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Mechanistic Relationship between Androgen Receptor Polyglutamine Tract Truncation and Androgen-dependent Transcriptional Hyperactivity in Prostate Cancer Cells*

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Androgen receptor (AR) signaling pathways mediate critical events in normal and neoplastic prostate growth. Shortening of the polymorphic N-terminal polyglutamine (poly(Q)) tract of the AR gene leads to transcriptional hyperactivity and has been correlated with an increased risk of prostate cancer. The underlying mechanisms for these effects are poorly understood. We show here that androgen-dependent cellular proliferation and transcription in prostate cancer cells is inversely correlated to the length of the AR poly(Q) region. We further show that AR proteins containing a shortened poly(Q) region functionally respond to lower concentrations of androgens than wild type AR. Whereas DNA binding activity is relatively unaffected by AR poly(Q) variation, we found that ligand binding affinity and the ligand-induced NH_2- to COOH-terminal intramolecular interaction is enhanced when the poly(Q) region is shortened. Importantly, we show that AR proteins containing a shortened poly(Q) region associate *in vivo* with higher levels of specific p160 coactivators and components of the SWI/SNF chromatin remodeling complex as compared with the wild type AR. Collectively, our findings suggest that the AR transcriptional hyperactivity associated with shortened poly(Q) length stems from altered ligand-induced conformational changes that enhance coactivator recruitment.

Given that androgens are essential for the normal growth and survival of the prostate gland (1, 2), aberrant androgen receptor (AR)[1] signaling pathways have long been suspected of playing a critical role in the onset and progression of prostate neoplasia (reviewed in Refs. 3 and 4). The AR is a member of the nuclear hormone receptor (NR) superfamily that mediates the action of lipophilic ligands including steroids, retinoids, vitamin D3 and thyroid hormone (5, 6). NRs share common structure and functional domains including a variable N-terminal domain (NTD), a centrally located and highly conserved DNA binding domain, a hinge region, and a C-terminal ligand binding domain (LBD) (5–7). Transcriptional activation by NRs is mediated by both a poorly conserved constitutive activation function 1 (AF-1) in the NTD (8, 9) and a highly conserved, ligand-inducible activation function 2 (AF-2) in the LBD (10, 11). Transcriptional activation by AR involves a ligand-induced intramolecular interaction between its NTD and its LBD (12–14). It was recently found that F*XX*LF and W*XX*LF motifs in the AR NTD facilitate direct interactions with the AF-2 domain in the LBD (14–16). Importantly, the NTD/LBD interaction is essential for AR transactivation and is believed to generate a composite binding site for the recruitment of transcriptional coregulatory factors (17).

AR can regulate transcription by modifying the chromatin structure near AR target genes and by facilitating the recruitment of the basal transcription machinery. Specific transcriptional coregulatory factors termed coactivators and corepressors are indispensable for these activities (reviewed in Ref. 18). The p160 family of proteins are among the best characterized NR coactivaors (19, 20) and function through their association with potent histone acetyltransferases like CREB-binding protein/p300 and p/CAF (21–23) and histone methyltransferases like CARM1 or PRMT1 (24, 25). For most NRs, the p160 proteins directly contact the AF-2 region in the NR-LBD through consensus L*XX*LL motifs (also termed NR boxes) (11, 19). Interestingly, AR is unique among other NRs in that its ligand-induced intramolecular NTD/LBD interaction appears to be essential for p160 binding and that, in contrast to other NRs, specific motifs in the AR NTD may provide part of the p160 binding surface (26, 27).

The multisubunit SWI/SNF and related complexes (28, 29) have also been implicated in playing an important coactivator role for AR (30–32) as well as for other NRs (31, 33–35). SWI/SNF-like complexes can actively mobilize nucleosomes in the vicinity of target genes in an ATP-dependent manner and are believed to generate a chromatin structure that promotes transcriptional initiation (29). Whereas the mechanisms by which SWI/SNF complexes are recruited to AR remain unclear, recent studies found that the core SWI/SNF ATPases Brg1 and hBRM are differentially required for AR-mediated transcription at different AR target promoters (30). In addition to the p160 and SWI/SNF complexes, a host of other AR-interacting coregulatory factors and complexes have been identified and proposed to play additional coregulatory roles including the multiprotein TRAP-Mediator complex (31, 36), the ARAs, FHL2, β-catenin, AES, SNURF, PDEF, ARIP3, BRCA1, and cyclin D1 (reviewed in Ref. 18).

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[1] The abbreviations used: AR, androgen receptor; NR, nuclear hormone receptor; NTD, N-terminal domain; LBD, ligand binding domain; AF-2, activation function 2; CREB, cAMP-response element binding protein; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; KSFM, keratinocyte-SFM; MMTV, murine mammary tumor virus; ARE, AR response element; DHT, dihydrotestosterone.

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The AR-NTD contains an inherited polymorphic glutamine repeat region (termed poly(Q)) that begins at amino acid 58 and can typically range in size from 5 to 33 Gln residues with an average length of 20 repeats (37–39). Short poly(Q) repeats (≤19) have been clinically correlated with a higher risk of prostate cancer, an earlier age of onset, and a higher grade and more advanced stage of prostate cancer at the time of diagnosis (37–44). Several studies have demonstrated an inverse correlation between the length of the poly(Q) region and AR transcriptional activity (45–48). Interestingly, truncation or complete deletion of the poly(Q) region can enhance AR-mediated transactivation in the presence of overexpressed p160 proteins (49, 50), consistent with the idea that a shortened poly(Q) region can promote coactivator recruitment. However, the detailed molecular mechanisms responsible for the hyperactive transcriptional activity in AR proteins containing shortened poly(Q) regions remain poorly defined.

In this study, we investigated the molecular and functional consequences of shortening the poly(Q) region in AR proteins expressed in primary malignant and metastatic prostate cancer cells. In agreement with previous studies, we found that androgen-dependent AR transactivation activity increases as the number of Gln repeats decreases. Interestingly, we show here an inverse correlation between androgen-dependent cellular proliferation and poly(Q) length. Furthermore, we found that AR proteins containing a shortened poly(Q) region functionally respond to lower levels of ligand than the wild type AR. Whereas DNA-binding activity appears to be unaffected by shortening the poly(Q) region, we found that AR ligand binding affinity and its ligand-induced NTD/LBD intramolecular interaction is enhanced when the poly(Q) region is shortened. Importantly, we show that AR proteins containing a shortened poly(Q) region are associated *in vivo* with higher levels of specific p160 coactivators and components of the SWI/SNF complex. Taken together, our findings suggest that several molecular mechanisms contribute to the AR functional hyperactivity inversely correlated with poly(Q) length.

EXPERIMENTAL PROCEDURES

Plasmid Construction—The pcDNA-AR-Q_9, pcDNA-AR-Q_{20} (wild type), and pcDNA-AR-Q_{44} constructs were provided by G. A. Coetzee (University of Southern California) (49) and were further confirmed by sequencing. The pMMTV-Luc reporter gene was described previously (51). The pGL3-PSA-Luc reporter gene was a gift from T. A. Libermann (Harvard Medical School) (52). To construct pBabe Hyg-f:AR-Q_9, a XmaI/AflII fragment from pcDNA-AR-Q_9 was first subcloned into the XmaI/AflII sites of pT$_7$-FLAG-AR (51), generating pT$_7$-FLAG-AR-Q_9. An NdeI/BamHI fragment from pT$_7$-FLAG-AR-Q_9 was then subcloned into the NdeI/BamHI sites of pTetCMV-F^0(S)-AR (51), thus generating pTetCMV-F^0(S)-AR-Q_9. A BglII/BamHI fragment from pTetCMV-F^0(S)-AR-Q_9 was inserted into BamHI-digested pBabe Hyg vector from C.-M. Chiang (Case Western Reserve University), generating pBabe Hyg-f:AR-Q_9. The pBabe Hyg-f:AR-Q_{20} (wild type) construct was generated by subcloning the BglII/BamHI fragment from pTetCMV-F^0(S)-AR into the BamHI site of pBabe Hyg vector. To generate pcDNA-AR-NTD-Q_{44} (amino acids 1–521), a point mutation of pcDNA-AR-Q_{44} was introduced at amino acid residue 522 to generate a stop codon (TGA) using the GeneEditor Mutagenesis system (Promega) and the mutagenic oligonucleotide 5′-CCT GAT GTG TGA TAC CCT GGC-3′. Briefly, the DNA template was alkaline-denatured and then hybridized with the appropriate selection and mutagenic oligonucleotide. After the annealing reaction, mutant strand synthesis and ligation were obtained by adding T4 DNA polymerase and T4 DNA ligase. The mutant was verified by sequencing. To construct pcDNA-AR-NTD-Q_9 (amino acids 1–488) and pcDNA-AR-NTD-Q_{20} (amino acids 1–499), the AflII/EcoRI fragments (containing a stop codon at amino acid 522) from pcDNA-AR-Q_{44} (amino acids 1–521) were subcloned into AflII/EcoRI sites of pcDNA-AR-Q_9 and pcDNA-AR-Q_{20}. The pcDNA-AR-LBD (amino acids 506–919) was generated by first creating a NheI site at residue 1662, a Kozak sequence (from residue 1670–1678), and a BamHI site at residue 2996 by PCR amplification using the 5′ primer 5′-GAT GTG TGC TAG CCT GCC GCC ATG GTG A-3′ and the 3′ primer 5′-GCA CTC AGA GGA TCC GTG CAG AG-3′. The PCR fragment was then digested with NheI and BamHI and subsequently subcloned into the NheI/BamHI sites of pcDNA 3.1(+) vector (Promega).

