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Molecular Cloning of Human and Rat Complementary DNA Encoding Androgen Receptors

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Complementary DNAs (cDNAs) encoding androgen receptors were obtained from human testis and rat ventral prostate cDNA libraries. The amino acid sequence deduced from the nucleotide sequences of the cDNAs indicated the presence of a cysteine-rich DNA-binding domain that is highly conserved in all steroid receptors. The human cDNA was transcribed and the RNA product was translated in cell-free systems to yield a 76-kilodalton protein. The protein was immunoprecipitable by human autoimmune antibodies to the androgen receptor. The protein bound androgens specifically and with high affinity.

IN TARGET CELLS, STEROID HORMONES can form specific complexes with intracellular receptors (*1*). The hormonal regulation of gene expression appears to involve interaction of steroid receptor complexes with certain segments of genomes and modulation of specific gene transcription (*2*). The structural and functional analyses of different steroid receptor domains that participate in steroid and DNA binding are now possible largely as the result of the successful cloning of complementary DNAs (cDNAs) that code for various steroid receptors (*3–8*). Isolation of a cDNA that encodes the androgen receptor (AR), however, has not been clearly demonstrated. Cloning of cDNA for the AR has been difficult because monospecific antibodies against the AR have not been available for screening cDNA libraries.

We report here the preparation and partial structural analysis of cDNA for human (h) and rat (r) AR. For this purpose, we used λGT11 cDNA libraries (*9*) constructed with polyadenylated RNA (*10*) from human testis and the ventral prostate of rats that had been castrated 3 days earlier. The cDNA libraries were initially screened with a 41-bp oligonucleotide probe (*11*) that was highly homologous to nucleotide sequences in the DNA-binding domain of glucocorticoid receptors (GR) (*3*), estrogen receptors (ER) (*4*), progesterone receptors (PR) (*5*), mineralocorticoid receptors (MR) (*6*), and the v-*erb*A oncogene product of avian erythroblastosis virus (*7*).

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We obtained 302 and 21 positive clones, respectively, from approximately 3×10^6 human testis recombinants and 6×10^5 rat ventral prostate recombinants. If AR also has a cysteine-rich DNA-binding domain that is highly homologous with the DNA-binding regions of other steroid receptors, some of these positive clones should contain cDNA for AR. To eliminate GR-cDNA clones, we screened these positive clones with two GR-specific 24-bp probes (*11*) that had nucleotide sequences identical to nucleotide segments immediately next to the 5′ end or the 3′ end of the DNA-binding region of hGR-cDNA (*3*). By this method we were able to eliminate 244 and 14 clones, respectively, as hGR- and rGR-cDNA clones. With the same procedure, we did not detect any ER- or PR-cDNA clone in the human testis library. No ER-cDNA clone was detected with the rat prostate library, but one positive clone was obtained with hPR-specific 24-bp probes (*11*). By sequence analysis, we were also able to identify four other clones as hMR-cDNA clones (*6*). All of the remaining clones could be separated into two groups: 30 human testis clones had sequences overlapping to form a 2.1-kb cDNA, whereas 24 human testis and 6 rat prostate clones had sequences overlapping to form a 2.7-kb cDNA. The 2.1-kb and 2.7-kb cDNAs were designated as TR2-type (*12*) and AR-type cDNA, respectively.

Because the distance between the putative polyadenylation signal (AATAAA) and the 5′ end in the TR2-type cDNA was only 2.0

