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DOMAIN FUNCTIONS OF THE ANDROGEN RECEPTOR

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Summary—Molecular cloning of the androgen receptor cDNA has facilitated analysis of structure/function relationships of this ligand activated transcription factor. Amplification of mutant androgen receptor DNA using the polymerase chain reaction has revealed single base and deletion mutations in the androgen receptor gene that cause the androgen insensitivity syndrome in rats and humans. Site directed mutagenesis of the NH_2-terminal and hinge regions indicates specific sequences required for full transcriptional activation and nuclear targeting of the androgen receptor. Finally, transient transfection systems have shown that the antiandrogen cyproterone acetate is both an agonist and antagonist, while hydroxyflutamide acts only as an antagonist and thus is a pure antiandrogen.

The androgen receptor (AR) is a member of the steroid receptor family and thus shares structural homology with other receptors in its functional domains. Like other members of the family, the AR has an NH_2-terminal domain required for transcriptional activation [1]. Within this NH_2-terminal region is a tandem CAG repeat encoding 21 sequential glutamine residues which is polymorphic in length within the normal population [2, 3]. Doubling in size of the polyglutamine repeat was recently shown to associate with degeneration of motor neurons that occurs in X-linked spinal and bulbar muscular atrophy [4]. The amino acid sequences of the NH_2-terminal regions of steroid receptors are the most diverse among receptor domains and thus this region probably has an important role in determining specificity in gene activation. The NH_2-terminal domain is likely involved in interactions with tissue specific transcription factors during transcriptional activity. This is important since only a limited degree of specificity can be rendered by the DNA-binding region, located centrally in the protein. DNA-binding domains of the steroid receptors have striking sequence similarities [5]. Evidence thus far indicates that the AR interacts with DNA sequences that do not differ greatly from the DNA response element of the glucocorticoid receptor [6, 7]. The carboxyl-terminal region of steroid receptors is required for hormone-binding and thus offers a degree of specificity to the activation process through ligand-binding.

Resolving the AR into functional domains and establishing amino acid residues critical for receptor function is facilitated by mutagenesis of the coding sequence. This can be achieved by site directed mutagenesis *in vitro* or by taking advantage of natural mutations that cause androgen insensitivity in humans and rodents. Since the AR is essential only for male sexual differentiation and development, loss of receptor function through gene mutations can result in a female phenotype without compromise of other life functions. The severity of the mutation is reflected in the degree of feminization with some mutations causing partial forms of androgen insensitivity characterized by a normal male phenotype. The syndrome of androgen insensitivity can result from partial [8] or complete [9] deletions of the AR gene, as well as from single base mutations as reviewed recently [10].

ANDROGEN INSENSITIVITY IN THE TESTICULAR FEMINIZED RAT

A single base mutation in the steroid-binding domain of the AR from the Tfm rat results in arginine 734 conversion to glutamine. The consequences of this mutation were investigated by site directed mutagenesis and expression in monkey kidney COS cells. The arginine 734 to glutamine change results in loss of androgen-binding capacity despite equivalent expression of receptor protein and retention of binding affinity [11]. This site was investigated as a potential phosphorylation site in the AR by

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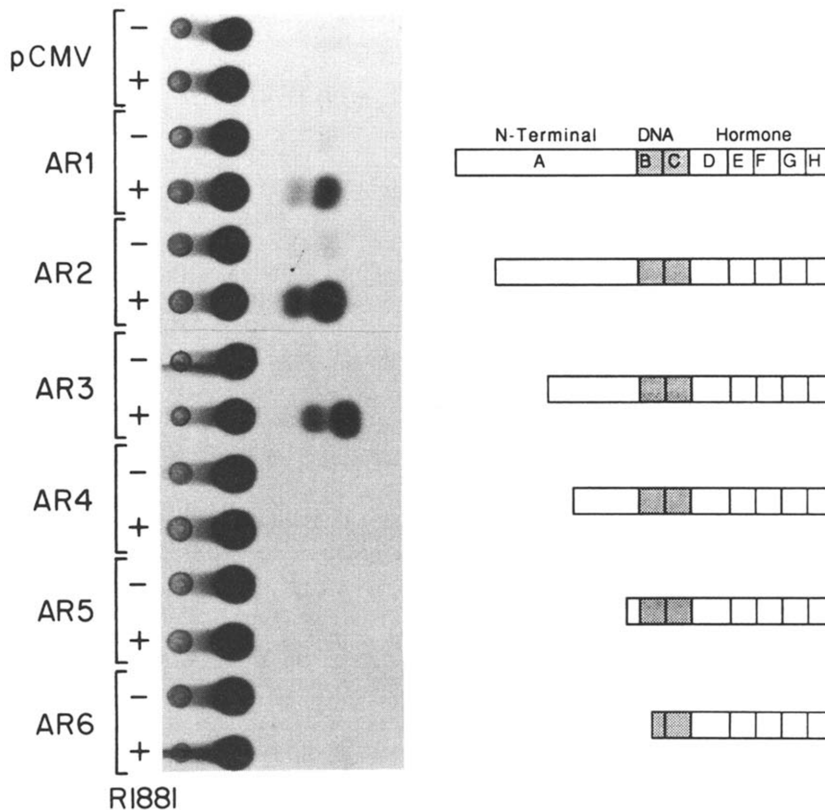


