The Androgen Receptor Interacts with Multiple Regions of the Large Subunit of General Transcription Factor TFIIIF*

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The androgen receptor (AR) is a ligand-activated transcription factor that regulates genes important for male development and reproductive function. The main determinants for the transactivation function lie within the structurally distinct amino-terminal domain. Previously we identified an interaction between the AR-transactivation domain (amino acids 142–485) and the general transcription factor TFIIIF (McEwan, I. J., and Gustafsson, J.-Å. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 8485–8490). We have now mapped the binding sites for the AR-transactivation domain within the RAP74 subunit of TFIIIF. Both the amino-terminal 136 amino acids and the carboxyl-terminal 155 amino acids of RAP74 interacted with the AR-transactivation domain and were able to rescue basal transcription after squelching by the AR polypeptide. Competition experiments demonstrated that the AR could interact with the holo-TFIIIF protein and that the carboxyl terminus of RAP74 represented the principal receptor-binding site. Point mutations within AR-transactivation domain distinguished the binding sites for RAP74 and the p160 coactivator SRC-1a and identified a single copy of a six amino acid repeat motif as being important for RAP74 binding. These data indicate that the AR-transactivation domain can potentially make multiple protein-protein interactions with coactivators and components of the general transcriptional machinery in order to regulate target gene expression.

The actions of the male sex hormones testosterone and dihydrotosterone are mediated by the intracellular androgen receptor (AR)¹ reviewed in Refs. 1 and 2. In the absence of hormone, the receptor is sequestered in the cytosol with molecular chaperone proteins, which dissociate upon hormone binding. The hormone-bound receptor translocates to the nucleus and is targeted to specific genes through the recognition and binding to the DNA response element, 5'-AGA/TACA/TnnnT/AGTTC/AG/C-3', which in turn leads to activation of gene transcription (3–10). The activated receptor also represses gene expression through protein-DNA interactions at negative response elements (11, 12) or through interactions with other transcription factors (13–17).

In addition to the well characterized DNA-binding domain (DBD) and ligand-binding domain (LBD), regions of the proteins important for transactivation have been mapped to the amino-terminal domain (NTD; 18–21). These studies have revealed a modular nature for the AR-transactivation domain, with the region between amino acids 142 and 485, containing the TAU-1/AF-1 and TAU-5/AF-5 determinants, being critical for receptor-dependent activation (20, 21). Sequences within the AR-NTD have been shown to mediate protein-protein interactions with the carboxyl-terminal LBD (22–28), the general transcription factors TFII (29) and TFIII (30), members of the p160 family of nuclear receptor coactivator proteins (31–34), and the general coactivator CREB-binding protein (35, 36).

TFIIF is a tetramer of two subunits, RAP30 and RAP74. TFIIIF recruits TFIIE and TFIIH to the preinitiation complex (PIC) and interacts directly with the RNA polymerase II enzyme and prevents pausing of the enzyme during subsequent transcription elongation (37–39). Previously, we have demonstrated that the isolated transactivation function of the human AR, amino acids 142 to 485, interacts with the large subunit of TFIIIF, termed RAP74, and that this interaction was capable of reversing AR-dependent squelching of basal transcription under cell-free conditions (29). More recently, we have shown that binding of RAP74 results in the AR-transactivation domain adopting a protease-resistant conformation (40).

In the present study we have extended these observations to map the region(s) of RAP74 involved in this interaction with the AR. Using a series of deletion constructs of RAP74 we show that sequences within both the amino- and carboxyl-terminal domains of the protein are sufficient to bind the AR-transactivation domain and to reverse receptor-dependent squelching of transcription. In the context of the holo-TFIIF, the carboxyl-terminal binding site may be the main binding site. Introduction of point mutations into the AR-transactivation domain revealed that sequences near the amino terminus are important for RAP74 binding. These mutations fail to disrupt the interaction of the AR with the p160 coactivator protein SRC-1a. Thus, TFIIIF and SRC-1a interact with distinct regions of the AR-transactivation domain. The implications of these findings for AR-dependent gene regulation are discussed.

**EXPERIMENTAL PROCEDURES**

**Plasmid Constructs**—Bacterial expression plasmids pET-AR4, encoding amino acids 142–485 of the human AR-NTD, and pET-AR4M5

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¹ The abbreviations used are: AR, androgen receptor; CREB, cAMP-response element-binding protein; CTD, carboxyl-terminal domain; NTD, amino-terminal domain; PIC, preinitiation complex.

