

The Prostatic Cell: Structure and Function

Part A, pages 381-389

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Cellular Dynamics of Androgen Receptor and Protein Induction in Rat Ventral Prostate

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Our working model for the major steps involved in the intracellular cycling of the androgen receptor and protein induction in the rat ventral prostate is shown in Figure 1. In this hypothetical scheme an intracellular receptor protein in a form (R^o) that cannot bind androgens is activated by an energy-dependent process into a form (R) that can bind active androgens. The androgen-receptor complex (AR) is then transformed by a temperature-dependent step to the form (AR^*) that can bind to nuclear chromatin. As the result of this interaction the synthesis of RNA is enhanced. The newly synthesized RNA is utilized in the production of certain early proteins that mediate later androgenic responses, such as reconstruction of cellular organelles and secretion of major proteins.

For the prostate cells to grow and function normally these processes must be regulated properly. Besides the obvious controlling factors, such as the availability of androgens and the levels of receptor proteins in the prostate cells, we have investigated, as the possible steps of control, the activation of inactive receptor protein and the interaction of the androgen-receptor complex with chromatin.

CELLULAR ENERGY SUPPLY

The importance of energy supply in the receptor binding of androgens was first recognized during our initial study of 5α -dihydrotestosterone (DHT) retention by prostate nuclei. We found that androgen retention could be virtually abolished if minced prostate was incubated with radioactive DHT in the presence of respiratory poisons, such as NaCN, 2,4-dinitrophenol, or NaN_3 [1,2]. Subsequently, we found that the amount of androgen-binding receptor as measured by a gradient centrifugation assay was greatly diminished by these inhibitors of cellular energy production [3]. These inhibitors did not affect the stability of the DHT-receptor complex in the cell-free system. The removal of the respiratory

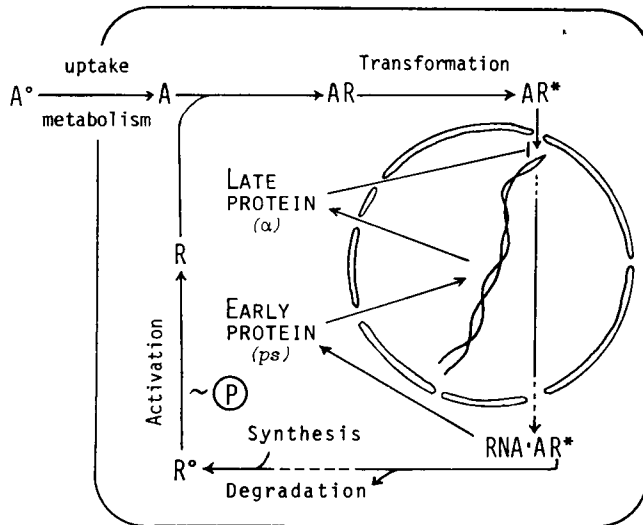


Fig. 1. A schematic view of intracellular androgen receptor cycling and action. A° , an androgen that can be metabolized to an active androgen (A), such as DHT; R° , a protein that can be activated by an energy-dependent process to a receptor (R) that can bind androgen; ps , a prostate spermine-binding protein induced within 1 hour of androgen treatment; α , a major secretory protein induced several hours after the initial androgen responses. See text for further details.

poison rapidly restores the androgen binding activity of the receptor protein in the incubated prostate and both inhibition and restoration occurred in the presence of cycloheximide [4]. These processes, therefore, appeared to represent a reversible inactivation and an energy-dependent activation and not degradation and resynthesis of the receptor protein.

Although not necessarily related to receptor activation, we also found that ATP and GTP at 1–5 mM can enhance the DHT-binding activity of the cytosol receptor fraction prepared from rat ventral prostate. These nucleoside triphosphates can stabilize the DHT-receptor complex at 20°C and can shift the sedimentation coefficient of the receptor complex [5]. Toft et al [6] also demonstrated that the chick oviduct progesterone-receptor complex, if activated to the nuclear form, can bind to ATP covalently linked to Sepharose.

The energy-dependent control of receptor binding of steroids has been the concern of several investigators. In 1968, Munck and his associates [7] showed, by adjusting the glucose and oxygen in the culture medium, that the extent of cortisol uptake by thymocytes was correlated with the ATP level in the cells. In 1972, they also suggested that ATP may be involved in the transformation of an inactive receptor protein to the glucocorticoid-binding form. Working on L-cells, Ishii et al [8] reached a similar conclusion.