Cell Culture—PC-3 and DU145 cells were obtained from the American Type Culture Collection (ATCC) (Manassas, VA) and were grown in DMEM/F-12 medium (Invitrogen) supplemented with 10% FBS and 100 units/ml penicillin and streptomycin. 1532T cells (malignant human primary prostate epithelial cells immortalized with human papilloma virus) from S. Topalian (NCI, National Institutes of Health) (53) were grown in keratinocyte-SFM (KSFM) (Invitrogen) supplemented with 10% FBS, 50 μg/ml bovine pituitary extract, 5 ng/ml epidermal growth factor, 100 units/ml penicillin and streptomycin, 2.5 μg/ml fungizone, and 10 mM HEPES. CV-1 cells (obtained from the ATCC) were maintained in DMEM (Invitrogen) supplemented with 10% FBS, 100 units/ml penicillin and streptomycin, and 10 mM HEPES.

Establishment of Stable Cell Lines—To generate 1532T cell lines stably expressing FLAG-tagged AR-Q_{20} or FLAG-tagged AR-Q_9, the amphotrophic retroviral packing cell line ϕNX-A from G. Nolan (Stanford University) was grown in DMEM plus 10% FBS and transfected with 15 μg of pBabe Hyg-f:AR-Q_{20} or pBabe Hyg-f:AR-Q_9 construct in a 10-cm plate using a modified calcium phosphate transfection method (54). The cultured supernatant containing viral particles was collected 48 h post-transfection and filtered through a 0.45-μm filter. 10–20 μl of filtered supernatant was added to 2 ml of KSFM medium containing polybrene (Sigma) (final concentration 5 μg/ml) and used to infect 1532T cells. Cells were incubated for 26 h before being split 1:3 for hygromycin selection (50 μg/ml). After 5 weeks of selection, individual hygromycin-resistant colonies were clonally expanded into cell lines and further characterized.

Western Blot—For detection of f:AR expression in 1532T cells stably transfected with f:AR-Q_{20} or f:AR-Q_9, equal numbers of cells were lysed with 150 μl of lysis buffer containing 1× Dulbecco's phosphate-buffered saline, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS supplemented with protease inhibitors (10 mg/ml phenylmethylsulfonyl fluoride, 30 μg/ml aprotinin, and 10 μl/ml 100 mM sodium orthovanadate). Cell lysate was prepared and transferred to cold microcentrifuge tubes and incubated on ice for 20 min. Cellular debris was pelleted by centrifugation at 10,000 rpm for 20 s, and the lysate was transferred to a new tubes. Equivalent amounts of lysate were added to sample loading buffer, boiled for 5 min, and then fractionated by 8% SDS-PAGE. The PAGE-fractionated protein extracts were then transferred to nitrocellulose membranes and probed with anti-AR monoclonal antibodies (anti-AR441; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) followed by peroxidase-conjugated anti-mouse IgG (Amersham Biosciences). Immunoblot signals were detected by enhanced chemiluminescence (ECL system; Amersham Biosciences) according to the manufacturer's instructions. For examination of AR-NTD-Q_n or AR-LBD expression in transient transfected CV-1 cells, protein extract was made using 1× lysis buffer (Promega) and was probed with either anti-AR441 or anti-AR C-19 (Santa Cruz Biotechnology).

Transient Transfections—Transient co-transfections of AR-Q_n (AR-Q_9, -Q_{20}, and -Q_{44}) constructs and reporter genes in prostate cancer cells (PC-3, DU 145, or 1532T cells) were carried out using LipofectAMINE PLUS™ reagent (Invitrogen) as recommended by the manufacturer. The cells were plated in 12-well plates (1.5×10^5 cells/well) in DMEM/F-12 medium containing 10% charcoal/dextran-stripped FBS (for PC-3 and DU 145 cells) or KSFM medium containing 5% charcoal/dextran-stripped FBS (for 1532T cells) 24 h prior to transfection. A DNA mixture containing 0.4 μg of AR plasmid (pcDNA-AR-Q_9, pcDNA-AR-Q_{20}, or pcDNA-AR-Q_{44}) or empty pcDNA-3.1(+) plasmid, 0.2 μg of reporter plasmid (pMMTV-Luc, or pPSA-Luc) and 0.2 μg of the internal control plasmid pSV-β-gal was combined with LipofectAMINE reagent and added to each well. Cells were incubated at 37 °C in 5% CO_2 for 3 h before replacing the media with fresh DMEM, 10% charcoal, dextran-stripped FBS lacking or containing different concentrations of dihydrotestosterone (DHT) (10^{-12}, 10^{-11}, 10^{-10}, and 10^{-7} M final). The cells were further incubated for 24 h and then harvested with a lysis buffer supplied in a luciferase kit (Promega). Luciferase activity was then measured in a Lumat LB 9507 luminometer (EG&G Wallac, Inc.) The β-galactosidase activity of the lysed transfected cells was determined using a kit (β-galactosidase enzyme assay system; Promega) according to the manufacturer's instructions.

Saturation Ligand Binding Assay—To determine the relative kinetics of androgen binding to f:AR-Q_9 and f:AR-Q_{20}, 1532T/f:AR-Q_9 and 1532T/f:AR-Q_{20}(b) cells were plated at 2×10^5 cells/well in 24-well plates in steroid-free medium (KSFM supplemented with 5% charcoal/dextran-stripped FBS, 50 μg/ml bovine pituitary extract, 5 ng/ml epidermal growth factor) and allowed to attach overnight. The following

day, the medium was aspirated and replaced with steroid-free, serum-free Iscove's modified Eagle's medium containing [17α-*methyl*-^{3}H]R1881 (specific activity 70–87 Ci/mmol) (PerkinElmer Life Sciences) (0.06–3.6 nM) in the presence or absence of a 500-fold excess of cold DHT to determine nonspecific binding and 1 μM R5020 (a synthetic progesterone) to saturate progesterone and glucocorticoid receptors. After an incubation period of 2 h at 37 °C, cells were washed twice with ice-cold Dulbecco's phosphate-buffered saline and solubilized in Dulbecco's phosphate-buffered saline containing 0.5% SDS and 20% glycerol. Extracts were removed, and cell-associated radioactivity was counted with a scintillation counter. The data were analyzed and kDa and B_{max} were determined by nonlinear regression using Graphpad Prism software.

Mammalian Two-hybrid Assay—Transient co-transfections of AR-NTD-Q_n (AR-NTD-Q_9, -Q_{20}, and -Q_{44}), AR-LBD constructs, and MMTV reporter gene in CV-1 cells were carried out using LipofectAMINE PLUS™ reagent (Invitrogen) as recommended by the manufacturer. The cells were plated in 12-well plates (1.5×10^5 cells/well) in DMEM containing 10% charcoal/dextran-stripped FBS 24 h prior to transfection. A DNA mixture containing 0.2 μg of AR-LBD plasmid (pcDNA-AR-LBD (amino acids 506–919)) with 0.2 μg of AR-NTD-Q_n plasmid (pcDNA-AR-NTD-Q_9 (amino acids 1–488), PC DNA-AR-NTD-Q_{20} (amino acids 1–499), or pcDNA-AR-NTD-Q_{44} (amino acids 1–521)) or 0.2 μg of empty pcDNA-3.1(+) plasmid, 0.1 μg of reporter plasmid (pMMTV-Luc) and 0.2 μg of the internal control plasmid pSV-β-gal was combined with LipofectAMINE reagent and added to each well. Cells were incubated at 37 °C in 5% CO_2 for 3 h before replacing the media with fresh DMEM plus 10% charcoal/dextran-stripped FBS lacking or containing different concentrations of DHT (10^{-10} and 10^{-7} M final). The cells were further incubated for 24 h and then harvested with a lysis buffer supplied in a kit as described above. Luciferase activity was then measured as described above.

Electromobility Shift Assay—The AR response element (ARE) located in the MMTV long terminal repeat promoter (5′-AGC TTT ATG GTT ACA AAC TGT TCT TAA AGT CGA-3′) (55) and its complement were annealed to obtain double-stranded templates with protruding BglII ends. The double-stranded ARE was then labeled by filling in with [α-^{32}P]dATP (50 μCi; Amersham Biosciences) and Klenow enzyme. For f:AR protein used in EMSA, f:AR was immunopurified from 1532T/f:AR-Q_9 and 1532T/f:AR-Q_{20} cell lines using anti-FLAG antibodies coupled to agarose beads (Sigma) and subsequently eluted using a FLAG peptide as described previously (51). f:AR was incubated for 15 min at room temperature in a binding buffer containing 10 mM Tris·Cl (pH 7.9), 50 mM KCl, 1 mM dithiothreitol, 10% glycerol, 1 μg/μl bovine serum albumin, 0.5 μg of poly(dI-dC), 1 mM EDTA, 0.1% Nonidet P-40 with 2 ng of labeled double-stranded ARE probe. The reactions were electrophoresed in a prerun 5% polyacrylamide gel, 0.5× Tris borate-EDTA at 100V for 3–4 h. Gel was dried and autoradiographed.

