJ, Krithivas K, Brown M, Brufsky A, Talcott J, Hennekens CH, Kantoff PW: The CAG repeat within the androgen receptor gene and its relationship to prostate cancer. Proc Natl Acad Sci USA 1997;94:3320–3323.
13. Stanford JL, Just JJ, Gibbs M, Wicklund KG, Neal CL, Blumenstein BA, Ostrander EA: Polymorphic repeats in the androgen receptor gene: Molecular markers of prostate cancer risk. Cancer Res 1997;57:1194–1198.
14. Hardy DO, Scher HI, Bogenreider T, Sabbatini P, Zhang ZF, Nanus DM, Catterall JF: Androgen receptor CAG repeat lengths in prostate cancer: Correlation with age of onset. J Clin Endocrinol Metab 1996;81:4400–4405.
15. Simental JA, Sar M, Lane MV, French FS, Wilson EM: Transcriptional activation and nuclear targeting signals of the human androgen receptor. J Biol Chem 1991;266:510–518.
16. Palvimo JJ, Kallio PJ, Ikonen T, Mehto M, Janne OA: Dominant negative regulation of trans-activation by the rat androgen receptor: Roles of the N-terminal domain and heterodimer formation. Mol Endocrinol 1993;7:1399–1407.
17. Yen PM, Liu Y, Palvimo JJ, Trifiro M ,Whang J, Pinsky L, Janne OA, Chin WW: Mutant and wild-type androgen receptors exhibit cross-talk on androgen- glucocorticoid- and progesterone-mediated transactivation. Mol Endocrinol 1997;11:162–171.
18. Jenster G, van der Korput HAGM, Trapman J, Brinkmann AO: Identification of two transcription activation units in the N-terminal domain of the human androgen receptor. J Biol Chem 1995;270:7341–7346.
19. Chamberlain NL, Whitacre DC, Miesfeld RC: Delineation of two distinct type 1 activation functions in the androgen receptor amino-terminal domain. J Biol Chem 1996;271:26772–26778.
20. Snoek R, Rennie PS, Kasper S, Matusik RJ, Bruchovsky N: Induction of cell-free, in vitro transcription by recombinant androgen receptor peptides. J Steroid Biochem Mol Biol 1996;59: 243–250.
21. Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM, French FS: Androgen receptor defects: Historical, clinical, and molecular perspectives. Endocr Rev 1995;16:271–321.
22. Jenster G, van der Korput HAGM, van Vroonhoven C, van der Kwast TH, Trapman J, Brinkmann AO: Domains of the human androgen receptor involved in steroid binding, transcriptional activation, and subcellular localization. Mol Endocrinol 1991;5: 1396–1404.
23. Langley E, Zhou Z-X, Wilson EM: Evidence for an anti-parallel orientation of the ligand-activated human androgen receptor dimer. J Biol Chem 1995;270:29983–29990.
24. Doesburg P, Kuil CW, Berrevoets CA, Steketee K, Faber PW, Mulder E, Brinkmann AO, Trapman J: Functional in vivo interaction between the amino-terminal, transactivation domain and the ligand binding domain of the androgen receptor. Biochemistry 1997;35:1052–1064.
25. Ikonen T, Palvimo JJ, Janne OA: Interaction between the amino- and carboxyl-terminal regions of the rat androgen receptor

modulates transcriptional activity and is influenced by nuclear receptor coactivators. J Biol Chem 1997;272:29821–29828.
26. Horwitz KB, Jackson TA, Bain DL, Richer JK, Takimoto GJ, Tung L: Nuclear receptor coactivators and corepressors. Mol Endocrinol 1996;10:1167–1177.
27. Heery DM, Kalkhoven E, Hoare S, Parker MG: A signature motif in transcriptional co-activators mediates binding to nuclear receptors. Nature 1997;387:733–736.
28. Yeh S, Chang C: Cloning and characterization of a specific coactivator, ARA_{70}, for the androgen receptor in human prostate cells. Proc Natl Acad Sci USA 1996;93:5517–5521.
29. Voegel JJ, Heine MJS, Zechel C, Chambon P, Gronemeyer H: TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. EMBO J 1996;15:3667–3675.