More recently, Pratt and his associates [9-11] were able to show that the glucocorticoid receptor from L-cells or lymphocytes which were not bound to a glucocorticoid could be inactivated by a mechanism that may involve dephosphorylation. This inactivation could be prevented by a phosphatase inhibitor, such as molybdate. They also succeeded in reactivating the inactive form by incubation with ATP in the presence of dithiothreitol and a heat stable factor prepared from thymocytes, L-cells, or liver. According to Toft, molybdate can also inhibit the transformation of the chick progesterone-receptor complex to the form that can be retained by nuclei [6]. These studies support the view that a phosphorylation process is required to maintain the steroid-binding activity of the receptor, but that a dephosphorylation process may be needed before the steroid-receptor complex can be retained by the target cell nuclei.

The same mechanism may exist in rat prostate for the androgen receptor. We have been able to use molybdate to stabilize the DHT binding activity of the prostate cytosol receptor and also to inhibit the nuclear retention of DHT-receptor complex in a cell-free system or during incubation of minced prostate [3,4]. It should be emphasized that no direct evidence is available at present to show whether the suggested phosphorylation or dephosphorylation process directly involves the receptor proteins or other closely related molecules in the receptor preparations.

α -PROTEIN AND RECEPTOR INTERACTION WITH CHROMATIN

As a model system to study the control of receptor interaction with nuclei, we have investigated a cytosol protein that can prevent the DHT-receptor complex from binding to nuclear chromatin. The protein, named α -protein, was first isolated in 1970 [12,13] as a nonreceptor steroid-binding protein. The protein binds various sex steroids (androgens, estrogens, and progestins) well, but not glucocorticoids. We have recently found that purified α -protein has about 0.7-1.0 mole of cholesterol per mole protein. No other steroids were detected. Cholesterol, therefore, may be a natural ligand [14].

α -Protein (Mr: about 50,000) can be dissociated by sodium dodecyl sulfate into two different subunits (A and B). As shown in Figure 2, subunit A is composed of components I (Mr: 10,000) and III (Mr: 15,000), and the B unit is composed of components II (Mr: 13,000) and III (Mr: 15,000). The two polypeptide chains in the individual subunits appear to be linked by disulfide bonds and can be dissociated from each other in the presence of β -mercaptoethanol. We have purified all components to homogeneity. Component III isolated from subunit A and that from subunit B are antigenically identical and have the same amino terminal sequences (Ser-Gly-Ser-Gly), suggesting that they are the same polypeptide. Of the three components only component III has carbohydrate (19%). The protein has no detectable sialic acid [14,15]. Although there

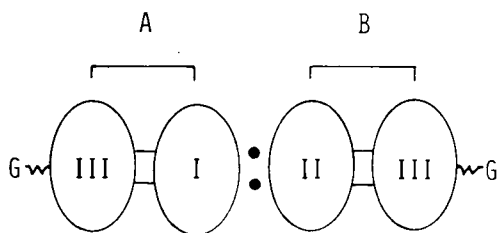


Fig. 2. A diagrammatic representation of the arrangement of subunits (A and B) and polypeptide component (I, II, and III) in α -protein of rat ventral prostate. Carbohydrate (G) is attached to component III. One mole of cholesterol can bind to 1 mole of α -protein.

are some distinct disagreements in the estimated sizes of the protein components, α -protein is probably identical to the "prostastein" of Lea et al [16], the "prostate-binding protein" of Heyns et al [17], and the estramustine binding protein of Forsgren et al, [18]. (See articles in this book by Wilson et al, Heyns et al, and Forsgren et al.)

To show inhibition of receptor interaction with chromatin, a prostate α -protein fraction is mixed with the androgen receptor complex and prostate cell nuclei. The inhibitor did not appear to cause irreversible destruction of the receptor complex or damage to the nuclear binding site. Besides acting as an inhibitor, α -protein can promote the release of the androgen-receptor complex already attached to chromatin. To show this, we first allowed the radioactive receptor complex to bind to prostate nuclei. The nuclei were then washed to removed the excess receptor complex and were incubated again either with or without the inhibitor. At a low concentration of the inhibitor, a significant loss of radioactivity occurred at 20°C, but not at 0°C. At a high concentration of the inhibitor, the release of radioactivity from the chromatin was evident even at 0°C, but was more obvious at 20°C. Without the inhibitor, there was no temperature-dependent loss of radioactivity released from the chromatin. By gradient centrifugation, we found that all radioactivity released from the nuclei was associated with protein that sedimented as 3S [19].