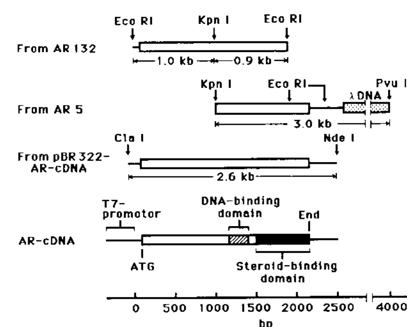

Fig. 1. Strategy used in the construction of cDNA for human androgen receptor. A human testis λGT11 cDNA library that was constructed with *Escherichia coli* Y1090 was screened with a 5′-end ^{32}P-labeled 41-bp oligonucleotide probe (*11*). Positive clones were probed with 5′-end and 3′-end ^{32}P-labeled 24-bp oligonucleotides that were specific for various steroid receptors (*11*). A less stringent hybridization condition was used first to eliminate most of the non-AR clones. The remaining clones were then probed again at the more stringent conditions. After clones for other steroid receptors were eliminated, the DNA inserts in the remaining clones were analyzed by restriction mapping and subcloned into M13 vectors for dideoxy sequence analysis (*10*). Two clones containing DNA inserts that overlapped to form a 2.7-kb cDNA were named AR 132 and AR 5. The cDNA of clone AR 132 was digested with Eco RI to obtain a 1.9-kb fragment that was then digested with Kpn I to obtain a 1-kb Eco RI–Kpn I fragment. This 1-kb fragment was ligated to a 3-kb fragment that was obtained by digestion of another cDNA clone (AR 5) with Kpn I and Pvu I. The ligated 4-kb fragment was inserted into Eco RI– and Pvu I–digested pBR322 vector and used to infect *E. coli* (DH5α). The transformed clones were selected by tetracycline resistance. The plasmid with DNA insert was digested with Cla I and Nde I to obtain a 2.6-kb fragment. The fragment was blunt-ended with the Klenow fragment of *E. coli* DNA polymerase I and ligated to the cloning vector pGEM-3Z plasmid DNA, which had also been blunt-ended by digestion with Sma I. The *E. coli* (DH5α) cells were transformed with the plasmid, and colonies containing the plasmid were selected and amplified. The restriction enzyme sites are shown with arrows.

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kb (which is considerably shorter than that in the cDNA of other steroid receptors), we suspected that the AR-type cDNA rather than the TR2-type cDNA was the putative cDNA that encoded AR. To obtain additional information, we probed a human X-chromosome library (*13*) with TR2-type cDNA and AR-type cDNA fragments. With TR2-type cDNA fragments we were not able to obtain positive clones, whereas with a 1.9-kb fragment (AR 132) of AR-type cDNA from a human testis clone, we obtained three positive clones; this suggested the presence of an AR-type cDNA sequence but not a TR2-type cDNA sequence on the X-chromosome. Because the X-chromosome is thought to be the chromosome that contains an AR gene (*14*), this information also suggested that AR-type cDNA but probably not TR2-type cDNA had the DNA sequence that could encode an AR.

In order to obtain a cDNA containing the amino acid–coding regions in different clones, we ligated two DNA fragments prepared by restriction enzyme digestion of two overlapping AR-type cDNAs. After restriction enzyme digestion, the DNA segments in these two clones were ligated, selected, and amplified by using pBR322 and pGEM-3Z vectors (Fig. 1). The plasmid DNA was isolated, and its structure was analyzed by restriction enzyme mapping and sequencing.

Since a T7 promoter, but not an SP6 promoter, was found ahead of the 5′ end of the ligated AR cDNA, T7 RNA polymerase was used in the transcription of the plasmid DNA. The transcribed RNA was isolated and then translated in a rabbit reticulocyte lysate system. By SDS–polyacrylamide gel electrophoresis (PAGE) (Fig. 2), we found that more than 85% of the translated products was a 76-kD protein.

To study the steroid-binding activity of the protein coded by the cloned cDNA, we incubated the reticulocyte lysate containing the newly synthesized protein with 17α-[^{3}H]methyl-17β-hydroxyestra-4,9,11-trien-3-one ([^{3}H]R1881), a potent synthetic androgen that binds AR with a high affinity (*15*). Using a hydroxylapatite filter assay method (*16*), we observed that one molecule of the ^{35}S-labeled 76-kD protein obtained from the lysate bound 0.9 ± 0.1 (SEM) molecule of the tritiated androgen at a saturating concentration of ligand. For this purpose, the binding assay was carried out with duplicate tubes containing receptor made in the presence of either [^{35}S]methionine or unlabeled methionine. By Scatchard plot analysis (*17*), the apparent dissociation constant was 0.3 ± 0.1 (SEM) n*M*, which is within the range of binding constants (0.2 to 4.0 n*M*) reported previously for AR of rat (*18*) and human prostates (*19*). The active natural androgen, 17β-hydroxy-5α-androstan-3-one (5α-dihydrotestosterone) (*15*) competed well with [^{3}H]R1881 binding, but the inactive 5β-isomer (*15, 18*) that does not tightly bind to AR did not compete well (Table 1). Testosterone was also active but to a lesser extent. Dexamethasone, hydrocortisone, progesterone, and 17β-estradiol did not compete well with the radioactive androgen for binding to the protein.