Cellular Proliferation Assay—To determine the effects of R1881 on the growth of 1532T/f:AR-Q_{20}(b) and 1532T/f:AR-Q_9 cells *in vitro*, 1532 T cells were grown as mentioned under "Cell Culture." Cells were plated at 1.2×10^4 cells/well in KSFM medium containing 5% charcoal/dextran-treated FBS for 24 h. Then R1881 (10^{-7} and 10^{-10} M) was added to the culture medium and the cells were incubated for 0, 12, 24, 48, and 72 h. The cells then were fixed in 1% glutaraldehyde and stained with 0.5% crystal violet. Plates were rinsed and air-dried, and the dye was eluted with Sorensen's solution. Absorbance at 590 nm was measured by a precision microplate reader (Fisher).

Limited Proteolytic Digestion Assay—[^{35}S]Methionine-labeled pcDNA-AR-Q_9 and pcDNA-AR-Q_{20} (wild type) were translated *in vitro* using a TNT T7 quick coupled transcription/translation kit (Promega Corp.) according to the manufacturer's instructions in the presence of 25 μM $ZnCl_2$. 5 μl of labeled translation product was incubated for 10 min in the presence or absence of 100 nM R1881 at 30 °C. The mixture was then digested with chymotrypsin (0.5 ng/μl final; Sigma) for 10 min at room temperature. The reaction was stopped by adding 10 μl of 2× sample buffer to 10 μl of digestion product and boiling at 95 °C for 5 min. Samples were analyzed on 10% SDS-PAGE gels. The gel was then dried and visualized by autoradiography.

Coimmunoprecipitation of f:AR-Coregulatory Factor Complexes—1532T/f:AR-Q_9 and 1532T/f:AR-Q_{20} (4×10^6) cells were grown in 15-cm plates in KSFM medium containing 5% charcoal/dextran-stripped FBS (HyClone) until 80% confluent. Cells were then starved for 24 h in serum-free KSFM, followed by treatment with R1881 (10^{-7} M) or vehicle (ethanol) for 6 h. After the treatment, cells were harvested for nuclear extract production. The entire procedure of nuclear extract preparation was performed at 4 °C. Cells were rinsed once with cold phosphate-buffered saline and once with cold buffer A (10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, and 0.5 mM dithiothreitol). Then 2 ml of cold buffer A containing 0.1% Nonidet P-40 was added to the plate. Cells were scraped, transferred to a 10-ml tube, and vigorously pipetted. The tube was rotated for 60 min. Nuclei were pelleted by centrifugation at 14,000 rpm for 10 min. The nuclei were then resuspended in 200 μl of buffer C (20 mM HEPES, pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, and 0.5 mM dithiothreitol) and incubated for 45 min. The extracts were centrifuged at 14,000 rpm for 15 min. FLAG-AR-cofactor complexes were immunoprecipitated by adding anti-FLAG antibodies coupled to agarose beads (20 μl packed) to the nuclear lysate. The mixture was rotated overnight at 4 °C. The beads were pelleted by gentle centrifugation and washed twice with 1.5 ml of ice-cold buffer A. After final wash, the precipitated protein complexes were resuspended in SDS-sample loading buffer, fractionated by 8% SDS-PAGE and then transferred to a nitrocellulose membrane. Western blotting was performed as described above using antibodies against Brg1, RAC-3, GRIP-1, and AR (Santa Cruz Biotechnology). The BAF-155 antibody is from Dr. G. Crabtree (Stanford University).

RESULTS

Inverse Relationship between AR Poly(Q) Length and AR Transactivation in Transiently Transfected Metastatic and Malignant Prostate Cancer Cells—Whereas previous studies revealed an inverse relationship between poly(Q) length and AR transcriptional activity in nonprostate cells (45–47), the experimental effects of poly(Q) variation on AR activity in prostate cancer cells are less defined. To investigate AR poly(Q) variation on prostate-specific transcription here, AR expression vectors containing variable poly(Q) regions (Q_9, Q_{20}, and Q_{44}) were transiently transfected into AR(−) metastatic prostate cancer cell lines PC-3 and DU145 together with the androgen-responsive reporter gene MMTV-Luc. As shown in Fig. 1*A*, AR transcriptional activity in PC-3 cells increased with decreasing Q-repeat length in the presence of both high and low concentrations of DHT (10^{-7} and 10^{-10} M, respectively). When DHT concentration was further decreased to 10^{-11} M, no significant transactivation differences were observed among the different AR variants. Similar to the results in PC-3 cells, AR transactivation in DU145 cells was also inversely correlated with the poly(Q) length (Fig. 1*B*). Notably, AR-Q_9 transactivation in DU145 cells was significantly higher than that of AR-Q_{20} in the presence of low concentrations of DHT (10^{-10} to 10^{-11} M). Taken together, these data show that an inverse relationship between the poly(Q) length of AR and its ability to activate transcription exists in metastatic prostate cancer cells and that AR proteins containing a shortened poly(Q) repeat (Q_9) respond to lower levels of ligand than the wild type AR (Q_{20}).

Since PC-3 and DU145 cells are derived from metastatic lesions, they limit our understanding of the effect of poly(Q) variation on AR function in cells derived from earlier stages of prostate cancer. 1532T is a papilloma virus immortalized cell line derived from a primary prostate adenocarcinoma resected from a prostate cancer patient (53). To investigate whether the observed inverse relationship between poly(Q) length and AR transcriptional activity also exists in prostate adenocarcinoma cells, we transiently transfected the 1532T line with the AR poly(Q)$_n$ expression vectors and measured transcription from either MMTV-Luc or a reporter gene containing the human prostate-specific antigen promoter (PSA-Luc) (52). Fig. 1, *C* and *D*, again shows an inverse relationship between AR poly(Q) length and androgen-dependent transactivation. Interestingly, and consistent with the results with the metastatic prostate lines, we again found that the AR-Q_9 protein is transcriptionally responsive to lower levels of ligand (10^{-10} M DHT) than AR-Q_{20}. In sum, these data demonstrate that AR transcriptional activity is inversely correlated to the length of the poly(Q) region and that AR proteins containing a shortened poly(Q) have a higher transcriptional responsiveness to low levels of androgen.

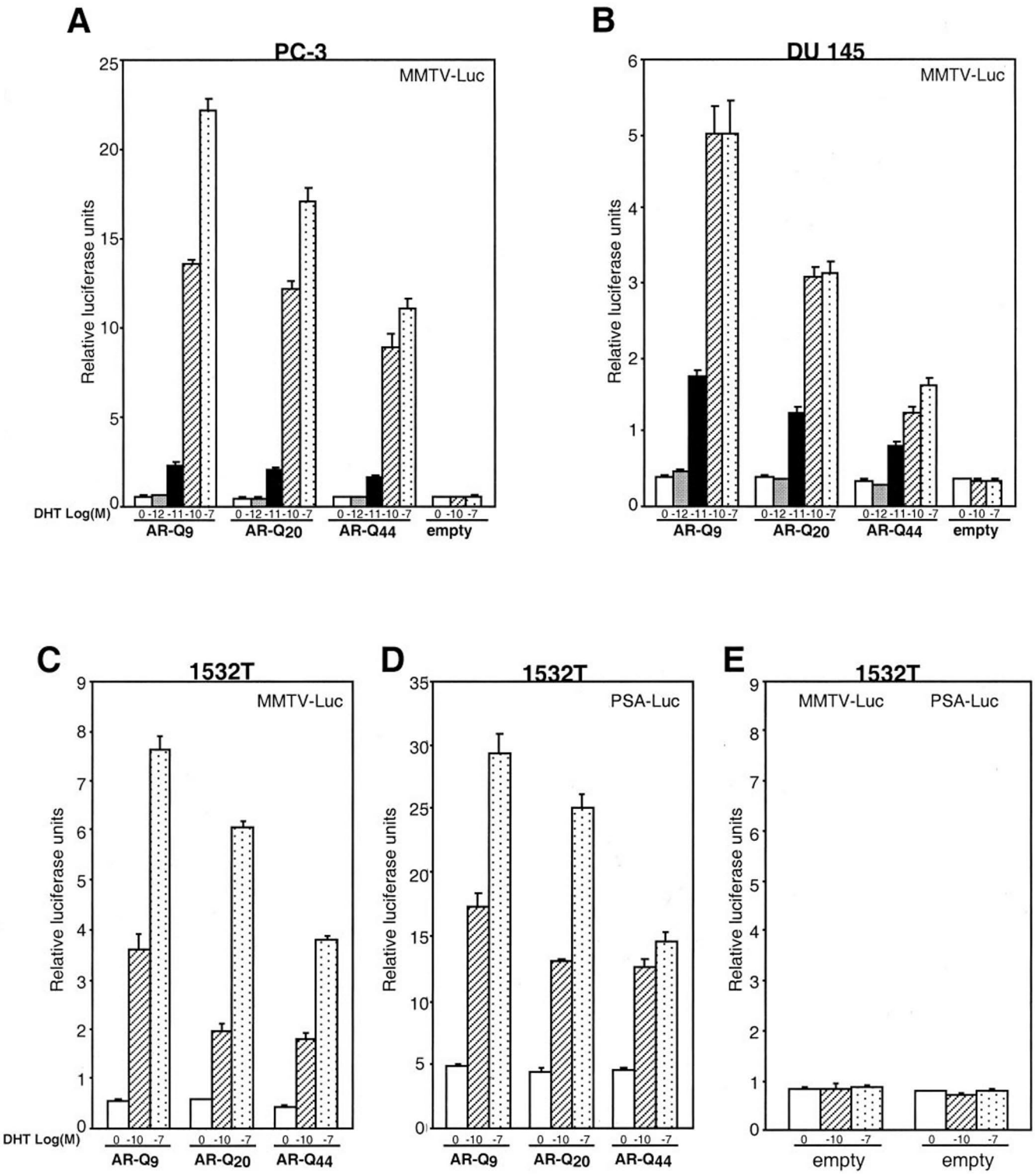
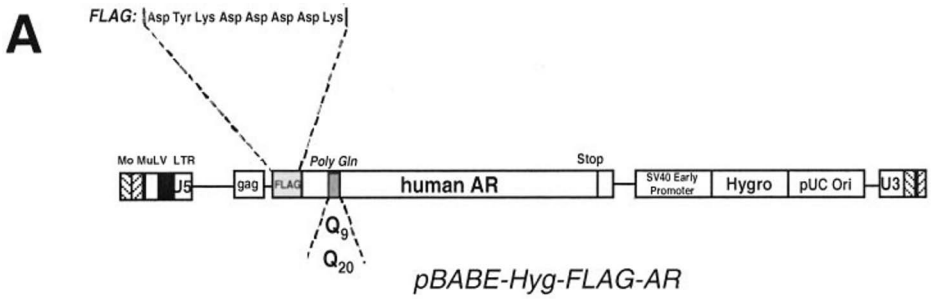
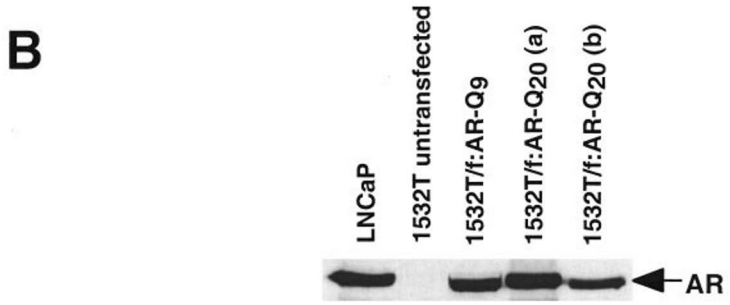