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have been described previously (29, 40). Bacterial expression plasmids for the AR4 mutant proteins M6 and M7 were constructed by site-directed mutagenesis using the oligonucleotides described in Table I and the QuikChange™ (Stratagene) system. The yeast expression plasmids were constructed by subcloning PCR products of the full-length AR-NTD (termed AR1, amino acids 1 to 528), AR4, and AR4M5 into pRS315-LexA (see Ref. 41; a gift from Prof. A. P. H. Wright, Södertörns Hogskola University College) containing introduced HindIII and XhoI sites. Fragments of the p160 steroid receptor coactivator, SRC-1a, were amplified by PCR using the plasmid pCR-hSRC-1a (a gift from Prof. B. W. O'Malley, Baylor College of Medicine) and subcloned into a modified pET-19b plasmid. All plasmids were confirmed by restriction enzyme digests and DNA sequencing of the insert. Expression plasmids pET-23d/RAP74 1,615–1,802, 1,802–2,068, and 2,068–2,348 were provided by Dr. Z. F. Burton (Michigan State University). Expression and Purification of Recombinant Proteins—AR4, AR4 mutations, and the RAP74 constructs, with the exception of RAP74 1–136, were expressed in Escherichia coli strains BL21 (pLys) or BL21 (DE3) by inducing with 1 mM isopropyl β-D-thiogalactoside and purified from the soluble fraction by nickel-nitrotriacetate (Ni²⁺-NTA)-agarose affinity chromatography. RAP74 1–136 was purified from the insoluble fraction by dissolving the cell pellet material in 8 M urea and subsequent Ni²⁺-NTA-affinity chromatography. The purified AR proteins were dialyzed against 25 mM HEPES (pH 7.6), 100 mM sodium acetate, 5% glycerol, and 1 mM dithiothreitol. The RAP74 proteins, except RAP74 1–136, were dialyzed against 25 mM HEPES (pH 7.6), 250 mM sodium acetate, 5% glycerol, and 1 mM dithiothreitol. RAP74 1–136 was dialyzed against 25 mM HEPES (pH 7.6), 500 mM sodium acetate, 5% glycerol, and 1 mM dithiothreitol. Protein concentrations were estimated against BSA standards using Bradford reagent (Bio-Rad).

Reconstitution of Holo-TFIIF Proteins—Untagged RAP30 and RAP74 full length or RAP74ΔC in buffer containing 8 M urea and then dialyzing successively against 20 mM Tris (pH 7.8), 500 mM NaCl, 5% glycerol, and 1 mM dithiothreitol, containing 4 or 0 M urea. Precipitated protein was removed by centrifugation, and the supernatant was passed through a Ni²⁺-NTA-agarose column. TFIIF was then eluted with 200 mM imidazole and checked before dialysis against 20 mM HEPES (pH 7.9), 250 mM sodium acetate, 5% glycerol, and 1 mM dithiothreitol. Microtiter Plate-based Protein-Protein Interaction Assay—RAP74 and SRC-1a polypeptides were synthesized in vitro using a coupled-rabbit reticulocyte lysate system (Promega). Note, the RAP74 polypeptides show anomalous mobility on SDS-polyacrylamide gels. This has been observed previously (42) and most likely reflects the high percentage of charged amino acids present in RAP74. Purified recombinant AR4 and mutant proteins in binding buffer (20 mM HEPES (pH 7.6), 10% glycerol, 100 mM KCl, 0.2 mM EDTA, 5 mM MgCl₂, 5 mM β-mercaptoethanol, 0.2 mM phenylmethylsulfonyl fluoride) were allowed to adsorb to the surface of a ScintiStrip microtiter plate (PerkinElmer Life Sciences) at a concentration of 200 nM per well. Control wells were incubated with 200 nM BSA in the same buffer. The solutions were subsequently removed, and the wells were blocked overnight with binding buffer + 5 mg/ml BSA before incubating with binding buffer containing 1 mg/ml BSA and 32S-labeled RAP74 or SRC-1a polypeptides. After extensive washing with binding buffer + 1% BSA, the bound radio-labelled polypeptides were counted directly using a PerkinElmer Life Sciences MicroBeta counter. For each labeled protein, binding to AR4 or receptor mutants was measured relative to the BSA control only. The relative binding was then plotted with BSA = 1.