We had found earlier that some older men with prostate cancers had high titers of autoimmune antibodies to AR in their serum samples (*20*). Therefore, we investigated the possibility that the human autoantibodies could recognize the protein made by the reticulocyte lysate system. For this study, we incubated the receptor protein made in the lysate system with [^{3}H]R1881 to allow the formation of radioactive AR complexes

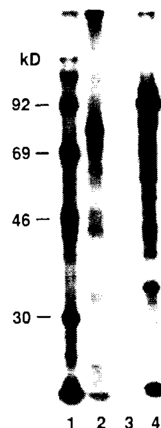

Fig. 2. SDS-PAGE analysis of AR synthesized by cDNA transcription and RNA translation in cell-free systems. pGEM-3Z vector (20 μg) containing the 2.6-kb hAR DNA segment was linearized with restriction enzyme Bam HI, extracted with phenol-chloroform, and precipitated with ethanol. For transcription and translation, the procedures described by the supplier (Promega Biotec) of the polymerase and reticulocyte lysate were followed. The linearized plasmid was transcribed by T7 RNA polymerase and translated in a micrococcal nuclease–treated rabbit reticulocyte lysate in the presence of 40 μCi of [^{35}S]methionine (800 Ci/mmol; Amersham). The radioactive protein products were precipitated by trichloroacetic acid and their radioactivity was determined. SDS-PAGE (8% acrylamide gel) analysis was performed as described elsewhere (*21*). (Lane 1) Standard molecular size proteins used were carbonic anhydrase (30 kD), ovalbumin (46 kD), bovine serum albumin (69 kD), and phosphorylase b (92 kD); (lane 2) lysate mixture containing proteins synthesized when RNA transcribed from AR cDNA was used in the translation system; (lane 3) lysate mixture in which translation was performed without additional RNA; and (lane 4) lysate mixture containing proteins translated from Brome mosaic virus RNA.

h-GR 419	Lys	Leu	Cys	Leu	Val	Cys	Ser	Asp	Glu	Ala	Ser	Gly	Cys	His	Tyr	Gly	Val	Leu	Thr	Cys	Gly	Ser	Cys	Lys
h-MR 601	Lys	Ile	Cys	Leu	Val	Cys	Gly	Asp	Glu	Ala	Ser	Gly	Cys	His	Tyr	Gly	Val	Val	Thr	Cys	Gly	Ser	Cys	Lys
h-PR 565	Lys	Ile	Cys	Leu	Ile	Cys	Gly	Asp	Glu	Ala	Ser	Gly	Cys	His	Tyr	Gly	Val	Leu	Thr	Cys	Gly	Ser	Cys	Lys
h-AR	Lys	Thr	Cys	Leu	Ile	Cys	Gly	Asp	Glu	Ala	Ser	Gly	Cys	His	Tyr	Gly	Ala	Leu	Thr	Cys	Gly	Ser	Cys	Lys
r-AR	Lys	Thr	Cys	Leu	Ile	Cys	Gly	Asp	Glu	Ala	Ser	Gly	Cys	His	Tyr	Gly	Ala	Leu	Thr	Cys	Gly	Ser	Cys	Lys
h-ER 183	Arg	Tyr	Cys	Ala	Val	Cys	Asn	Asp	Tyr	Ala	Ser	Gly	Tyr	His	Tyr	Gly	Val	Trp	Ser	Cys	Glu	Gly	Cys	Lys
h-TR2	Asp	Leu	Cys	Val	Val	Cys	Gly	Asp	Lys	Ala	Ser	Gly	Arg	His	Tyr	Gly	Ala	Val	Thr	Cys	Glu	Gly	Cys	Lys
v-erbA35	Glu	Gln	Cys	Val	Val	Cys	Gly	Asp	Lys	Ala	Thr	Gly	Tyr	His	Tyr	Arg	Cys	Ile	Thr	Cys	Glu	Gly	Cys	Lys
c-VDR	Arg	Ile	Cys	Gly	Val	Cys	Gly	Asp	Arg	Ala	Thr	Gly	Phe	His	Phe	Asn	Ala	Met	Thr	Cys	Glu	Gly	Cys	Lys