FIG. 1. **AR transcriptional activity increases with decreasing poly(Q) length in transiently transfected prostate cancer cell lines.** PC-3 cells (*A*), DU145 cells (*B*), or 1532T cells (*C–E*) were transiently cotransfected with the MMTV-Luc (*A–C* and *E*) or PSA-Luc (*D* and *E*) reporters and the AR-Q_9, AR-Q_{20}, or AR-Q_{44} constructs or empty vector and then incubated with varying concentrations of DHT (0.001, 0.01, 0.1, and 100 nM) or vehicle for 24 h as indicated. Relative luciferase units were determined from three independent transfections. Results are presented as the mean ± S.E. of the triplicate transfections.

AR Poly(Q) Truncation Enhances Androgen-dependent Transactivation in Stably Transfected Prostate Cancer Cells—To date, nearly all functional studies examining poly(Q) variation on AR function have made use of AR poly(Q) variants transiently transfected into cultured cells. The drawback of this approach is that transient AR expression is not necessarily physiologic, since the ectopic AR protein is typically grossly overexpressed and subsequently lost after only a few days in culture (13, 56, 57). To study the mechanisms by which poly(Q) variation effects AR function under more pathophysiological conditions, we used a retrovirus-mediated gene transfer approach to generate 1532T prostate cell lines that stably express FLAG epitope-tagged AR-Q_9 or AR-Q_{20} (f:AR-Q_9 and f:AR-Q_{20}, respectively) (Fig. 2*A*). As shown in Fig. 2*B*, quantitative im-





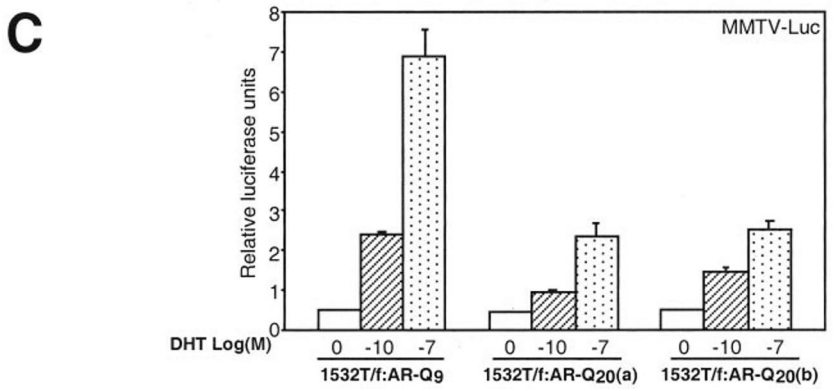


FIG. 2. **AR poly(Q) truncation enhances androgen-dependent transactivation in stably transfected prostate cancer cells.** *A*, schematic diagram of the retroviral vector expressing FLAG-AR-Q9 (f:AR-Q_9) or FLAG-AR-Q20 (f:AR-Q_{20}). *B*, relative f:AR expression levels in stably transfected 1532T cells. Whole cell lysate was prepared from equal numbers (1×10^6) of LNCaP cells or the different stable 1532T/f:AR-Q_n cell lines and probed by Western blot with an anti-AR monoclonal antibody. *C*, the transcriptional activity of 1532T/f:AR-Q_9 is higher than that of 1532T/f:AR-Q_{20}. Each of the stably transfected cell lines were transiently transfected with the pMMTV-Luc reporter and grown in the presence or absence of varying concentrations of DHT (0.1 and 100 nM) or vehicle for 24 h as indicated. Relative luciferase activities were determined from three independent transfections. Results are presented as the mean ± S.E. of the triplicate transfections.

munoblotting revealed relatively comparable f:AR expression levels in each of the three representative lines 1532T/f:AR-Q_{20} and 1532T/f:AR-Q_9(a) and 1532T/f:AR-Q_9(b). The expression levels were also less than or comparable with the expression of endogenous AR in prostate LNCaP cells, thus suggesting that the f:AR expression in the stable 1532T lines was not supraphysiological.

To compare androgen-dependent transactivation mediated by f:AR-Q_{20} *versus* f:AR-Q_9 stably expressed in 1532T cells, we measured transcription from the MMTV-Luc reporter gene. As shown in Fig. 2*C*, and in agreement with the transient AR-Q_n transfection studies, androgen-dependent transactivation in the stable 1532T/f:AR-Q_9 line was significantly greater than that observed in both of the 1532T/f:AR-Q_{20} lines in the presence of either a high or low DHT concentration (10^{-7} *versus* 10^{-10} M). It is interesting to note that the -fold difference in f:AR-Q_9 *versus* f:AR-Q_{20} transactivation in the stably transfected 1532T cells is significantly greater than that observed for 1532T cells transiently transfected with AR-Q_9 *versus* AR-Q_{20} (compare Figs. 1*C* and 2*C* at 10^{-7} M DHT). Whereas we do not fully understand the reason for this difference, it is plausible that differences in the overall AR expression levels in transient *versus* stably transfected 1532T cells result in different stoichiometric ratios between AR and limiting coregulatory factors.

AR Poly(Q) Truncation Enhances Androgen-dependent Prostate Cancer Cell Growth—Given the inverse relationship between AR poly(Q) length and androgen-dependent transcription, we tested whether poly(Q) truncation affects androgen-dependent prostate cell growth. Fig. 3 shows that DHT stimulated the proliferation of 1532T cells stably expressing wild type AR-Q_{20} between 25 and 50% over a 72-h period when compared with unstimulated cells. By contrast, DHT stimulated the growth of 1532T cells stably expressing the AR-Q_9 variant greater than 100% over the same time period. Interestingly, and in agreement with the transcription studies, 1532T cells expressing AR-Q_9 again responded to relatively low levels of DHT (Fig. 3*C*). These findings suggest that AR-mediated transcription is essential for androgen-dependent cell growth and that prostate cells expressing the transcriptionally hyperactive AR-Q_9 variant display a greater growth response to androgens than do cells expressing wild type AR.