30. Rennie PS, Bruchovsky N, Leco KJ, Sheppard PC, McQueen SA, Cheng H, Snoek R, Hamel A, Bock ME, MacDonald BS, Nickel BE, Chang C, Liao S, Cattini PA, Matusik RJ: Characterization of two *cis*-acting DNA elements involved in the androgen regulation of the probasin gene. Mol Endocrinol 1993;7:23–36.
31. Antras J, Lasnier F, Pairsult J: Adipsin gene expression in 3T3-F442A adipocytes is postranscriptionally down-regulated by retinoic acid. J Biol Chem 1991;266:1157–1161.
32. Claessens F, Alen P, Devos A, Peeters B, Verhoeven G, Rombauts W: The androgen-specific probasin response element 2 interacts differentially with androgen and glucocorticoid receptors. J Biol Chem 1996;271:19013–19016.
33. Tan JA, Joseph DR, Quarmby VE, Lubahn DB, Sar M, French FS, Wilson EM: The rat androgen receptor: Primary structure, autoregulation of its messenger ribonucleic acid, and immunocytochemical localization of the receptor protein. Mol Endocrinol 1988;2:1276–1285.
34. Culig Z, Klocker H, Eberle J, Kaspar F, Hobisch A, Cronauer MV, Bartsch G: DNA sequence of the androgen receptor in prostatic tumor cell lines and tissue specimens assessed by means of the polymerase chain reaction. Prostate 1993;22:11–22.
35. Tilley WD, Bentel JM, Aspinall JO, Hall RE, Horsfall DJ: Evidence for a novel mechanism of androgen resistance in the human prostate cancer cell line, PC-3. Steroids 1995;60:180–186.
36. Berns EMJJ, De Boer W, Mulder E: Androgen-dependent growth regulation of and release of specific protein(s) by the androgen receptor containing human prostate tumor cell line LNCaP. Prostate 1986;9:247–259.
37. Hasenson M, Lundh B, Stege R, Carlstrom K, Pousette A: PAP and PSA in prostatic carcinoma cell lines and aspiration biopsies: Relation to hormone sensitivity and to cytological grading. Prostate 1989;14:83–90.
38. Dodd JG, Sheppard PC, Matusik RJ: Characterization and cloning of rat dorsal prostate mRNAs. Androgen regulation of two closely related abundant mRNAs. J Biol Chem 1983;258:10731–10737.
39. Wang MC, Valenzuela LA, Murphy GP, Chu TM: Purification of a human prostate specific antigen. Invest Urol 1979;17:159–163.
40. Cleutjens KBJM, van Eekelen CCEM, van der Korput HAGM, Brinkmann AO, Trapman J: Two androgen response regions cooperate in steroid hormone regulated activity of the prostate-specific antigen promoter. J Biol Chem 1996;271:6379–6388.
41. Greenberg NM, DeMayo FJ, Sheppard PC, Barrios R, Lebovitz R, Finegold M, Angelopoulou R, Dodd JG, Duckworth ML, Rosen JM, Matusik RJ: The rat probasin gene promoter directs hormonally and developmentally regulated expression of a heterologous gene specifically to the prostate in transgenic mice. Mol Endocrinol 1994;8:230–239.
42. Kasper S, Rennie PS, Bruchovsky N, Sheppard PC, Cheng H, Lin L, Shiu RPC, Snoek R, Matusik RJ: Cooperative binding of androgen receptors to two DNA sequences is required for androgen induction of the probasin gene. J Biol Chem 1994;269:31763–31769.
43. Chamberlain NL, Driver ED, Miesfeld RL: The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acids Res 1994;22:3181–3186.
44. Jenster G, de Ruiter PE, van der Korput HAGM, Kuiper GGJM, Trapman J, Brinkmann AO: Changes in the abundance of androgen receptor isotypes: Effects of ligand treatment, glutamine-stretch variation, and mutation of putative phosphorylation sites. Biochemistry 1994;33:14064–14072.
45. Gordon DA, Chamberlain NL, Flomerfelt FA, Miesfeld RL: A cell-specific and selective effect on transactivation by the androgen receptor. Exp Cell Res 1995;217:368–377.