h-GR 439	Val	Phe	Phe	Lys	Arg	Ala	Val	Glu	Gly	Gln	His	Asn	Tyr	Leu	Cys	Ala	Gly	Arg	Asn	Asp	Cys	Ile	Ile	Asp
h-MR 625	Val	Phe	Phe	Lys	Arg	Ala	Val	Glu	Gly	Gln	His	Asn	Tyr	Leu	Cys	Ala	Gly	Arg	Asn	Asp	Cys	Ile	Ile	Asp
h-PR 589	Val	Phe	Phe	Lys	Arg	Ala	Met	Glu	Gly	Gln	His	Asn	Tyr	Leu	Cys	Ala	Gly	Arg	Asn	Asp	Cys	Ile	Val	Asp
h-AR	Val	Phe	Phe	Lys	Arg	Ala	Ala	Glu	Gly	Lys	Gln	Lys	Tyr	Leu	Cys	Ala	Ser	Arg	Asn	Asp	Cys	Thr	Ile	Asp
r-AR	Val	Phe	Phe	Lys	Arg	Ala	Ala	Glu	Gly	Lys	Gln	Lys	Tyr	Leu	Cys	Ala	Ser	Arg	Asn	Asp	Cys	Thr	Ile	Asp
h-ER 207	Ala	Phe	Phe	Lys	Arg	Ser	Ile	Gln	Gly	His	Asn	Asp	Tyr	Met	Cys	Pro	Ala	Thr	Asn	Gln	Cys	Thr	Ile	Asp
h-TR2	Gly	Phe	Phe	Lys	Arg	Ser	Ile	Arg	Lys	Asn	Leu	Val	Tyr	Ser	Cys	Arg	Gly	Ser	Lys	Asp	Cys	Ile	Ile	Asn
v-erbA59	Ser	Phe	Phe	Arg	Arg	Thr	Ile	Gln	Lys*	His	Pro	Thr	Tyr	Ser	Cys	Thr	Tyr	Asp	Gly	Cys	Cys	Val	Ile	Asp
c-VDR	Gly	Phe	Phe	Arg	Arg	Ser	Met	Lys	Arg	Lys	Ala	Met	Phe	Thr	Cys	Pro	Phe	Asn	Gly	Asp	Cys	Lys	Ile	Thr

h-GR 463	Lys	Ile	Arg	Arg	Lys	Asn	Cys	Pro	Ala	Cys	Arg	Tyr	Arg	Lys	Cys	Leu	Gln	Ala	Gly	Met	Asn	Leu	Glu	Ala
h-MR 649	Lys	Ile	Arg	Arg	Lys	Asn	Cys	Pro	Ala	Cys	Arg	Leu	Gln	Lys	Cys	Leu	Gln	Ala	Gly	Met	Asn	Leu	Gly	Ala
h-PR 613	Lys	Ile	Arg	Arg	Lys	Asn	Cys	Pro	Ala	Cys	Arg	Leu	Arg	Lys	Cys	Cys	Gln	Ala	Gly	Met	Val	Leu	Gly	Gly
h-AR	Lys	Phe	Arg	Arg	Lys	Asn	Cys	Pro	Ser	Cys	Arg	Leu	Arg	Lys	Cys	Tyr	Glu	Ala	Gly	Met	Thr	Leu	Gly	Ala
r-AR	Lys	Phe	Arg	Arg	Lys	Asn	Cys	Pro	Ser	Cys	Arg	Leu	Arg	Lys	Cys	Tyr	Glu	Ala	Gly	Met	Thr	Leu	Gly	Ala
h-ER 231	Lys	Asn	Arg	Arg	Lys	Ser	Cys	Gln	Ala	Cys	Arg	Leu	Arg	Lys	Cys	Tyr	Glu	Val	Gly	Met	Met	Lys	Gly	Gly
h-TR2	Lys	His	His	Arg	Asn	Arg	Cys	Gln	Tyr	Cys	Arg	Leu	Gln	Arg	Cys	Ile	Ala	Phe	Gly	Met	Lys	Gln	Asp	Cys
v-erbA85	Lys	Ile	Thr	Arg	Asn	Gln	Cys	Gln	Leu	Cys	Arg	Phe	Lys	Lys	Cys	Ile	Ser	Val	Gly	Met	Ala	Met	Asp	Leu
c-VDR	Lys	Asp	Asn	Arg	Arg	His	Cys	Gln	Ala	Cys	Arg	Leu	Lys	Arg	Cys	Val	Asp	Ile	Gly	Met	Met	Lys		

Fig. 3. Amino acid sequence alignment of the cysteine-rich DNA binding domain of human AR, GR, MR, PR, ER, TR2, rat AR, c-VDR, and v-*erb*A which is identical with a thyroid hormone receptor (*7*). The numbers represent the positions of amino acid residues in the individual receptors. Common residues are boxed with solid lines. The residues in dotted boxes represent those not in common with others in the solid boxes. In the sequence for v-*erb* A, there are two more amino acids at the position marked with an asterisk.

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and then mixed them with a serum containing autoantibodies. The results indicated a quantitative immunoprecipitation of the radioactive AR complexes in the presence of both the high titer human serum and a rabbit antibody to human immunoglobulin G (Table 2). By SDS-PAGE, we also observed that the immunoprecipitated protein was the 76-kD protein.