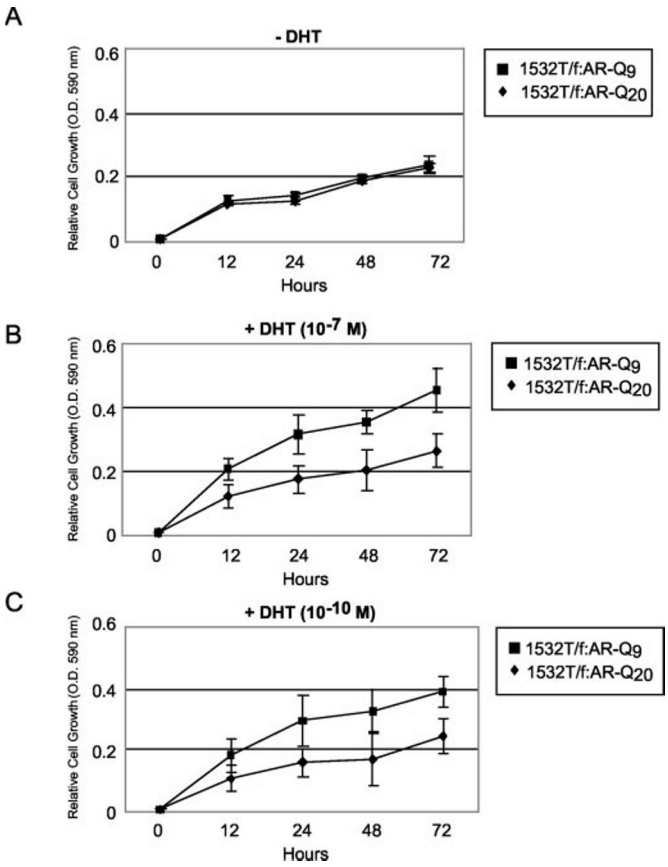


FIG. 3. **AR poly(Q) truncation enhances androgen-dependent prostate cancer cell growth.** Dose-dependent effects of R1881 on the growth of 1532T prostate cancer cells stably transfected with f:AR-Q_{20} or f:AR-Q_9 as determined by crystal violet assay. Cells were grown in medium containing charcoal/dextran-treated FBS for 24 h and then stimulated with R1881 (10^{-7} or 10^{-10} M) as indicated for 0, 12, 24, 48, and 72 h prior to cell number quantitation. Data represent the mean of three values repeated at least three times ± S.E.

AR Poly(Q) Truncation Enhances Ligand Binding Affinity—To begin to investigate the mechanisms that underlie the inverse correlation between AR poly(Q) length and androgen-dependent transcription and cell growth, we asked whether shortening the AR poly(Q) region affects ligand binding affinity. To address this question, saturation ligand binding assays were performed using the synthetic androgen methyltrienolone R1881 and 1532T cells stably expressing either AR-Q_9 or AR-Q_{20} (see "Experimental Procedures"). As shown in Fig. 4, [H^3]R1881 binding affinity for AR-Q_9 (K_d = 44.03 ± 3.40 pM) was almost double that for AR-Q_{20} (K_d = 79.67 ± 2.22 pM) ($p < 0.0001$), thus suggesting that AR proteins containing a truncated poly(Q) region can bind specific ligands more tightly than wild type AR proteins. Importantly, this finding may, at least in part, account for the higher transcriptional activity and higher responsiveness to low levels of ligand associated with the AR-Q_9 variant.

AR Poly(Q) Truncation Does Not Alter DNA Binding—Given that AR homodimerization and subsequent DNA binding is triggered by androgen (58, 59), it appeared plausible that AR proteins containing a truncated poly(Q) region might bind DNA more strongly in response to ligand than wild type AR, possibly accounting for their transcriptional hyperactivity. To investigate this possibility, 1532T cells stably expressing either f:AR-Q_9 or f:AR-Q_{20} were treated with 10^{-10} or 10^{-7} M DHT. The f:AR proteins were then immunoprecipitated from cell lysates using anti-FLAG antibodies coupled to agarose beads and subsequently eluted with a synthetic FLAG peptide (NH_2-DYKDDDDK-COOH) as previously described (51). As shown in Fig. 5*A*, f:AR-Q_9 and f:AR-Q_{20} purified from either 10^{-7} or 10^{-10} M DHT-treated cells bound to a radiolabeled androgen response element with relatively equal efficiency. These findings suggest that variation within the AR poly(Q) region does not have a significant effect on AR-DNA binding affinity and are consistent with a previous study showing no increased

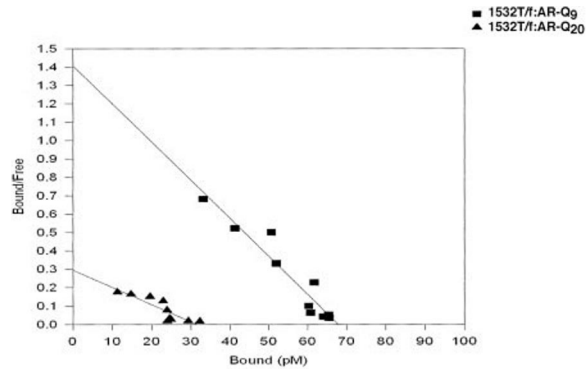


FIG. 4. **AR poly(Q) truncation enhances ligand binding affinity.** 1532T cells stably expressing f:AR-Q_9 or f:AR-Q_{20} were cultured in steroid-free medium overnight. The following day, the cells were cultured in serum-free medium containing [^{3}H]R1881 (0.06–3.6 nM) in the presence or absence of a 500-fold excess of cold DHT to determine nonspecific binding. 1 μM R5020 was additionally added to saturate progesterone and glucocorticoid receptors. Extracts were prepared, and cell-associated radioactivity was measured with a scintillation counter. The data were analyzed, and K_D and B_{max} were determined by nonlinear regression using Graphpad Prism software. $K_{D(1532T/f:AR\text{-}Q9)}$ = 44.03 ± 3.40 pM; $K_{D(1532T/f:AR\text{-}Q20)}$ = 79.67 ± 2.22 pM. $B_{max(1532T/f:AR(Q)9)}$ = 101,211 ± 2440 receptors/cell; $B_{max(1532T/f:AR(Q)20)}$ = 44,272 ± 265 receptors/cell.

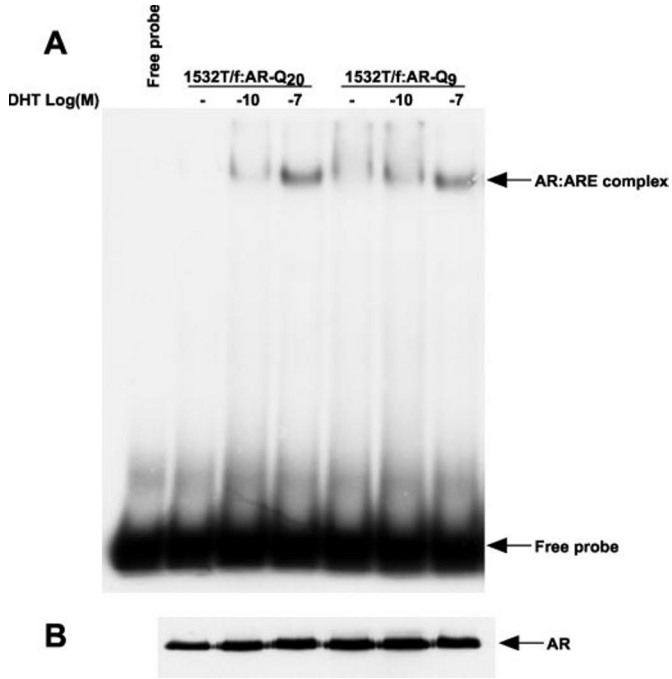


FIG. 5. **AR poly(Q) truncation does not alter DNA binding.** 1532T cells stably expressing either f:AR-Q_9 or f:AR-Q_{20} were grown in the presence or absence of DHT (10^{-7} or 10^{-10} M). The f:AR protein was then purified from whole cell lysates using anti-FLAG immunoresin followed by elution with a synthetic FLAG peptide. *A*, equal amounts of eluted protein were incubated with a ^{32}P-labeled ARE probe and then fractionated on a nondenaturing 5% polyacrylamide gel. The f:AR-ARE complexes and free probe are indicated by the *arrowheads*. *B*, Western blot quantitation of f:AR proteins used in *A*. Equal quantities of the eluted f:AR proteins from *A* were fractionated by SDS-PAGE and probed with the anti-AR monoclonal antibody.

