Regulatory regions of androgen-responsive genes

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Androgens are presumed to influence gene transcription by modifying the association of the androgen receptor with specific nuclear sites. However, the details of this process are still obscure. Elucidation of the mechanisms involved is complicated by the diversity of androgen binding within target-cell nuclei and by a lack of understanding of the interaction of androgen receptors with androgen-responsive genes, and is hampered by the unavailability as yet of androgen receptor protein purified to homogeneity. This report describes our endeavours in this area, with special reference to our studies on androgen mechanisms in rat ventral prostate and the interaction of androgen-receptor complexes with regions of the prostate binding protein (PBP) C3(1) gene (see Parker et al., 1983).

The androgen receptor used in these studies was derived from rat ventral prostate cytosol by a modification of a procedure previously published (Davies & Thomas, 1984). This method employed chromatography on Cibacron Blue-agarose, heparin-Sepharose, precipitation with and resuspension from 0–40% (w/v) ammonium sulphate and affinity chromatography on dihydrotestosterone 17-hemisuccinate linked to Sephadex. The major modification was chromatography on mixed triazinyl dyes (Matrex Red, Matrex Blue, Sigma) at the first stage, which gave a level of purification which was the product rather than the sum of using the dyes separately. This procedure has been used as an initial step in the processing of both non-transformed and transformed receptor (Fig. 1); non-transformed receptor not being retained (Fig. 1a) and transformed receptor being retained and eluted from the matrix with NaCl (Fig. 1b). Recently, Bruchovsky et al. (1987) reported that another triazinyl dye, Green A, was more efficient than either Red or Blue at purifying nuclear receptor.

The procedure outlined above produced androgen receptor purified > 35 000-fold and at a yield of < 20%. An initial phosphocellulose "flow through" step increases the yield without affecting the purification. In practice, owing to the large amounts of starting material required to produce small quantities of receptor, it is convenient to use receptor at an earlier stage of purification when all proteolytic and nucleolytic activities have been eliminated (Rushmere et al., 1987). This androgen receptor has a sedimentation coefficient of 4.2 \pm 0.3 \times 10^{-13} - 4.5 \times 10^{-13} s, Stokes radius 4.6-5 nm and \textit{M}_{i} (by calculation) 78 000-82 000. This value of \textit{M}_{i} is in accord with

\begin{figure}
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\includegraphics[width=\textwidth]{fig1.pdf}
\caption{Receptor purification}
\end{figure}

\begin{itemize}
\item (a) Non-transformed (maintained at 0–4°C) and (b) transformed (warmed to 25°C) androgen-receptor complexes from rat ventral prostate cytosol were applied to mixed resins (Matrex Red, Matrex Blue) and material eluted therefrom without NaCl, with a linear gradient of 0–2 m-NaCl and with 2 m-NaCl, as shown (continuous line). Receptor was assessed using [\textit{H}]dihydrotestosterone (DHT, 20 nmol/l) without (○) and with (●) unlabelled DHT (2 μmol/l). Eluted proteins were assessed spectro-photometrically at 280 nm (△).
\end{itemize}
with that reported by Chang et al. (1983), but is somewhat smaller than that reported more recently, i.e. 109 000–121 000 by Johnson et al. (1987) for a consensus androgen-receptor monomer, and somewhat larger than that quoted for the largest major proteolytic fragment, i.e. 52 000–59 000. However, the receptor derived by our purification procedure binds to DNA, and tissue specifically to chromatin and nuclear matrices (Davies & Thomas, 1984; Davies et al., 1986).

Several studies have highlighted the diversity of androgen-receptor binding within the prostate nucleus. Both DNA (Mainwaring & Mangan, 1971; Rennie, 1979; Davies et al., 1982) and non-histone proteins (Mainwaring & Peterken, 1971; Davies & Griffiths, 1973; Klyszeko-Stefanowicz et al., 1976) have been implicated as essential components of acceptor sites. At least two classes of binding sites have been demonstrated on the basis of differing affinities (Mainwaring et al., 1976; Davies et al., 1980, 1982, 1986) and by covalent cross-linking of receptor proteins to acceptor sites using formaldehyde (Foekens et al., 1985). These studies (Foekens et al., 1985) showed that 20% of androgen receptors were in direct contact with DNA, whereas the vast majority (80%) were in contact with acceptor proteins. The associations of androgen receptors with nuclear matrices (Barrack & Coffey, 1980; Rennie et al., 1983) is not directly relevant to this communication.

A diversity of intranuclear involvement of androgen-receptor complexes could be indicative of a diversity of function. It is now accepted that in many cases steroid receptors are trans-acting regulatory factors creating an enhancer function in the area of susceptible genes (Yamamoto, 1985). These effects necessarily involved association directly with nucleotide sequences. Intuitively, androgen receptors function similarly, but to attribute all the effects of androgens to direct interaction of receptor to DNA requires dismissal of the evidence listed above and the assumption that all effects of androgens in the prostate are direct, which they are not (Bruchovsky et al., 1975), and that all transcriptional effects are mediated through initiation, which they do not appear to be (Mainwaring & Jones, 1975; Thomas et al., 1977, 1978).