Of the two subunits, only subunit A was inhibitory. Component I was at least five-fold more active than subunit A, when the concentrations required to show 50% inhibition were compared. Components II and III were inactive. Component I, therefore, may be the inhibitory component of α -protein [14,15].

Figure 3 shows the complete amino acid sequence of component I [20]. This component is rich in acidic amino acids. Most of the glutamic acid and lysine are localized in the amino terminal half, whereas all aspartic acid and almost all aromatic amino acids are in the carboxyl terminal half of the protein. These

Amino acid sequence

NH₂- Ser-Gln-Ile-Cys-Glu-Leu-Val-Ala-His-Glu-Thr-Ile-Ser-Phe-Leu-Met-Lys-Ser-Glu-Glu-Glu-Leu-
 10 20
 Lys-Lys-Glu-Leu-Glu-Met-Tyr-Asn-Ala-Pro-Pro-Ala-Ala-Val-Glu-Ala-Lys-Leu-Glu-Val-Lys-Arg-
 30 40
 Cys-Val-Asp-Gln-Met-Ser-Asp-Gly-Asp-Arg-Leu-Val-Val-Ala-Glu-Thr-Leu-Val-Tyr-Ile-Phe-Leu-
 50 60
 Glu-Cys-Gly-Val-Lys-Gln-Trp-Val-Glu-Thr-Tyr-Tyr-Pro-Glu-Ile-Asp-Phe-Tyr-Tyr-Asp-Met-Asn-OH
 70 80 88

Amino acid composition:

Molecular weight:

Glu₁₃, Gln₃, Asp₅, Asn₂, Lys₆, Arg₂, His₁,
 Val₉, Leu₈, Ala₆, Ser₄, Ile₄, Thr₃, Gly₂,
 Tyr₆, Phe₃, Pro₃, Trp₁, Met₄, Cys₃
 10,191

Fig. 3. Complete amino acid sequence of component I of α-protein of rat ventral prostate.

features may be important in the inhibition but this activity may simply be due to a small oligopeptide stretch and not dependent on a complex structure.

INTERACTION OF RNA WITH ANDROGEN-RECEPTOR COMPLEX

Another aspect we have explored is the interaction of RNA with steroid-receptor complexes. In 1969, we suggested that RNA made in the nuclei may bind to the receptor complex and facilitate the release of the complex from nuclei [2]. We proposed that the receptor complex, in turn, may play an important role in processing, stabilization, and/or utilization of RNA [2,21]. Although direct evidence supporting this idea is still lacking, our studies have shown that both the estrogen- and androgen-receptor complexes can bind to certain ribonucleo-protein particles in the uterus and in the prostate [22,23].

Since the proposed scheme suggests that certain RNA molecules can promote the release of DHT-receptor complex from DNA or chromatin, we have tested this possibility by using DNA-cellulose column chromatography [24]. These studies indicated that for a polynucleotide to be active in releasing the receptor complex from DNA-cellulose the polymer appears to need nonhydrogen-bonded bases with an oxygen or a sulfur atom at C-6 of purines or C-4 of pyrimidines. Thus, poly(U), poly(G), poly(X), and poly(I) were active but poly(C) and poly(A) were inactive. Poly(U,G) was more active than poly(G), poly(U), or equivalent mixtures of poly(G) and poly(U), indicating that the activity was dependent on the nucleotide sequence. The minimum length of the oligonucleo-

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tion needed to show activity appeared to be about 10–20 nucleotides but this may be dependent on the type of nucleotide in the oligomer.

The RNA-dependent release of the steroid-receptor complex from DNA could also be demonstrated by gradient centrifugation. As shown in Figure 4, the radioactive androgen-receptor complex stayed near the top of the tube after gradient centrifugation if no nucleic acid was present. If SV-40 DNA was added to the tube, a large quantity of the radioactivity was found to associate with DNA that sedimented at the bottom of the tube. When poly(U_1, G_1) (5S) was added to the receptor complex and SV-40 DNA before centrifugation, the radioactivity was not found with DNA in the bottom of the tube, but was found associated with poly(U_1, G_1). Poly(A,C) was able to bind to the receptor complex if no DNA was present; however, it could not release the receptor complex from the viral DNA.