In Vitro Transcription Assay—A cell-free transcription assay based on the yeast one-hybrid system was used to assay AR-mediated transcriptional activity. The yeast strain W303-1A (MATa, ade2-1, can1-100, his3-11, 15, leu2-3, 112, trp1-1, ura3-1) was transformed with the reporter plasmid pLGZ-2LexA (see Refs. 41 and 43; a gift from Prof. A. P. H. Wright, Södertörns Hogskola University College) and pRS315-LexA, pRS315-AR4-LexA, or pRS315-AR4M5-LexA using the lithium acetate method (44). Transformants were selected on synthetic defined medium—leucine, uracil agar plates. Colonies were then selected and inoculated into 10 ml of synthetic defined medium containing 2% galactose to induce expression of recombinant proteins and grown at 30 °C. After 24-48 h, cells were harvested by centrifugation and lysed using glass beads and mechanical shaking in Z buffer (100 mM phosphate buffer, pH 7, 10 mM KCl, 1 mM MgSO₄.7H₂O), supplemented with 1 mM phenylmethylsulfonyl fluoride and 1 mM dithiothreitol. The soluble protein fraction was then recovered by centrifugation and protein concentration determined by the method of Bradford (Bio-Rad). β-Galactosidase activity was measured using the substrates o-nitrophenol β-D-galactopyranoside as previously described (41, 43). A405 was measured at 0, 10, and 20 min using microplate reader (Molecular Devices, Sunnyvale, California), and β-galactosidase activity was ex-

| Name  | Mutation | Sequence                          |
|-------|----------|-----------------------------------|
| M6    | S159A/S162A | Sense   |
| M7    | S340A/S343A | Sense   |

Fig. 1. The AR-NTD amino acids 142 to 485 represent the main transactivation determinants for the AR. A, schematic representation of the human AR and the receptor constructs used in the present study. LBD, ligand binding domain; DBD, DNA binding domain; TAD, transactivation domain representing the AF-1 and AF-5 activities; Q and G represent the glutamine and glycine repeats, respectively. B, yeast reporter gene assay. The full length AR-NTD (AR1) and the AR-TAD (AR4) robustly activate the LexA reporter gene driven by two LexA response elements when fused to the heterologous LexAop1. This activity of AR4 is abrogated by the double point mutation H181N, L182N (see Ref. 19). The results presented are for at least five independent transformants, assayed in triplicate, and the means ± S.D. are shown. The activity for the AR-NTD (AR1) has been set at 100%.
pressed as nmol of o-nitrophenol β-D-galactopyranoside converted per minute per mg protein. Response was calculated as specific activity = (reaction volume (ml) × ΔA<sub>405</sub>) ÷ (0.0016 × extract volume (ml) time (min) × protein (mg/ml)).

RESULTS

Multiple Sequences within RAP74 Mediate Interactions with the AR-transactivation Domain—Work from a number of laboratories has highlighted the importance of the AR-NTD in gene activation (see Introduction). A region of the AR-NTD, amino acids 142–485 (termed AR4), retains at least 65% of the activity of the full length NTD when fused to a heterologous DNA-binding domain in a yeast reporter gene assay (Fig. 1). This activity can be abrogated by the introduction of two point mutations, I181N/L182N, originally described by Miesfeld and

FIG. 2. The AR-TAD interacts with the N and C-terminal regions of RAP74. A, Coomassie Blue-stained SDS-polyacrylamide gel of recombinant AR4 purified by nickel-NTA-affinity chromatography. B, the RAP74 polypeptide (amino acids 1–517) and deletion fragments transcribed and radiolabelled in vitro in a rabbit reticulocyte lysate system (Promega). C, binding of RAP74 polypeptides to immobilized AR4 is shown relative to BSA controls set at 1. The results are the means ± S.D. for at least four observations from two or more independent experiments.