Fig. 1. Functional analysis of NH_2-terminal deletion mutants of the human AR. Deletion mutagenesis was performed using unidirectional exonuclease III digestion of wild type human AR. Monkey kidney CV1 cells were cotransfected with mutant AR plasmids and an androgen responsive reporter vector as described previously [1]. Shown are the following deletion constructs: pCMV, the parent vector lacking AR sequence; AR1, full-length human AR cDNA; AR2, amino acids (aa) 73–919, AR3, aa 141–919; AR4, aa 338–919; AR5, aa 507–919; AR6, aa 566–919. CAT activity was assessed by thin layer chromatography of cell extracts incubated with [^{14}C]chloramphenicol as described previously [1]

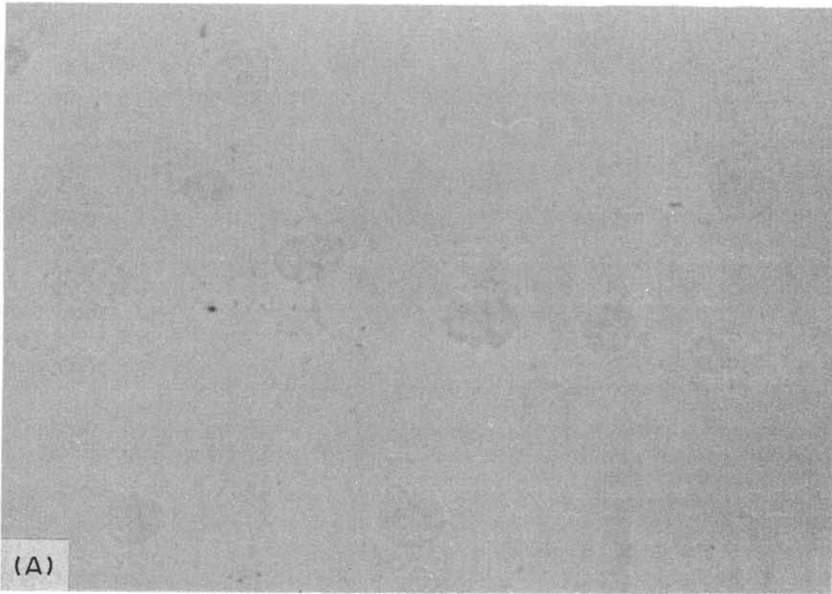
mutagenesis to mimic a negative charge, replacing a contiguous serine residue with aspartic or glutamic acid. Both of these changes resulted in loss of androgen-binding activity. Further investigations are required to elucidate the role of phosphorylation in this site and to account for what appear to be two populations of receptor, 10% retains normal binding affinity and the remaining has lost detectable binding activity.