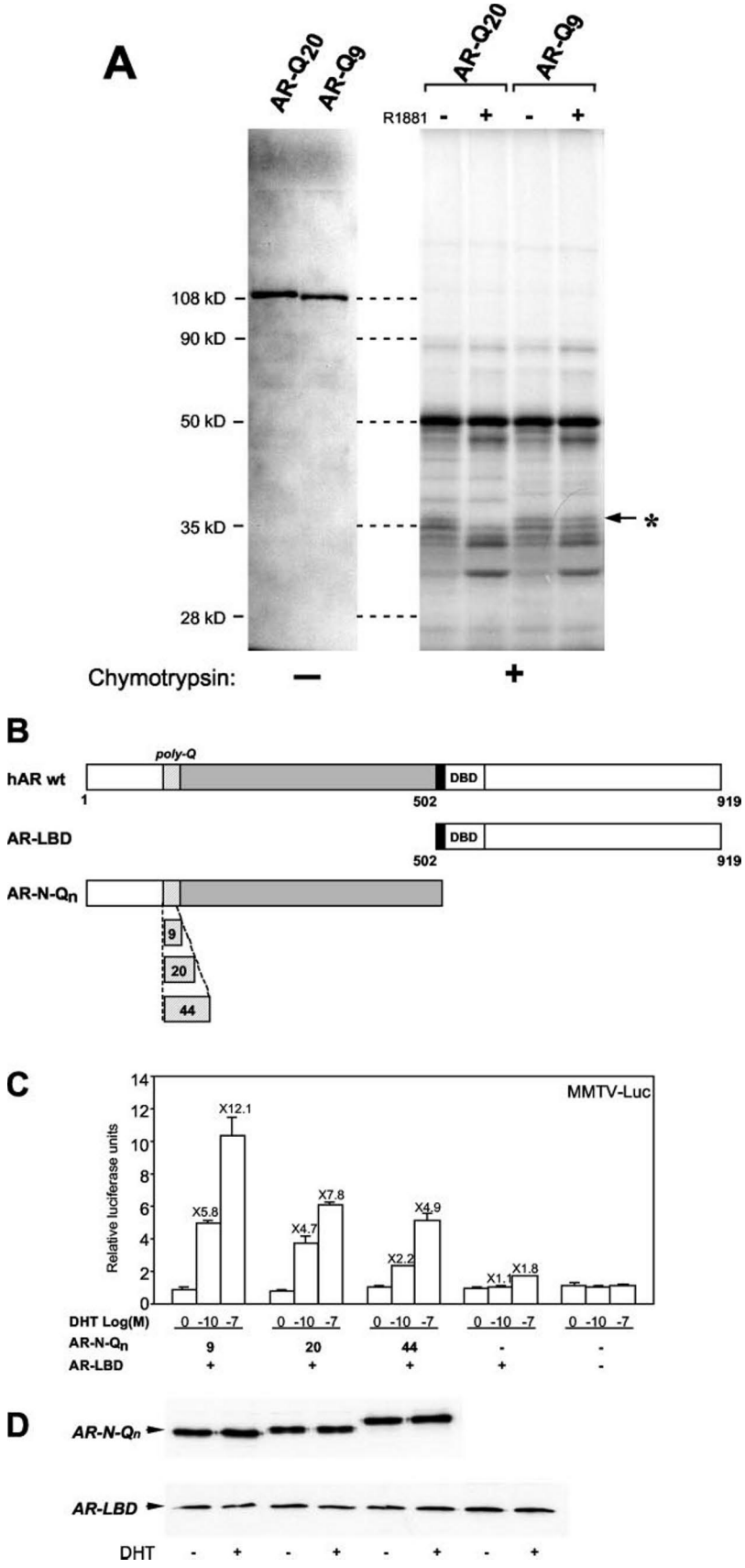


FIG. 6. **AR poly(Q) truncation confers distinct ligand-induced conformational changes and enhances the NTD/LBD interaction.** *A*, limited chymotrypsin digestion of f:AR-Q_9 and f:AR-Q_{20} in the presence or absence of ligand. *In vitro* synthesized [^{35}S]f:AR was incubated in the presence or absence of 10^{-7} M R1881 as indicated. The mixture was then digested with chymotrypsin (0.5 ng/μl final) for 10 min and then analyzed on a 10% SDS-acrylamide gel. The presence of a 37-kDa proteolysis-resistant fragment in the f:AR-Q_9 reaction is indicated by an *asterisk*. *B*, schematic representation of the AR-LBD and AR-NTD expression constructs. *C*, strength of the AR-NTD/LBD interaction is inversely proportional to the length of the poly(Q) region. CV-1 cells were transiently transfected with AR-LBD together with either AR-NTD-Q_9, AR-NTD-Q_{20}, or AR-NTD-Q_{44}. Transcription was measured from a cotransfected MMTV-Luc reporter gene. Three hours post-transfection, cells were stimulated with DHT (0.1 or 100 nM) or vehicle. Following a 24-h incubation, luciferase activities were determined from three independent transfections and are presented as the mean ± S.E. *D*, Western blot analysis of transfected AR-NTD-Q_n and AR-LBD. Equivalent amounts of the cellular lysate used in luciferase assays (*C*) was fractionated by SDS-PAGE and subsequently probed by Western blot with an anti-AR-NTD or anti-AR-LBD antibody.

DNA binding by an AR variant containing a shortened poly(Q) region (60).

AR Poly(Q) Truncation Confers Distinct Ligand-induced Conformational Changes and Enhances the NTD/LBD Interaction—The addition of ligand induces conformational changes in the AR that can be detected by limited proteolytic digestion (61). To examine whether truncation within the AR poly(Q) region alters ligand-induced conformation changes in the full-length AR protein, limited chymotrypsin digestion was performed on f:AR-Q_9 *versus* wild type f:AR-Q_{20} in the presence or

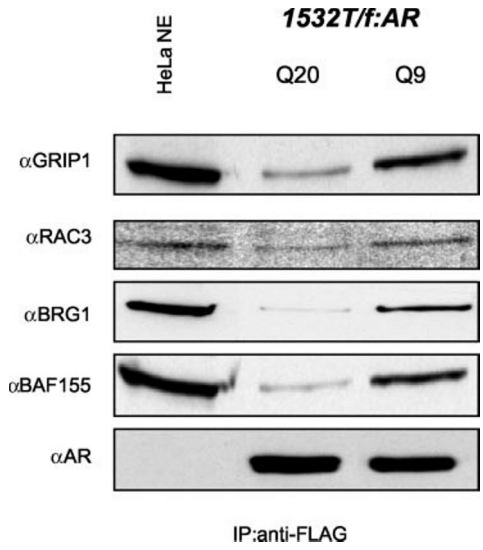


FIG. 7. **AR poly(Q) truncation enhances ligand-dependent association with coregulatory factors.** 1532 cells stably expressing either f:AR-Q_9 or f:AR-Q_{20} were expanded in medium containing charcoal/dextran-stripped FBS until 80% confluent. Cells were then starved for 24 h in serum-free medium, followed by treatment with R1881 10^{-7} M or vehicle (ethanol) for 6 h. Nuclear extract was then prepared, and f:AR-cofactor complexes were isolated using anti-FLAG immunochromatography (see "Experimental Procedures"). The isolated protein complexes were fractionated by 8% SDS-PAGE and then transferred to a nitrocellulose membrane. The same membrane was successively probed, stripped, and reprobed with the antibodies indicated on the *left*.

absence of R1881 (Fig. 6*A*). Interestingly, whereas both f:AR-Q_9 and f:AR-Q_{20} exhibited similar digestion patterns in the absence of ligand, f:AR-Q_9 differentially displayed a 37-kDa proteolysis-resistant fragment in the presence of R1881 that was not observed with wild type AR (Fig. 6*A*). These findings are consistent with the notion that truncation in the AR poly(Q) region confers distinct ligand-induced changes in the structural conformation of the AR protein. Given that truncated AR poly(Q) variants concomitantly exhibit transcriptional hyperactivity, it is intriguing to speculate that these ligand-induced conformational alterations are mechanistically important for transcriptional activation.

It has been reported that the ligand-induced intramolecular interaction between the AR-NTD and -LBD is indispensable for AR transactivation *in vivo* (12–14). Given the findings in Fig. 6*A*, it is conceivable that truncated poly(Q) length in the AR-NTD might positively influence this interaction. To test this hypothesis more thoroughly, we expressed a series of AR-NTD constructs containing different poly(Q) lengths (9, 20, or 44) and tested their ability to interact with the wild type AR-LBD using a modified mammalian two-hybrid assay (Fig. 6*B*) (see "Experimental Procedures"). In agreement with the modest AF-2 activity intrinsically associated with the AR-LBD (13, 27, 62, 63), transfection of AR-LBD alone exhibited very weak (or no) transactivation from an MMTV reporter gene in the presence of high (10^{-7} M) or low (10^{-10} M) levels of DHT (Fig. 6*C*). By contrast, and in accordance with a crucial role for the intramolecular NTD/LBD interaction in mediating AR transactivation, coexpression of the wild type AR-NTD-Q_{20} together with AR-LBD increased transactivation 7.8- and 4.7-fold in the presence 10^{-7} or 10^{-10} M DHT, respectively (Fig. 6*C*).

Importantly, cotransfection of AR-LBD together with the shortened AR-NTD-Q_9 variant significantly raised the ligand-induced transactivation (12.1- and 5.8-fold at 10^{-7} or 10^{-10} M DHT, respectively), whereas cotransfection with the elongated AR-NTD-Q_{44} variant lowered the level of induction (Fig. 6*C*). As shown in Fig. 6*D*, the expression levels of the various AR-NTD proteins were nearly identical from transfection to transfection, as was the expression of AR-LBD, thus indicating that the differences in -fold transactivation are due to differences in poly(Q) length and not fluctuating AR-NTD-Q_n or AR-LBD expression. Taken together, these data suggest that the relative strength of the ligand-induced intramolecular AR-NTD/LBD interaction is inversely proportional to the length of the poly(Q) region with the AR-NTD. Considering that the NTD/LBD interaction is critical for AR transactivation, these findings are consistent with our parallel observations showing that AR proteins containing a shortened poly(Q) region are transcriptionally hyperactive.

AR Poly(Q) Truncation Promotes Association with Coregulatory Factors—The ligand-induced AR-NTD/LBD interaction is believed to generate a composite binding surface for the recruitment of transcriptional coactivators that modulate the functional activity of AR (17, 26, 27). Since poly(Q) truncation strengthens and/or stabilizes the NTD/LBD interaction, we investigated whether AR poly(Q) truncation concomitantly affects ligand-dependent AR association with coregulatory factors. Toward this end, f:AR-Q_9- *versus* f:AR-Q_{20}-associated protein complexes were immunopurified from the 1532T/f:AR-Q_9 and 1532T/f:AR-Q_{20} stable cell lines stimulated with R1881 and then probed by Western blot with antibodies against known NR cofactors (Fig. 7). Interestingly, we observed an ~3-fold increase in the amount of GRIP-1, a member of the p160 family of coactivators, that was associated with f:AR-Q_9 as compared with that associated with f:AR-Q_{20}. Similarly, there was an ~2-fold increase in the amount of the p160 coactivator RAC3 associated with f:AR-Q_9 relative to that associated with f:AR-Q_{20} (Fig. 7).