FIG. 3. Reversal of AR4-dependent squelching by RAP74 polypeptides. A, Coomassie Blue-stained SDS-polyacrylamide gel of recombinant RAP74 polypeptides purified by nickel-NTA-affinity chromatography. B, basal transcription was squelched to levels of 35% of control by the addition of 15 pmol of AR4, and the ability of 40 pmol of RAP74 polypeptides to rescue transcription was measured. The results shown are the means ± S.D. of at least three observations and the level of transcription was quantified using a phosphoimager (BioRad). (C) Effect of RAP74 polypeptides (40 pmol) on basal transcription in the absence of AR4. The results shown are the means ± S.D. of at least three independent observations; except for the full length RAP74 only two experiments were performed. D, ratio of basal transcription in the presence or absence of AR4.
co-workers (21) as impairing the activity of the full-length rat AR (Fig. 1B). The reduction in activity of AR4M5 was not caused by reductions in the level of protein synthesized (data not shown). In a protein-protein proximity-based assay, significant binding was observed between the subunits of the basal transcription factor TFIIF and the AR4 polypeptide (29). In an attempt to better understand the mechanism of gene activation by the AR we have mapped the regions of RAP74, the large subunit of TFIIF, involved in this interaction. The AR transactivation function (AR4, amino acids 142–485) was expressed in bacteria and purified too greater than 80% (Fig. 2A). Full length RAP74 or deletion fragments were synthesized in vitro and radiolabeled with [35S]methionine and cysteine (Fig. 2B) and incubated with AR4 or BSA control, previously adsorbed to the surface of a scintillant-impregnated microtiter plate. After

**Fig. 4.** The AR-TAD interacts with holo-TFIIF. A, Coomassie Blue-stained gel of reconstituted and purified holo-TFIIF containing full length RAP74 (IIIF) or a carboxyl terminus-deleted RAP74 polypeptide (IIIFAC). B, binding of RAP74-NTD (amino acids 1–136) and RAP74-CTD (amino acids 363–517) to immobilized AR4 in the absence or presence of 200 nM TFIIF or TFIIFAC. Binding to AR4 in the absence of competitor has been set at 100%, and the results are the means ± S.D. for four observations.

**Fig. 5.** Interaction of the AR-TAD with the coactivator SRC-1a. A, schematic representation of SRC-1a showing the location of the NR boxes (LXXLL) and the binding sites for the coactivators CBP/p300 and CARM1 (methyltransferase). The location of deletion fragments of the protein are illustrated below. B, full length SRC-1a and deletion fragments synthesized and radiolabeled in vitro. C, binding of SRC-1a and deletion fragments to immobilized AR4. Means ± S.D. for at least four independent observations are plotted relative to a BSA control. Right panel shows SDS-PAGE analysis of bound proteins stripped from control and AR4 wells. Input is equivalent of 5% of starting material.

**Fig. 6.** The binding site for RAP74 maps to the amino terminus of AR4. A, schematic representation of point mutations introduced into the AR4 polypeptides. B, Coomassie Blue-stained SDS-polyacrylamide gel of the purified AR4 and AR4 mutants M5, M6, and M7. C, binding of radiolabeled RAP74 or SRC-CTD (amino acids 977–1441) to immobilized AR4 and AR4 mutant polypeptides. Binding to wild-type AR4 has been set at 100%. The results represent means ± S.D. of at least eight observations for RAP74 and four for SRC-CTD.
through coactivators (not shown) and TFIIF leading to enhanced initiation/promoter escape and/or transcription elongation. See “Discussion” for details.

Fig. 7. Model for AR-dependent gene regulation. The DNA-bound AR makes multiple interactions with the transcriptional machinery through coactivators (not shown) and TFIIF leading to enhanced initiation/promoter escape and/or transcription elongation. See “Discussion” for details.

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extensive washing, the bound radioactivity was measured directly and the relative binding calculated for each fragment. Fig. 2C shows, as expected, binding of the full length RAP74 (amino acids 1–517) to AR4. Significantly, equally strong binding was seen with the amino-terminal 136 amino acids (1–136) and the carboxyl-terminal 155 amino acids (363–517) of RAP74, whereas fragments corresponding to central portions of the protein, amino acids 136–258 and 258–356, showed reduced or no binding to AR4, respectively. Thus, the AR-transactivation function (AR4) is capable of interacting with multiple regions of RAP74.

