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

Qianben Wang‡, T. S. Udayakumar‡, Tadas S. Vasaitis§, Angela M. Brodie§, and Joseph D. Fondell¶

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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 regulation 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 coactivators (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 LXXLL 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 hBHRM 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 ARs, FHL2, β-catenin, AES, SNURF, PDEF, ARIP3, BRCA1, and cyclin D1 (reviewed in Ref. 18).
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 transactivation 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 transactivation 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 relationship 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-Q9, pcDNA-AR-Q20 (wild type), and pcDNA-AR-Q44 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 pC3OS-Luc reporter gene was a gift from A. Libermann (Harvard Medical School) (52). To construct pBabe Hyc-fAR-Q9, a Xmal/AflIII fragment from pcDNA-AR-Q9 was first subcloned into the Xmal/AflIII sites of pt-TFLAG-AR (51), generating ptT-FLAG-AR-Q9. An Ndel/BamHI fragment from ptT-FLAG-AR-Q9 was then subcloned into the Ndel/BamHI sites of ptetCMV-F-PS-AR-Q. A BglII/BamHI fragment from ptetCMV-F-PS-AR-Q9 was inserted into BamHI-digested pBabe Hyg vector from C.-M. Chang (Case Western Reserve University), generating pBabe Hyc-fAR-Q9. The pBabe Hyc-fAR-Q20 (wild type) construction was generated by subcloning the BglII/BamHI fragment from ptetCMV-F-PS-AR-Q9 into the BamHI site of pBabe Hyc vector. To generate pcDNA-AR-NTD-Q44 (amino acids 1–251), a point mutation of pcDNA-AR-Q44 was generated 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 template 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-Q9 (amino acids 1–488) and pcDNA-AR-NTD-Q39 (amino acids 1–489), the AflII/EcoRI fragments (containing a stop codon at amino acid 522) from pcDNA-AR-Q9 and pcDNA-AR-Q39, respectively, were ligated into AflII/EcoRI sites of pcDNA-AR-Q9 and pcDNA-AR-Q39, respectively. The pcDNA-AR-NTD-Q9 and pcDNA-AR-NTD-Q39 were then subcloned into pcDNA-AR-Q9 (amino acids 1–251) were subcloned into AflII/EcoRI sites of pcDNA-AR-Q9 and pcDNA-AR-Q39, respectively. The pcDNA-AR-NTD-Q9 and pcDNA-AR-NTD-Q39 were 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 relationship between androgen-dependent cellular proliferation and poly(Q) length. Furthermore, we found 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.
Truncated CAG Repeat Facilitates AR Hyperactivity

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day, the medium was aspirated and replaced with steroid-free, serum-free Iscove’s modified Eagle’s medium containing [17β-methyl-3H]R1881 (specific activity 70–87 Ci/nmol) (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 progestrone) 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 Bmax were determined by nonlinear regression using Graphpad Prism software.

Coimmunoprecipitation of AR-Coregulatory Factor Complexes—Transient co-transfections of AR-NTD-Q9 (AR-NTD-Q9, AR-NTD-Q9, and 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 × 105 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 plasmid (pcDNA-AR-N/TQ9, amino acids 506–919) with 0.2 μg of AR-NTD-Q9, plasmid (pcDNA-AR-NTD-Q9, amino acids 1–488), PC DNA-AR-NTD-Q9o (amino acids 1–489), or pcDNA-AR-NTD-Q4 (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-gal was cotransfected with LipofectAMINE reagent and added to each well. Cells were incubated at 37 °C in 5% CO2 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). 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 CTT AAC ACA TGT TCT TAA AAT CGA-3′) (55) and its complement were annealed to double-stranded templates with protruding BglIII ends. The double-stranded ARE was then labeled by filling in with [α32P]dCTP (Amersham Biosciences) and Klenow enzyme. The fAR protein used in EMSA, fAR, was immunopurified from 152T/AR-Q9, and 152T/AR-Q20 cells lines using anti-FLAG antibodies coupled to agarose beads (Sigma) and subsequently eluted using a FLAG peptide as described previously (51). fAR was incubated for 15 min at room temperature in a binding buffer containing 10 mM Tris (pH 7.9), 50 mM KCl, 1 mM dithiothreitol, 10% glycerol, 1 μg/ml 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.

Limited Proteolytic Digestion Assay—To determine the effects of R1881 on the growth of 152T/AR-Q9(b) and 152T/AR-Q20 cells in vitro, 1532 T cells were grown as mentioned under “Cell Culture.” Cells were plated at 1.2 × 106 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, 24, 12, 48, and 72 h. The cells were then fixed in 1% glacial acetic acid 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).

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 (Q9, Q20, and Q44) 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. 1A, AR transcriptional activity in PC-3 cells increased with decreasing Q-repeat length in the presence of both high and low concentrations of DHT (10−10 and 10−7 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 transcription in DU145 cells was also inversely correlated with the poly(Q) length (Fig. 1B). Notably, AR-Q20 transcription in DU145 cells was significantly higher than that of AR-Q9, 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 (Q9) respond to lower levels of ligand than the wild type AR (Q20).