Of note, we also observed an ~5-fold increase in the amount of Brg1, a core ATPase of the SWI/SNF chromatin remodeling complex, that was associated with f:AR-Q_9 *versus* f:AR-Q_{20} (Fig. 7). Another component of the SWI/SNF complex, Brg1-associated factor 155 (BAF155), was also differentially associated with f:AR-Q_9, consistent with the notion that AR is associated with the holo-SWI/SNF complex in the presence of ligand (30–32). In contrast to these findings, we observed no significant differences between the binding affinity of f:AR-Q_9 *versus* f:AR-Q_{20} with other reported AR cofactors (*e.g.* ARA70, FHL2, and β-catenin; data not shown). These results thus show that AR proteins containing a shortened poly(Q) region are associated *in vivo* with higher levels of specific p160 coactivators and components of the SWI/SNF complex in prostate cancer cells. Importantly, and taken together with the data presented earlier, these findings may provide insights into the potential molecular mechanisms that, at least in part, account for the transcriptional hyperactivity inversely correlated with AR poly(Q) length.

DISCUSSION

Several epidemiological studies suggest that the inheritance of an AR allele containing an abnormally low number of Q repeats within the AR-NTD poly(Q) tract may predict for a higher risk of prostate cancer, an earlier age of onset, and a higher grade and more advanced stage of prostate cancer at the time of diagnosis (reviewed in Refs. 37 and 64). It has been proposed that an enhanced AR transcriptional activity associated with the shortened poly(Q) region may overstimulate prostate cell growth, thus leading to an increased risk of prostate cancer (44, 65). Consistent with this hypothesis, a number of reports clearly revealed an inverse correlation between the length of the poly(Q) region and ligand-dependent transcriptional activity of AR (45–48). However, the detailed mechanisms that trigger these putative changes in AR activity as a function of poly(Q) length are poorly understood.

In this study, we examined the affect of poly(Q) length variation on AR functional activity in metastatic and primary malignant prostate cancer cell lines. In agreement with the earlier reports, we found that ligand-dependent transcription by AR at both high (10^{-7} M) and low (10^{-10} to 10^{-11} M) DHT concentrations is inversely correlated to the length of the poly(Q) tract. Here, we extend the previous studies and further show an inverse correlation between androgen-dependent cellular proliferation and AR poly(Q) length. Interestingly, we found that AR proteins containing a truncated poly(Q) region (AR-Q_9) were more responsive to low concentrations of DHT (10^{-11} to 10^{-10} M) than the wild type AR (AR-Q_{20}). Relevant to this finding, we also found that the ligand binding affinity of AR-Q_9 for radiolabeled R1881 was almost double that for AR-Q_{20}. Thus, our results suggest that AR proteins containing a truncated poly(Q) region bind specific types of androgenic-like compounds *in vivo* more tightly than the wild type AR.

Given that the ligand binding pocket is localized predominantly to polypeptide sequences within the C-terminal end of AR (66, 67), the question arises as to how variation within the N-terminal poly(Q) tract might influence AR ligand binding affinity. One potential clue may come from the ligand-induced NTD/LBD interaction that appears to be crucial for ligand-dependent transactivation (12–14). It is conceivable that the NTD/LBD interaction serves to stabilize ligand binding, and in turn, variation within the poly(Q) region influences the relative strength of the NTD/LBD interaction. Indeed, we found that the strength of the ligand-induced NTD/LBD interaction is inversely proportional to the length of the poly(Q) region within the AR-NTD. These findings are in agreement with a recent study showing that deletion of the entire AR poly(Q) tract positively affects the NTD/LBD interaction (50). However, our findings contrast an earlier study showing that deletion of the entire AR poly(Q) tract resulted in only a slight increase in ligand binding (8). The reason for this difference might be accounted for by the fact that in addition to the entire poly(Q) tract, several flanking residues in the NTD were also deleted. In addition, the AR variants used in this study were transiently transfected in CV-1 cells (8) as opposed to stably expressed in prostate cells as carried out here.

Two regions in the AR-NTD are directly involved in interactions with the LBD. The first region is located between amino acids 3 and 36, and the second region is located between residues 370 and 494 (13). He *et al.* (15) precisely mapped a ^{23}FQNLF^{27} sequence and a ^{433}WHTLF^{437} motif that appear to be critical for mediating interactions with the AF-2 domain in the LBD. Although the poly(Q) tract, spanning amino acids 58–78 in the wild type AR, does not necessarily contact the LBD, shortening of the poly(Q) tract changes the relative spacing between the F*XX*LF and W*XX*LF motifs and probably alters their spatial orientation in the presence of ligand. These changes presumably result in a structural conformation that promotes the NTD/LBD interaction. Alternatively, truncation of the poly(Q) tract may generate a novel structure in which the poly(Q) region directly interacts with the LBD. Additional biochemical and crystallography studies will be needed to clearly investigate the affect of poly(Q) variation on the stereospecificity of the NTD and LBD in the presence of ligand.

The ligand-induced NTD/LBD interaction is believed to generate a composite binding surface for the recruitment of transcriptional coactivators essential for the functional activity of AR (17, 26, 27), and as shown here, the relative strength of this interaction increases as the length of the poly(Q) tract decreases. Importantly, we found that AR proteins containing a shortened poly(Q) region are associated *in vivo* with higher levels of p160 family members and components of the SWI/SNF chromatin remodeling complex when compared with wild type AR. Therefore, shortening the poly(Q) tract not only strengthens the intramolecular NTD/LBD interaction but also appears to generate a conformational structure that enhances the binding of specific transcriptional coactivators. The Tau1 and Tau5 motifs in the AR-NTD (residues 101–360 and 370–494, respectively) as well as the AF-2 motif in the AR-LBD have been implicated in directly contacting p160 proteins (16, 26, 27, 68). Hence, it is likely that a shorter poly(Q) tract generates a more stable p160 binding surface, possibly via stabilization of the NTD/LBD interaction, whereas an expansion of the poly(Q) tract may inhibit p160 binding possibly via a steric hindrance mechanism (37).

Whereas direct interactions between AR and p160 proteins have been clearly established, the manner by which AR contacts the SWI/SNF complex is less defined. The BAF57 and BAF60a subunits of the SWI/SNF complex have been shown to directly interact with the estrogen and glucocorticoid receptors (33, 34) and may play analogous roles with AR. Thus, similar to the situation with p160 proteins, shortening of the AR poly(Q) tract may generate a composite NTD/LBD interaction surface that promotes the binding of one of these SNF/SWI subunits. Alternatively, it is intriguing to speculate that the truncated poly(Q) tract itself might act as novel binding surface that directly interacts with specific components of the SNF/SWI complex. Finally, the possibility exists that the SNF/SWI complex might be targeted to AR indirectly via its direct association with p160 proteins (31, 34). Such a scenario would still be consistent with the findings here in that an enhanced recruitment of p160 coactivators to AR proteins containing a truncated poly(Q) tract would concomitantly increase the recruitment of the SNF/SWI complex. Future binding experiments should establish whether the SWI/SNF complex is directly or indirectly targeted to AR and more clearly determine the affect of poly(Q) variation on this interaction. Taken together, our findings suggest that the transcriptional hyperactivity associated with AR proteins containing shortened poly(Q) tracts stems from altered ligand-induced conformational changes that result in a higher ligand binding affinity, a stronger ligand-induced NTD/LBD interaction, and an enhanced recruitment of specific transcriptional coregulatory factors.

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REFERENCES

1. Quigley, C. A., De Bellis, A., Marschke, K. B., el-Awady, M. K., Wilson, E. M., and French, F. S. (1995) *Endocr. Rev.* **16,** 271–321
2. Trapman, J., and Brinkmann, A. O. (1996) *Pathol. Res. Pract.* **192,** 752–760
3. Jenster, G. (1999) *Semin. Oncol.* **26,** 407–421
4. Sadar, M. D., Hussain, M., and Bruchovsky, N. (1999) *Endocr. Relat. Cancer* **6,** 487–502
5. Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R. M. (1995) *Cell* **83,** 835–839
6. Tsai, M. J., and O'Malley, B. W. (1994) *Annu. Rev. Biochem.* **63,** 451–486
7. Parker, M. G. (1993) *Curr. Opin. Cell Biol.* **5,** 499–504
8. Chamberlain, N. L., Whitacre, D. C., and Miesfeld, R. L. (1996) *J. Biol. Chem.* **271,** 26772–26778
9. Jenster, G., van der Korput, H. A., Trapman, J., and Brinkmann, A. O. (1995) *J. Biol. Chem.* **270,** 7341–7346
10. Danielian, P. S., White, R., Lees, J. A., and Parker, M. G. (1992) *EMBO J.* **11,** 1025–1033
11. Moras, D., and Gronemeyer, H. (1998) *Curr. Opin. Cell Biol.* **10,** 384–391
12. Ikonen, T., Palvimo, J. J., and Janne, O. A. (1997) *J. Biol. Chem.* **272,** 29821–29828
13. Berrevoets, C. A., Doesburg, P., Steketee, K., Trapman, J., and Brinkmann, A. O. (1998) *Mol. Endocrinol.* **12,** 1172–1178
14. He, B., Kemppainen, J. A., Voegel, J. J., Gronemeyer, H., and Wilson, E. M. (1999) *J. Biol. Chem.* **274,** 37219–37225
15. He, B., Kemppainen, J. A., and Wilson, E. M. (2000) *J. Biol. Chem.* **275,** 22986–22994
16. He, B., and Wilson, E. M. (2003) *Mol. Cell. Biol.* **23,** 2135–2150
17. Saitoh, M., Takayanagi, R., Goto, K., Fukamizu, A., Tomura, A., Yanase, T., and Nawata, H. (2002) *Mol. Endocrinol.* **16,** 694–706