AR4 Binding Correlates with the Ability of RAP74 Fragments to Reverse Receptor-dependent Squelching—Previously we showed that the addition of the isolated receptor transactivation function to a cell-free transcription system results in a concentration-dependent squelching of basal transcription and that recombinant TFIIF (RAP30 + RAP74) could rescue transcriptional activity (29). To test the functional significance of the interactions observed with the RAP74 fragments in the proximity assay, recombinant RAP74 and deletion fragments were expressed in bacteria and purified by nickel-affinity chromatography (Fig. 3A). The ability of these proteins to reverse AR4-dependent squelching was then tested. Fig. 3B shows that addition of 15 pmol of AR4 alone squelches basal transcription by up to 65%. In the presence of the full length RAP74 or the carboxyl-terminal fragment (amino acids 363–517) of RAP74, these fragments corresponding to central portions of the protein, amino acids 136–258 and 258–356, showed reduced or no binding to AR4, respectively. Thus, the AR-transactivation function (AR4) is capable of interacting with multiple regions of RAP74.

RAP74 Interacts with the Amino-terminal Region of the AR Transactivation Function—We (45) and others (31–34) have shown that the AR-NTD interacts with members of the p160 steroid receptor coactivator family. We have mapped the binding of the AR4 polypeptide to the CTD of SRC-1a (Fig. 5, A and B). Modest, but reproducible binding of the full-length SRC-1a to AR4 was observed, whereas the carboxyl-terminal 465 amino acids showed a robust interaction (Fig. 5C). The amino-terminal and central region of SRC-1a failed to show any significant binding with AR4 (Fig. 5C). These data are in good agreement with Bevan et al. (33) and Irvine et al. (46) and emphasize that the interaction of SRC-1a with the AR-NTD is independent of the NR boxes (LXXLL motifs).

In an attempt to identify the residues within the receptor transactivation function that are involved in the binding to RAP74 and SRC-1a, a series of point mutations was created within AR4 (Fig. 6, A and B). M5 is equivalent to a double point mutation originally described by Chamberlain et al. (21), which significantly disrupted the transactivation activity of the full-length rat AR and the AR4 polypeptide (Fig. 1B). Mutations M6 and M7 represent double serine mutations within a six-amino acid repeat motif, PSTLSL. Mutation of the serines in the amino-terminal repeat (Ser-159/Ser-162) significantly impaired the interaction with RAP74, reducing binding by 65% (Fig. 6C). In contrast, mutation of the carboxyl-terminal repeat (Ser-340/Ser-343) or the hydrophobic residues isoleucine 181 and leucine 182 had only a modest (30% reduction) or no effect on RAP74 binding, respectively (Fig. 6C). None of the mutations tested disrupted interactions of AR4 with the coactivator protein SRC-1a-CTD (Fig. 6C). Taken together, these data indicate that the amino-terminal region of AR4 is important for TFIIF (RAP74) binding and that a repeat motif, PSTLSL, plays a role in this interaction. Furthermore, TFIIF and SRC-1a interact with distinct regions of the AR-transactivation domain.
DISCUSSION

Steroid receptors and related proteins have been shown to regulate transcription at multiple steps through a diverse range of protein-protein interactions. Thus, the DNA-bound receptor recruits complexes with enzymatic activity that result in alterations in chromatin structure through ATP hydrolysis or histone modifications (see Ref. 47 and references therein). In addition, these receptors have been shown to directly enhance preinitiation complex assembly through interactions with co-activators and/or basal transcription factors. In the present report we further characterize the interaction between the AR-NTD and the general transcription factor TFIIF. Mapping studies revealed that sequences in both the amino- and carboxyl-terminal regions of RAP74 are capable of interacting with the receptor transactivation function. Although both regions reversed AR-dependent squelching of transcription, the carboxyl-terminal fragment appeared more efficient. Because the amino-terminal of RAP74 is important for binding to the small subunit of TFIIF, RAP30, it is tempting to speculate that the carboxyl-terminal interacting site is the more relevant, and competition experiments with reconstituted TFIIF support this conclusion.

Point mutations introduced into the AR4 polypeptide implicated an amino-terminal six-amino acid repeat sequence as being important for RAP74 binding. Interestingly, the wild-type motif is predicted to be β-sheet, whereas the mutated sequence is α-helical. In contrast, RAP74 binding was relatively refractory to mutations in a more carboxyl-terminal repeat of this motif, which is predicted to be helical in nature even for the wild-type sequence, suggesting that the conformation of this motif is a critical determinant in RAP74 binding. Recently, we have reported that the AR-transactivation domain folds into a more compact, protease-resistant conformation in the presence of structure-stabilizing solutes (40). Significantly, a similar protease-resistant conformation is adopted upon binding RAP74, consistent with a protein-protein induced conformational change (40).