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 cell line with the AR poly(Q) 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-Q9 protein is transcriptionally responsive to lower levels of ligand (10−10 to DHT) than AR-Q20. 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.
**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-Q9 or AR-Q20 (f:AR-Q9 and f:AR-Q20, respectively) (Fig. 2A). As shown in Fig. 2B, quantitative im-
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 9 versus f:AR-Q 20 stably expressed in 1532T cells, we measured transcription from the MMTV-Luc reporter gene. As shown in Fig. 2C, and in agreement with the transient AR-Q 9a transfection studies, androgen-dependent transactivation in the stable 1532T/f:AR-Q 9a 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. 1C and 2C 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. 3C). 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.
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-Q9 or AR-Q20 (see "Experimental Procedures"). As shown in Fig. 4, [3H]R1881 binding affinity for AR-Q9 (K_d = 44.03 ± 3.40 pM) was almost double that for AR-Q20 (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-Q9 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-Q9 or f:AR-Q20 were treated with 10^{-7} or 10^{-10} M DHT. The f:AR proteins were then immunoprecipitated from whole cell lysates using anti-FLAG immunoresin followed by elution with a synthetic FLAG peptide. As shown in Fig. 5, equal amounts of eluted protein were incubated with a 32P-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. Western blot quantitation of f:AR proteins used in A were fractionated by SDS-PAGE and probed with the anti-AR monoclonal antibody.

**Fig. 5. AR poly(Q) truncation does not alter DNA binding.** 1532T cells stably expressing either f:AR-Q9 or f:AR-Q20 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 32P-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. 4. AR poly(Q) truncation enhances ligand binding affinity.** 1532T cells stably expressing f:AR-Q9 or f:AR-Q20 were cultured in serum-free medium containing [3H]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-Q9) = 44.03 ± 3.40 pM; K_d(1532T/f:AR-Q20) = 79.67 ± 2.23 pM. B_max(1532T/f:AR-Q9) = 101,211 ± 2440 receptors/cell; B_max(1532T/f:AR-Q20) = 44,272 ± 265 receptors/cell.
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-Q9 versus wild type f:AR-Q20 in the presence or absence of ligand. In vitro synthesized [35S]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-Q9 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-Q9, AR-NTD-Q20, or AR-NTD-Q44. 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-Qn 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.
activation, coexpression of the wild type AR-NTD-Q20 together
By contrast, and in accordance with a crucial role for the
plexes were fractionated by 8% SDS-PAGE and then transferred to a
matography (see “Experimental Procedures”). The isolated protein com-
plexes were fractionated by 8% SDS-PAGE and then transferred to a
itrocellulose membrane. The same membrane was successively
probed, stripped, and reprobed with the antibodies indicated on the
absence of R1881 (Fig. 6A). Interestingly, whereas both f:AR-Q9 and f:AR-Q20 exhibited similar digestion patterns in the
absence of ligand, f:AR-Q9 differentially displayed a 37-kDa proteolysis-resistant fragment in the presence of R1881 that
was not observed with wild type AR (Fig. 6A). 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 trun-
cated 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 transcription in vivo (12–14). Given the findings in Fig.
6A, 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. 6B) (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. 6C).
By contrast, and in accordance with a crucial role for the
intramolecular NTD/LBD interaction in mediating AR trans-
activation, coexpression of the wild type AR-NTD-Q20 together
with AR-LBD increased transactivation 7.8- and 4.7-fold in the
presence 10^{-7} or 10^{-10} M DHT, respectively (Fig. 6C).

Importantly, cotransfection of AR-LBD together with the
shortened AR-NTD-Q9 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-Q44 variant lowered the level of induction (Fig. 6C).
As shown in Fig. 6D, the expression levels of the various AR-
NTD proteins were nearly identical from transfection to trans-
fection, 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-, 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 find-
ings are consistent with our parallel observations showing that
AR proteins containing a shortened poly(Q) region are tran-
scriptionally hyperactive.

AR Poly(Q) Truncation Promotes Association with Coregula-
tory Factors—The ligand-induced AR-NTD/LBD interaction is
believed to generate a composite binding surface for the re-
cruitment 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 af-
facts ligand-dependent AR association with coregulatory factors.
Toward this end, f:AR-Q9 versus f:AR-Q20-associated protein
complexes were immunopurified from the 1532T/f:AR-Q9
and 1532T/f:AR-Q20 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-Q9
as compared with that associated with f:AR-Q20. Similarly,
there was an −2-fold increase in the amount of the p160 coac-
tivator RAC3 associated with f:AR-Q9 relative to that associ-
ated with f:AR-Q20 (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-Q9 versus f:AR-Q20
(Fig. 7). Another component of the SWI/SNF complex, Brg1-
associated factor 155 (BAF155), was also differentially associ-
ated with f:AR-Q9 consistent with the notion that AR is asso-
ciated 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-Q9
versus f:AR-Q20 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 coactiva-
tors and components of the SWI/SNF complex in prostate
cancer cells. Importantly, and taken together with the data pre-
sented 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 associ-
ated with the shortened poly(Q) region may overstimulate pro-
state 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 transcrip-
tional activity of AR (45–48). However, the detailed mecha-
nisms that trigger these putative changes in AR activity as a
function of poly(Q) length are poorly understood.

**Fig. 7.** AR poly(Q) truncation enhances ligand-dependent as-

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**Fig. 7.** AR poly(Q) truncation enhances ligand-dependent as-
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^{-11} to 10^{-14} 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-Q20) were more responsive to low concentrations of DHT than the wild type AR (AR-Q80). Relevant to this finding, we also found that the ligand binding affinity of AR-Q20 for radiolabeled R1881 was almost double that for AR-Q80. Thus, our results suggest that AR proteins containing a truncated poly(Q) region bind specific types of androgenic-like compounds in vitro 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 proliferative AR poly(Q) length. 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 23FQNLFLG sequence and a 43WIHTLAF 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 FXXLF and WXXL 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 vitro 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 SWI/SNF 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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Mechanistic Relationship between Androgen Receptor Polyglutamine Tract Truncation and Androgen-dependent Transcriptional Hyperactivity in Prostate Cancer Cells

Qianben Wang, T. S. Udayakumar, Tadas S. Vasaitis, Angela M. Brodie and Joseph D. Fondell

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