The steroid-binding affinity and specificity as well as the immunological property of the translated protein product strongly support our contention that the cDNA clone we prepared codes for a putative AR. The cDNA (Fig. 1) had an ATG, located approximately 80 bases from the 5′ end, that was preceded by the T7 promoter. The open reading frame was about 2.2 kb, which was sufficient to code for a protein with more than 700 amino acids. Near the middle of the protein, there was a cysteine-rich region with 72 amino acids whose sequence was highly homologous to the putative DNA-binding regions of other steroid receptors (Fig. 3). In this region, the human and rat cDNA for AR had identical amino acid sequences, although different codons were used for some amino acids. Also in this region, the homology between hAR or rAR and other receptors was: GR, 76%; MR, 76%; PR, 79%; ER, 56%; TR2, 46%; chicken vitamin D receptor (cVDR), 40%; and v-*erb*A, 40%. In the putative region for steroid binding, which has about 200 amino acids near the carboxyl terminal of steroid receptors (*3–8*), the homology between hAR (or rAR) and hGR, hMR, or hPR was 50% to 55%, whereas the homology between AR and hER was less than 20%. Thus, AR appears to be more closely related to GR, MR, and PR, than to v-*erb*A, TR2, or to receptors for estrogen, vitamin D, and thyroid hormones.

Note added in proof: Since submission of this report, we have isolated an rAR cDNA that is capable of encoding a 94-kD protein as well as a 76-kD protein. The extra amino acids in the 94-kD protein are present at the amino terminal of the 76-kD protein.

Table 1. Androgen-specific binding of human AR coded by cloned cDNA. RNA transcribed from the cloned cDNA was translated in a rabbit reticulocyte lysate system (see Fig. 2). Aliquots of the lysate were then incubated with 5 n*M* [^{3}H]R1881 (87 Ci/mmol) in the absence or presence of 25 n*M*, 50 n*M*, or 250 n*M* unlabeled steroids. The final incubation volume was 100 μl. Binding of ^{3}H-labeled androgen was measured by the hydroxylapatite filter method (*16*). The result was expressed as a percentage of the label bound in the control tube (5000 dpm) without additional unlabeled steroid. The experiment was repeated three times to assure the steroid specificity. The values of duplicate assay tubes were within 10% of the average shown in the table.

Unlabeled steroid added	[^{3}H]R1881-bound (% of control)		
	25 n*M*	50 n*M*	250 n*M*
R1881	13	10	1
Testosterone	38	28	*
5α-dihydrotestosterone	25	17	6
5β-dihydrotestosterone	89	89	81
17β-estradiol	91	91	86
Progesterone	100	91	92
Dexamethasone	100	93	93
Hydrocortisone	96	90	90

*Not examined.

Table 2. Antiserum to human immunoglobulin was used to precipitate human AR made by the translation of RNA transcribed from cloned cDNA. The experiment was carried out as described elsewhere (*20*). Reticulocyte lysate containing translated AR was incubated with [^{3}H]R1881 as shown in Table 1. The mixture was then incubated again in the presence or absence of a human male serum containing antibodies to AR (anti-AR) and rabbit antiserum to human immunoglobulins (anti-IgG). After incubation, the mixture was centrifuged and the radioactivity associated with the precipitate was estimated. A human female serum containing no anti-AR was also used for comparison. The standard errors among duplicate tubes were within 10% of the average values shown. The experiment was repeated twice to assure reproducibility.

Sample incubated with [^{3}H]R1881	Antiserum addition	Immunoprecipitable radioactivity (dpm)
AR coded by cDNA*	None	32
	Anti-AR + anti-IgG	8212
	Female serum + anti-IgG	430
	Anti-IgG	8
Heated AR†	Anti-AR + anti-IgG	42
BMV-lysate‡	Anti-AR + anti-IgG	204

*8500 dpm of the ^{3}H-labeled AR complexes were used. †The ^{3}H-labeled AR complexes were heated at 50°C for 20 minutes to inactivate receptor and release the radioactive androgen bound before the addition of antiserum. ‡Brome mosaic virus (BMV) RNA was used in the reticulocyte lysate translation system instead of RNA transcribed from cloned cDNA.

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11. The oligonucleotide probes used were: 41-bp probe for the highly homologous DNA-binding domain (TGTGGAAGCTGT/CAAAGTC/ATTCTTTAAA-AGG/AGCAA/GTGGAAGG); 24-bp probe for 5′ end of GR (TGTAAGCTCTCCTCCATCCAG-CTC); 24-bp probe for 3′ end of GR (CAGCAG-GCCACTACAGGAGTCTCA); 24-bp probe for 5′ end of PR (CCGGATTCAGAAA/GCCAGT/CCA-GAGC); and 24 bp for 3′ end of ER (GCA/CGA-CCAGATGGTCAGTGCCTTG).
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