18. Heinlein, C. A., and Chang, C. (2002) *Endocr. Rev.* **23,** 175–200
19. Glass, C. K., and Rosenfeld, M. G. (2000) *Genes Dev.* **14,** 121–141
20. McKenna, N. J., Lanz, R. B., and O'Malley, B. W. (1999) *Endocr. Rev.* **20,** 321–344
21. Bannister, A. J., and Kouzarides, T. (1996) *Nature* **384,** 641–643
22. Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H., and Nakatani, Y. (1996) *Cell* **87,** 953–959
23. Blanco, J. C., Minucci, S., Lu, J., Yang, X. J., Walker, K. K., Chen, H., Evans, R. M., Nakatani, Y., and Ozato, K. (1998) *Genes Dev.* **12,** 1638–1651
24. Chen, D., Ma, H., Hong, H., Koh, S. S., Huang, S. M., Schurter, B. T., Aswad, D. W., and Stallcup, M. R. (1999) *Science* **284,** 2174–2177
25. Wang, H., Huang, Z. Q., Xia, L., Feng, Q., Erdjument-Bromage, H., Strahl, B. D., Briggs, S. D., Allis, C. D., Wong, J., Tempst, P., and Zhang, Y. (2001) *Science* **293,** 853–857
26. Alen, P., Claessens, F., Verhoeven, G., Rombauts, W., and Peeters, B. (1999) *Mol. Cell. Biol.* **19,** 6085–6097
27. Bevan, C. L., Hoare, S., Claessens, F., Heery, D. M., and Parker, M. G. (1999) *Mol. Cell. Biol.* **19,** 8383–8392
28. Becker, P. B., and Horz, W. (2002) *Annu. Rev. Biochem.* **71,** 247–273
29. Kingston, R. E., and Narlikar, G. J. (1999) *Genes Dev.* **13,** 2339–2352
30. Marshall, T. W., Link, K. A., Petre-Draviam, C. E., and Knudsen, K. E. (2003) *J. Biol. Chem.* **278,** 30605–30613
31. Huang, Z. Q., Li, J., Sachs, L. M., Cole, P. A., and Wong, J. (2003) *EMBO J.* **22,** 2146–2155
32. Inoue, H., Furukawa, T., Giannakopoulos, S., Zhou, S., King, D. S., and Tanese, N. (2002) *J. Biol. Chem.* **277,** 41674–41685
33. Hsiao, P. W., Fryer, C. J., Trotter, K. W., Wang, W., and Archer, T. K. (2003) *Mol. Cell. Biol.* **23,** 6210–6220
34. Belandia, B., Orford, R. L., Hurst, H. C., and Parker, M. G. (2002) *EMBO J.* **21,** 4094–4103
35. Lemon, B., Inouye, C., King, D. S., and Tjian, R. (2001) *Nature* **414,** 924–928
36. Wang, Q., Sharma, D., Ren, Y., and Fondell, J. D. (2002) *J. Biol. Chem.* **277,** 42852–42858
37. Clark, P. E., Irvine, R. A., and Coetzee, G. A. (2003) *Methods Mol. Med.* **81,** 255–266
38. Choong, C. S., and Wilson, E. M. (1998) *J. Mol. Endocrinol.* **21,** 235–257
39. Ingles, S. A., Ross, R. K., Yu, M. C., Irvine, R. A., La Pera, G., Haile, R. W., and Coetzee, G. A. (1997) *J. Natl. Cancer Inst.* **89,** 166–170
40. Giovannucci, E., Stampfer, M. J., Krithivas, K., Brown, M., Dahl, D., Brufsky, A., Talcott, J., Hennekens, C. H., and Kantoff, P. W. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 3320–3323
41. Hardy, D. O., Scher, H. I., Bogenreider, T., Sabbatini, P., Zhang, Z. F., Nanus, D. M., and Catterall, J. F. (1996) *J. Clin. Endocrinol. Metab.* **81,** 4400–4405
42. Stanford, J. L., Just, J. J., Gibbs, M., Wicklund, K. G., Neal, C. L., Blumenstein, B. A., and Ostrander, E. A. (1997) *Cancer Res.* **57,** 1194–1198
43. Montgomery, J. S., Price, D. K., and Figg, W. D. (2001) *J. Pathol.* **195,** 138–146
44. Casella, R., Maduro, M. R., Lipshultz, L. I., and Lamb, D. J. (2001) *Urology* **58,** 651–656
45. Chamberlain, N. L., Driver, E. D., and Miesfeld, R. L. (1994) *Nucleic Acids Res.* **22,** 3181–3186
46. Jenster, G., de Ruiter, P. E., van der Korput, H. A., Kuiper, G. G., Trapman, J., and Brinkmann, A. O. (1994) *Biochemistry* **33,** 14064–14072
47. Mhatre, A. N., Trifiro, M. A., Kaufman, M., Kazemi-Esfarjani, P., Figlewicz, D., Rouleau, G., and Pinsky, L. (1993) *Nat. Genet.* **5,** 184–188
48. Tut, T. G., Ghadessy, F. J., Trifiro, M. A., Pinsky, L., and Yong, E. L. (1997) *J. Clin. Endocrinol. Metab.* **82,** 3777–3782
49. Irvine, R. A., Ma, H., Yu, M. C., Ross, R. K., Stallcup, M. R., and Coetzee, G. A. (2000) *Hum. Mol. Genet.* **9,** 267–274
50. Callewaert, L., Christiaens, V., Haelens, A., Verrijdt, G., Verhoeven, G., and Claessens, F. (2003) *Biochem. Biophys. Res. Commun.* **306,** 46–52
51. Wang, Q., and Fondell, J. D. (2001) *Anal. Biochem.* **289,** 217–230
52. Oettgen, P., Finger, E., Sun, Z., Akbarali, Y., Thamrongsak, U., Boltax, J., Grall, F., Dube, A., Weiss, A., Brown, L., Quinn, G., Kas, K., Endress, G., Kunsch, C., and Libermann, T. A. (2000) *J. Biol. Chem.* **275,** 1216–1225
53. Bright, R. K., Vocke, C. D., Emmert-Buck, M. R., Duray, P. H., Solomon, D., Fetsch, P., Rhim, J. S., Linehan, W. M., and Topalian, S. L. (1997) *Cancer Res.* **57,** 995–1002
54. Parker, B. A., and Stark, G. R. (1979) *J. Virol.* **31,** 360–369
55. Claessens, F., Alen, P., Devos, A., Peeters, B., Verhoeven, G., and Rombauts, W. (1996) *J. Biol. Chem.* **271,** 19013–19016
56. Kemppainen, J. A., Langley, E., Wong, C. I., Bobseine, K., Kelce, W. R., and Wilson, E. M. (1999) *Mol. Endocrinol.* **13,** 440–454
57. Lobaccaro, J. M., Poujol, N., Terouanne, B., Georget, V., Fabre, S., Lumbroso, S., and Sultan, C. (1999) *Endocrinology* **140,** 350–357
58. Wong, C. I., Zhou, Z. X., Sar, M., and Wilson, E. M. (1993) *J. Biol. Chem.* **268,** 19004–19012
59. Karvonen, U., Kallio, P. J., Janne, O. A., and Palvimo, J. J. (1997) *J. Biol. Chem.* **272,** 15973–15979
60. Beilin, J., Ball, E. M., Favaloro, J. M., and Zajac, J. D. (2000) *J. Mol. Endocrinol.* **25,** 85–96
61. Kuil, C. W., Berrevoets, C. A., and Mulder, E. (1995) *J. Biol. Chem.* **270,** 27569–27576
62. Simental, J. A., Sar, M., Lane, M. V., French, F. S., and Wilson, E. M. (1991) *J. Biol. Chem.* **266,** 510–518
63. Langley, E., Zhou, Z. X., and Wilson, E. M. (1995) *J. Biol. Chem.* **270,** 29983–29990
64. Nelson, K. A., and Witte, J. S. (2002) *Am. J. Epidemiol.* **155,** 883–890
65. Kantoff, P., Giovannucci, E., and Brown, M. (1998) *Biochim. Biophys. Acta* **1378,** C1–C5
66. Vivat, V., Gofflo, D., Garcia, T., Wurtz, J. M., Bourguet, W., Philibert, D., and Gronemeyer, H. (1997) *J. Mol. Endocrinol.* **18,** 147–160
67. Zhu, Z., Becklin, R. R., Desiderio, D. M., and Dalton, J. T. (2001) *Biochemistry* **40,** 10756–10763
68. Christiaens, V., Bevan, C. L., Callewaert, L., Haelens, A., Verrijdt, G., Rombauts, W., and Claessens, F. (2002) *J. Biol. Chem.* **277,** 49230–49237