There is an increasing list of proteins that interact with the AR-NTD and that may play important roles in androgen-dependent gene regulation. These include the CREB-binding protein (35, 36); members of the p160 steroid receptor coactivator family (31–34); the androgen receptor-associated protein ARA160 (48); the cdk-activating kinase subcomplex of the general transcription factor TFIIF (30); the positive elongation factor b (49); SMAD3 (50, 51); the tumor-suppressor gene products BRCA1 (52, 53); caevesolin-1 (54); the cell cycle regulatory proteins cyclins E (55) and D1 (56); a novel coactivator termed ART-27 (57); the transcription factor signal transducers and activators of transcription STAT3 (58); as well as the negative regulators of transcription, amino-terminal enhancer of split (59) and the nuclear receptor corepressor SMRT (60). Regions within the amino-terminal domain have also been shown to mediate intradomain interactions between the AR-NTD and the LBD (22–28). The principal sequences appear to map with the main transactivation function. However, Alen et al. (31) found that mutating the isoleucine and leucine residues corresponding to M5 to alanine appeared to disrupt amino- and carboxyl-terminal interactions. It is worth noting that the alanine mutations will not alter structure whereas the asparagine double mutation, used in the present study, disrupts the helical structure in this region (21, 40). This result may explain why the AR4M5 mutant polypeptide is compromised for receptor-dependent transactivation in the absence of the LBD (Fig. 1B).

The AR-transactivation domain can potentially regulate gene expression at multiple stages of the eukaryotic transcription cycle, including initiation, promoter escape, and elongation. TFIIF stabilizes the binding of the RNA polymerase during PIC assembly and recruits the general transcription factors TFIIE and TFIIF (see 37 and references therein). In an elegant series of cross-linking studies, TFIIF was shown to mediate bending of the promoter DNA around the PIC, and this may be important for open complex formation by allowing access for TFIIE helicase activity (38). Recent studies have revealed a role for TFIIF in cooperation with TFIIB and TFIIF to overcome stalling of the RNA polymerase after the formation of the initial phosphodiester linkage (39). Thus, by targeting RAP74, the AR can potentially regulate gene expression at multiple stages of transcription and may act to recruit TFIIF to PIC and/or early elongating complex (Fig. 7). Alternatively, because the AR-binding sites within RAP74 map to regions involved in protein-protein and/or protein-DNA interactions, it is tempting to speculate that the receptor may compete for TFIIF interactions with components of the PIC and/or DNA and thus lead to release of the RNA polymerase during initiation. Recently we have shown a role for the AR during the initiation and/or promoter escape steps and subsequently during transcription elongation. Interestingly, the interaction with TFIIF was found to be important for the early steps during the transcription cycle (initiation and/or promoter escape) but not for elongation per se. Ongoing experiments are addressing the functional consequences of the AR-TFIIF interaction during the early steps of the transcription.

Recently, Brown and co-workers (65) using a ChiP assay demonstrated the agonist-dependent recruitment of the AR, p160 coactivator, CREB-binding protein, and RNA polymerase II to the promoter and enhancer of the PSA gene. In contrast, in the presence of the antagonist Bicalutamide, the AR, the corepressors SMRT and N-CoR, and HDAC2 are recruited to the promoter (65). In the present study, we have mapped the interactions of the AR-transactivation domain with both the amino- and carboxyl-terminal regions of RAP74. Significantly, in a competition assay with holo-TFIIF the carboxyl-terminal fragment of RAP74 appeared to be the principal site of inter-

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2 J. Reid and I. McEwan, unpublished observations.

3 A. Ball and I. McEwan, manuscript in preparation.
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action. Mutational analysis of the AR-transactivation domain identified a six-amino acid repeat as playing a role in receptor binding. Taken together, the above studies reveal the potential for the AR-transactivation domain to form multi-protein complexes involving general transcription factors and coactivators, which may be disrupted by corepressor binding. The precise composition of a given activator complex may depend on the promoter and/or cell type, providing an opportunity for specificity and fine regulation of gene expression by androgens.

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