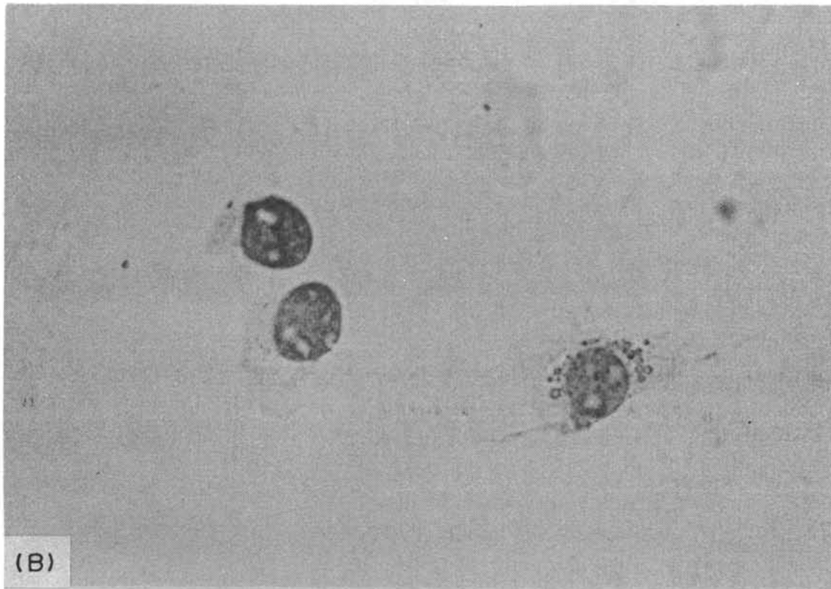
MUTAGENESIS OF THE NH_2-TERMINAL DOMAIN

In vitro mutagenesis was used to delineate domains within the NH_2-terminal region of the AR. A series of truncated forms of the AR was created by restriction enzyme digestion, polymerase chain reaction (PCR) mutagenesis and by exonuclease III digestion as described previously [1]. Shown in Fig. 1 are NH_2-terminal domain deletions of increasing size, some of which result in loss of receptor transcriptional activity. Functional analysis was performed in monkey kidney CV1 cells by cotransfection with an androgen responsive promoter linked to the chloramphenicol acetyltransferase gene [1]. Loss of transcriptional activity occurs with deletion of 337 amino acids from the NH_2-terminus. Smaller deletions of 72 and 140 amino acids do not significantly alter receptor activity. Therefore a region between amino acids 141 and 338 is required for transcriptional activation by the AR.

(Fig. 2 Opposite)

Fig. 2. Immunocytochemical localization of the full-length human AR transiently expressed in COS cells. COS cells were transfected with the pCMV parent vector lacking AR sequence (A), or the full-length AR DNA in cells incubated in the presence (B) or absence (C) of 50 nM R1881, a synthetic androgen. Cells were fixed and stained using the avidin biotin peroxidase procedure described previously [1, 20, 21]