We have also found that radioactive estradiol-receptor and progesterone-receptor complexes from rat or calf uterus and dexamethasone-receptor complex

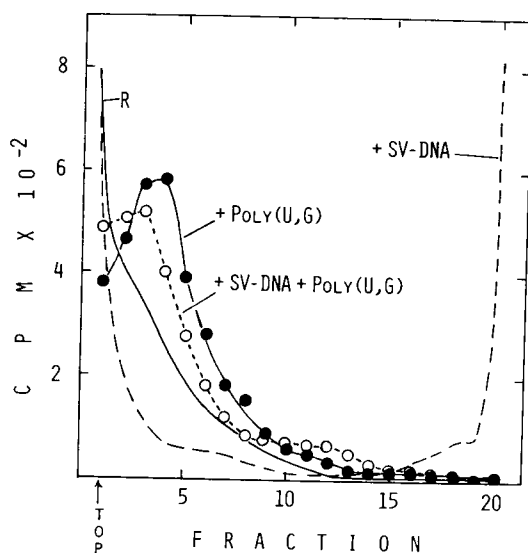


Fig. 4. Effect of poly(U,G) on binding of the [3 H]-DHT-receptor complex to SV-40 DNA. The radioactive receptor complex (5,000 cpm) was mixed with 3 μ g of SV-40 DNA. Poly(U,G) (3 μ g) was then added (o) and the mixture incubated at 0°C for 10 minutes. The incubated mixture was layered on top of the sucrose gradient and centrifuged at 50,000 rpm for 105 minutes at 0°C. After centrifugation, the contents of the tube were fractionated and the radioactivity in the individual fractions was determined and is shown on the ordinate. Parallel tubes contained receptor complex alone (R) or the complex mixed with SV-40 DNA (---) or with poly(U,G) (●). Monitoring the absorbance at 260 nm showed that after centrifugation SV-40 DNA sedimented to the bottom of the tube, whereas poly(U,G) was found near the top (fractions 2 to 5) of the tube.

from rat liver could also be removed from DNA by various polyribonucleotides as discussed above. Although we have not been able to demonstrate polymer specificity toward different receptor complexes, it is conceivable that certain natural RNA with specific nucleotide sequences may be more active than the synthetic polymers we have tested and can demonstrate such a specificity.

Since the concentration of RNA needed (1–5 $\mu\text{g/ml}$) to release receptor from DNA may be within the range expected in intact cell nuclei, preferential RNA binding of the steroid-receptor complexes in the nuclei is not inconceivable. Such a process may be important in the recycling of the receptor protein from nuclei to cytoplasm. The removal of RNA from DNA may also make the genetic template available for further transcription while receptor binding of RNA may be involved in post transcriptional control processes [2,21–24]. In this scheme, different RNA molecules may contain identical or similar nucleotide sequences so that more than one RNA species can be selected, although with some preference, by the same steroid-receptor complex. These diversified specificities together with other cellular factors may provide the selectivity and multiplicity observed in the induction of different proteins by steroid hormones.

CONCLUSIONS

Various steroid hormones can induce specific proteins in target cells. Hormonal induction of many of these proteins has been found to follow increases in the level of mRNA molecules specific for these proteins. Nevertheless, it has not been possible to prove that steroid or the steroid-receptor complexes can act directly on the genes for these protein products. In some well-studied cases there are reasons to believe that such inductions may not represent the primary effect of steroid hormones on the target cells. For example, increases in the cellular levels of chick oviduct ovalbumin mRNA by estrogen and rat liver α_{2u} globulin mRNA by androgen were seen after a lag phase and a protein synthesis inhibitor could inhibit the mRNA accumulation, suggesting that stimulation of mRNA synthesis or accumulation may be secondary to the synthesis of certain 'early' proteins [25]. In the rat ventral prostate, it is not clear whether spermine binding protein and α -protein represent an "early" mediatory protein and a "late" protein, respectively. Further studies are also necessary to determine whether a component of a "late" protein has a role in maintaining the chromatin interaction with androgen-receptor complex at a normal level as we have suggested.

REFERENCES

1. Anderson KM: Selective retention of dihydrotestosterone by prostatic nuclei in vivo and in vitro. PhD thesis, University of Chicago, 1969.
2. Liao S, Fang S: Receptor proteins for androgens and the mode of action of androgens on gene transcription in ventral prostate. *Vitam Horm* 27:17–90, 1969.