There are several lines of evidence indicating a functional involvement of androgen receptors with chromatin rather than with DNA. Apart from a structurally distinct diversity of binding, androgen receptors are heterogeneously distributed within chromatin, as revealed by digestion with micrococcal nuclease (Davies et al., 1980, 1982; Davies, 1983; Davies & Thomas, 1984). Micrococcal nuclease recognizes active chromatin structure and under certain conditions may additionally detect chromatin features related to the rates of transcription of the associated genes. Prostate chromatin was optimally cleaved at the hexanucleosome level, suggesting this structure reflects an architecture conferring transcriptional activity (Davies et al., 1986). At increasing times after castration, oligonucleosomes containing acceptor sites and PBP gene sequences become decreasingly susceptible to micrococcal nuclease (Davies et al., 1982, 1985, 1986). Administration of androgen in vivo or incubation of intact nuclei with androgen-receptor complexes in vitro reversed...
this trend (Davies et al., 1985, 1986). Thus, after castration, chromatin regions containing acceptor sites and androgen-responsive genes are withdrawn into more compact structures inaccessible to micrococcal nuclease, but accessible to androgen-receptor complexes. Two stages of control are therefore possible. First, androgen-receptor complexes alter chromatin structure into a transcriptionally favourable form in which genes are available for direct transcriptional enhancement. If a separate factor is involved, the androgen receptor acts as a competence factor (which may be its role in influencing growth processes). In the case of secretory-protein genes, the action of androgen receptors is probably more direct.

Androgen-receptor complexes are retained preferentially by restriction fragments derived from outside and within the PBP C3(1) gene (Perry et al., 1985; Rushmere et al., 1987).

Two fragments have been defined, one comprising mainly 5'-flanking sequence (−200 to +80) and another from the first intron. It is interesting to note that in terms of nucleotides these regions are approximately a hexanucleosome distance apart. Regions of increased affinity for steroid hormones have frequently been reported to occur upstream of responsive genes and to have functional promoter activity (Yamamoto, 1985). Intronic regions of increased affinity may or may not show similar functional activity (Slater et al., 1985; ten Heggeler-Bordier et al., 1987). Intronic regions may be involved in mRNA processing. Upstream and intronic regions together may anchor the C3 gene to the nuclear matrix, where it is enriched when active transcriptionally (Buttyan & Olsson, 1986). Alternatively, the two elements could behave co-operatively, analogous to the situation observed in the glucocorticoid-responsive tyrosine amino-

![Image](https://example.com/fig3.png)

**Fig. 3. Nuclease protection assay**

The non-coding strand of an intronic fragment, with increased affinity for androgen-receptor complexes, derived from the PBP C3(1) gene by cleavage with BglII and SstI (see Perry et al., 1985; Rushmere et al., 1987) was labelled at the 3'-hydroxy terminus with 32P (from γ-32P/ATP) using T4 polynucleotide kinase, and DNA (approx 10 000 c.p.m.) was incubated with DNAase I without (a) and with (b) prior incubation with androgen-receptor complexes. DNA was recovered by extraction in phenol and chloroform and electrophoresed on 8% (w/v) acrylamide gels containing 7 M-urea. Gels were washed in 10% (v/v) glacial acetic acid, dried overnight and autoradiographed for 72 h using direct-exposure film. Both panels show marker lanes: M, HpaII digest of pBR322; A, 123 bp ladder; B, EcoRI/HindIII digest of phage DNA: fragment sizes are given in terms of bases. (a) Lanes 1–10, increasing concentrations of DNAse I in multiples of 0.83 μg/ml (lane 1) to 8.3 μg/ml (lane 10). (b) Lanes 1–10, increasing input of androgen-receptor complex in integer volumes of 1–10 μl, inclusive.
transferase gene (Jantzen et al., 1987), as the upstream region of the C3 gene alone conferred only moderate promoter activity on a heterologous coding sequence (Parker et al., 1984).

The two fragments under discussion which showed high affinity for androgen receptors also competed effectively for progesterone and glucocorticoid receptors (not shown), in a DNA-cellulose competitive-binding assay (Rushmere et al., 1987). Based on levels of competitiveness, affinities for progesterone and glucocorticoid receptors did not differ significantly, and were approximately 55–60% of that for the androgen receptor, suggesting that both receptors were recognizing and binding to the same sequence element, as was found to be the case for the mouse mammary tumour virus genome and the glucocorticoid receptors did not differ significantly, and were estrogen receptors (not shown), in a DNA-cellulose competitive-binding assay (Rushmere et al., 1987). These observations support the rationale behind the search for putative androgen-receptor binding sequences based on homologies with consensus sequences for progesterone and glucocorticoid response elements (Perry et al., 1985). These authors proposed five such elements in the upstream sequence and six in the intronic sequence. Of particular interest may be the upstream element 5'-AGTTTTCTTTGG-3' and the intronic element 3'-ATTCTTTTGG-5'. Their relative reverse relationship could predispose the second element to belong to such a palindrome capable of stabilization by receptor–receptor, receptor–DNA, receptor–protein or protein–protein interactions (see, e.g. Théveny et al., 1987).

These suppositions have yet to be proven. Our attempts at defining androgen-receptor recognition sequences at the nucleotide level by nuclease protection assays have not produced unambiguous results (Fig. 3). Patterns obtained have indicated either non-optimization of assay conditions or multiple binding sites for androgen receptors in the intronic segment. It is to be hoped that the resolution of these sequences will rapidly advance the understanding of androgenic mechanisms.

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