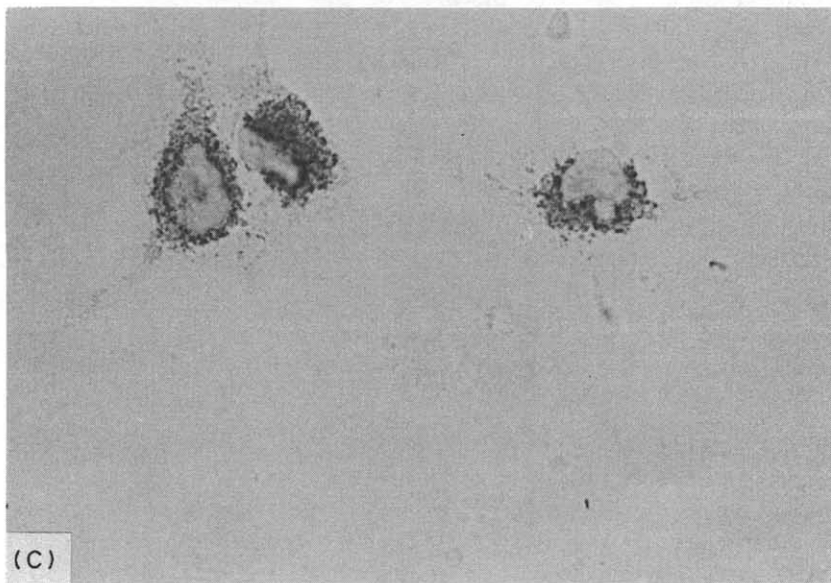


Fig. 2—*legend opposite.*

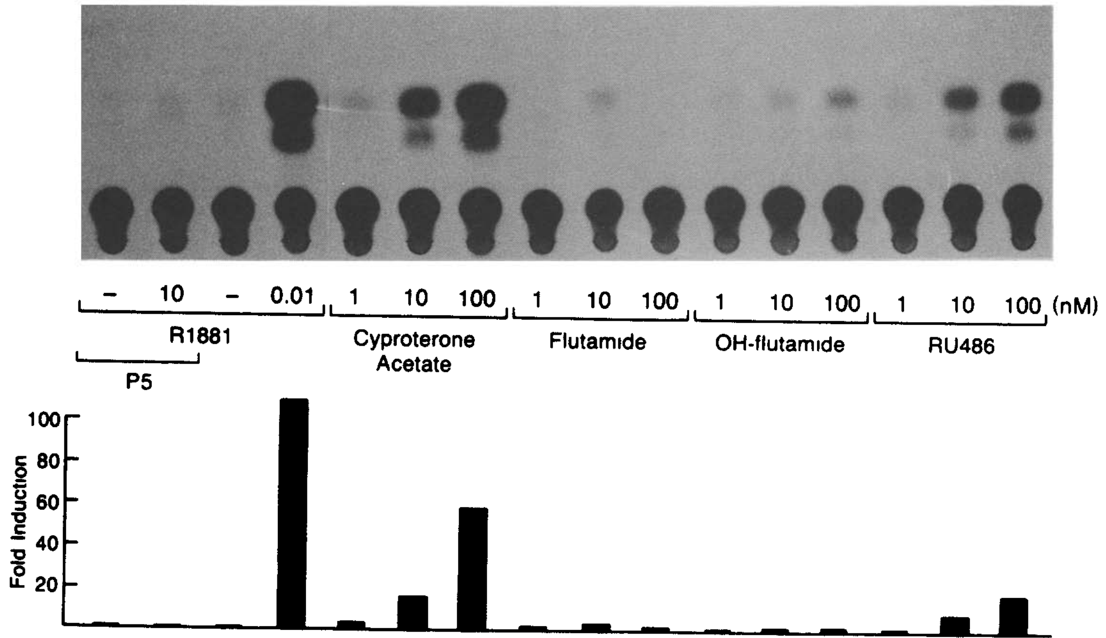


Fig. 3. Transcriptional activity of the human AR in the presence of antiandrogens and RU486. CAT activity was determined by cotransfection of the full-length human AR expression vector with an androgen responsive reporter vector as described previously [22]. Transfected CV1 cells were incubated with increasing concentrations of hormone as indicated

NUCLEAR TRANSPORT OF THE AR

Expression of the full-length AR in COS cells in the presence and absence of androgen was performed to determine the hormone dependence of nuclear transport. Shown in Fig. 2 is immunoperoxidase staining of mock transfected COS cells [Fig. 2(A)], and cells transfected with an AR expression vector in the presence [Fig. 2(B)] and absence [Fig. 2(C)] of androgen. While the receptor shows strong nuclear staining in the presence of androgen, a striking perinuclear distribution is observed in the absence of androgen. Deletion of a short basic sequence containing several lysine residues provided evidence for a nuclear targeting signal in the hinge region [1]. Ongoing mutagenesis studies in this laboratory indicate that additional sequences within the DNA-binding domain are required for full nuclear transport.

ANTIANDROGEN EFFECTS WITH THE TRANSIENTLY EXPRESSED AR

Cyproterone acetate and hydroxyflutamide inhibit androgen induced male sexual development and block the effects of androgen administered to castrated animals [12, 13]. The agonist and antagonist effects of the antiandrogens were tested in the cotransfection assay. Interestingly, cyproterone acetate stimulated an increase in CAT activity at hormone concentrations of 10 and 100 nM (Fig. 3). Similar androgenic activity of other progestins was observed previously *in vivo* [12]. Flutamide and hydroxyflutamide at these concentrations failed to stimulate transcriptional activity by the AR (Fig. 3). A hormone antagonist for the glucocorticoid and progesterone receptors, RU486 [14, 15], displayed agonist activity with the AR at concentrations of 10 nM or greater (Fig. 3).

It is interesting that the AR gene in the human prostate cancer cell line, LNCaP, has a single base mutation that changes the encoded amino acid threonine 877 to alanine [16]. This mutation alters the affinity of the receptor for progesterone and hydroxyflutamide and causes hydroxyflutamide to act as an agonist [17]. Certain single base mutations in the steroid-binding domain of the AR in cases of androgen insensitivity alter steroid specificity [18, 19]. These results have implications for antiandrogen and hormone withdrawal therapy in prostate cancer.

CONCLUSION

The AR is a ligand activated transcription factor required for male sexual development and function. Mutagenesis studies have revealed

important functional domains in the receptor for hormone-binding, transcriptional activation and nuclear transport. Transcriptional activity is activated not only by androgens, but also by the antiandrogen, cyproterone acetate. Hydroxyflutamide acts as a true antiandrogen because it lacks agonist activity but can inhibit the action of androgen. In certain prostate cancer cells and in androgen insensitivity, single base mutations alter steroid specificity which may influence considerations of hormonal therapy.

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