3. Liao S, Rossini GP, Hiipakka RA, Chen C: Factors that can control the interaction of the androgen-receptor complex with the genomic structure in the rat prostate. In Bresciani F (ed): "Perspectives in Steroid Receptor Research," New York: Raven Press, 1980, pp 99-112.
4. Rossini GP, Liao S: unpublished observation.
5. Liao S, Tymoczko JL, Castaneda E, Liang T: Androgen receptors and androgen-dependent initiation of protein synthesis in the prostate. *Vitam Horm* 33:297-313, 1975.
6. Nishigori H, Toft DO: Inhibition of progesterone receptor activation by sodium molybdate. *Biochemistry* 19:77-83, 1980.
7. Munck A, Wira C, Young DA, Mosher KM, Hallahan C, Bell PA: Glucocorticoid-receptor complexes and the earliest steps in the action of glucocorticoids on thymus cells. *J Steroid Biochem* 3:567-578, 1973.
8. Ishii DN, Pratt WB, Aronow L: Steady-state level of the specific glucocorticoid binding component in mouse fibroblasts. *Biochemistry* 11:3896-3904, 1972.
9. Sando JJ, LaForest AC, Pratt WB: ATP-dependent activation of L cell glucocorticoid receptors to the steroid binding form. *J Biol Chem* 254:4772-4778, 1979.
10. Sando JJ, Hammond ND, Stratford CA, Pratt WB: Activation of thymocyte glucocorticoid receptors to steroid binding form. *J Biol Chem.*, 254:4779-4789, 1979.
11. Wheeler RH, Leach KL, LaForest AC, O'Toole TE, Wagner R, Pratt WB: Glucocorticoid receptor activation and inactivation in cultured human lymphocytes. *J Biol Chem* 256:434-441, 1981.
12. Liao S, Fang S: Factors and specificities involved in the formation of 5 α -dihydrotestosterone-nuclear receptor protein complex in rat ventral prostate. In Griffiths K, Pierrepoint CG (eds): "Some Aspects of the Aetiology and Biochemistry of Prostate Cancer." Cardiff: Alpha Omega Alpha Publishing, 1970, pp 105-108.
13. Fang S, Liao S: Androgen receptors: Steroid- and tissue-specific retention of a 17 β -hydroxy-5 α -androstan-3-one-protein complex by cell nuclei of ventral prostate. *J Biol Chem* 246:16-24, 1971.
14. Chen C, Schilling K, Hiipakka RA, Huang I-Y, Liao S: Prostate α -protein: Isolation and characterization of the polypeptide components and cholesterol binding. *J Biol Chem* (in press).
15. Chen C, Hiipakka RA, Liao S: Prostate α -protein: Subunit structure, polyamine binding, and inhibition of nuclear chromatin binding of androgen-receptor complex. *J Steroid Biochem* 11:401-405, 1979.
16. Lea OA, Petruz P, French FS: Prostatein, a major secretory protein of the rat ventral prostate. *J Biol Chem* 254:6196-6202, 1979.
17. Heyns W, Peeters B, Mous J, Rombaut W, DeMoor P: Purification and characterization of prostatic binding protein and its subunits. *Eur J Biochem* 89:181-186, 1978.
18. Forsgren B, Bjork P, Carlstrom K, Gustafsson J-A, Pousette A, Hogberg B: Purification and distribution of a major protein in rat prostate that binds estramustine, a nitrogen mustard derivative of estradiol-17 β . *Proc Natl Acad Sci USA*, 76:3149-3153, 1979.
19. Shyr C-I, Liao S: A protein factor that inhibits binding and promotes the release of the androgen-receptor complex. *Proc Natl Acad Sci USA* 75:5969-5973, 1978.
20. Liao S, Chen C, Huang I-Y: Prostate α -protein: Complete amino acid sequence of the component that inhibits nuclear retention of androgen receptor complex. *J Biol Chem* (in press).
21. Liao S, Tymoczko JL, Howell DK, Lin AH, Shao T-C, Liang T: Interaction of ribonucleoprotein particles and sex-steroid-receptor complexes: A model for receptor cycling and possible function. *Int Congr Ser Excerpta Med* 219:434-440, 1970.
22. Liao S, Liang T, Tymoczko JL: Ribonucleoprotein binding of steroid-receptor complexes. *Nature New Biol* 241:211-213, 1973.
23. Liang T, Liao S: Association of the uterine 17 β -estradiol-receptor complex with ribonucleoprotein in vivo and in vitro. *J Biol Chem* 249:4671-4678, 1974.

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24. Liao S, Smythe S, Tymoczko JL, Rossini GP, Chen C, Hiipakka RA: RNA-dependent release of androgen and other steroid-receptor complexes from DNA. *J Biol Chem* 245:5545--5551, 1980.
25. Liao S, Hiipakka RA: Mechanism of action of steroid hormones at the subcellular level. In Makin HLJ (ed): "Biochemistry of Steroid Hormones." Oxford: Blackwell Scientific Publications (in press).

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