# FURTHER EVIDENCE FOR AN ANDROGEN-DEPENDENT INTERMEDIATE WITH A SHORT HALF LIFE REQUIRED FOR MAXIMAL RIBOSOMAL RNA SYNTHESIS IN THE RAT VENTRAL PROSTATE

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## 1. Introduction

Nucleolar ribosomal RNA (rRNA) synthesis in the rat ventral prostate is exquisitely androgen-dependent [1]. After castration, the activity of the DNA-dependent RNA polymerase (EC 2.7.7.6, nucleoside triphosphate:RNA nucleotidyl transferase) responsible for rRNA synthesis rapidly declines. Administration of testosterone propionate leads to an abrupt increase in activity, detectable within 1 hr. Prolonged treatment with hormone increases the activity further. In isolated nuclei, ribosomal RNA synthesis can be estimated by incubation at reduced ionic strength, in the presence of $Mg^{2+}$, with and without low concentrations of actinomycin D ($\simeq$ 0.1 $\mu$g/100 $\mu$g DNA) [2].

Dihydrotestosterone (DHT: 5$\alpha$-androstan-17$\beta$-ol-3-one) is the major androgen retained by isolated prostate nuclei [3,4]. Metabolism and binding of androgen to prostate cytoplasmic proteins precedes transfer of DHT–protein complexes to nuclear "acceptors", numbering 2000–3000 per nucleus, which selectively bind the complexes [5,6]. Cyproterone acetate (CypOAc), a synthetic progestational compound exhibiting potent antiandrogenic activity, inhibits the *in vivo* and *in vitro* association of DHT with target organ cytoplasmic receptors, and their transfer to nuclei [7,8]. Injection of cyproterone acetate for 3 days reduced prostate RNA polymerase activity, protein and RNA content, and was accompanied by regression of the organ [9,10]. Testicular feminization and androgen-insensitivity syndromes in man, mouse, and rat, associated in mice with defective cytosol receptor–DHT interactions, are further evidence of their importance [11].

We examined the effect of brief *in vivo* challenge with cyproterone acetate on the *in vitro* synthesis of rRNA or its precursors, by isolated prostate nuclei. The rapid target organ-specific fall in activity that was observed provides further evidence for the role of androgens in the provision to prostate nuclei, of an intermediate with a short half life, continuously required for maximal androgen-dependent rRNA synthesis.

## 2. Materials and methods

Long Evans rats, from 3–5 months of age were maintained on a standard diet and water ad lib. Cyproterone acetate (6.6 or 13.2 mg/kg) suspended in sesame oil or dissolved in a small volume of ethanol, was injected subcutaneously or intraperitoneally, and animals sacrificed 2 hr later. Controls received an equal amount of the vehicle. Ventral prostate and liver nuclei were isolated in hypertonic sucrose solution [12]. Reactions with isolated nuclei were carried out in a final volume of 0.5 ml containing 0.5 $\mu$moles each of ATP, CTP and GTP; 3 $\mu$Ci of [$^3$H] UTP (>1 Ci/mM) (and, in most experiments, 0.05 $\mu$moles of UTP); 2.5 $\mu$moles of $MgCl_2$; 0.5 $\mu$moles of $MnCl_2$; 3 $\mu$moles of KF; 10 $\mu$moles of $\beta$-mercaptoethanol; 30 $\mu$moles of KCl; 60 $\mu$moles of Tris-HCl buffer, pH 8.2; and an amount of prostate nuclear preparation containing from 50–100 $\mu$g DNA. After 20 min at 37°, precipitates were washed on Millipore filters (DA 25, 0.45 $\mu$) with trichloroacetic acid, and the radioactivity measured in a Packard Tri-

Table 1

Effect of cyproterone acetate on rat ventral prostate RNA polymerase activity measured in isolated nuclei under conditions promoting the synthesis of rRNA.

| | Prostate | | Liver | |
|---|---|---|---|---|
| CypOAc | (N) | ±CypOAc cpm/100 μg DNA | (N) | ±CypOAc cpm/100 μg DNA |
| 2 mg | 2160 | 1740 (−20) | 2100 | 2203 (0) |
| | 1962 | 1472 (−25) | 885 | 775 ( −9) |
| | 2080 | 1410 (−32) | | ( −4) |
| | 2232 | 1530 (−31) | | |
| | 286* | 222 (−23) | | |
| | | (−26 ± 5) | | |
| 4 mg | 376* | 224 (−40) | 278 | 298 (+10) |
| | 2360 | 1435 (−39) | 542 | 452 (−16) |
| | 1090 | 660 (−39) | | ( −3) |
| | | (−39 ± 1) | | {−4 ± 9.5} |

Rats received 2 or 4 mg of CypOAc in ethanol intraperitoneally, or in sesame oil subcutaneously, and were sacrificed 2 hr later. ( ) = percent change from control; { } = average of both experimental groups; * = >0.05 μmole of unlabelled UTP was employed; ± = standard deviation.

Carb liquid scintillation spectrometer. Zero time controls of 50 cpm were usual.

## 3. Results

It was established that maximum incorporation of labelled nucleoside triphosphates was dependent upon the presence of 4 bases, and inhibited by actinomycin D. Hot TCA or KOH at 37° degraded the radioactive product. These properties characterize DNA-dependent RNA synthesis.

Under conditions of low ionic strength, in the presence of $Mg^{2+}$ ions, synthesis of a ribosomal-like product, high in guanine and cytosine, is favored [13]. This was demonstrated by the inhibition resulting from increasing concentrations of actinomycin D (fig. 1, ref. [2]). Amounts of the antibiotic from 0.10 to 0.35 μg/54 μg DNA caused a >80% reduction in incorporation of [$^3$H] UTP into RNA. The template capacity for RNA synthesis mediated by DNA with a high deoxyG and deoxyC content, such as the DNA serving as a template for ribosomal RNA formation, characteristically is inhibited by low concentrations of actinomycin D. For comparable inhibition of deoxyA, deoxyT-rich DNA templates, coding for non-rRNA (dRNA) synthesis, much larger amounts of actinomycin D are required [2,14].

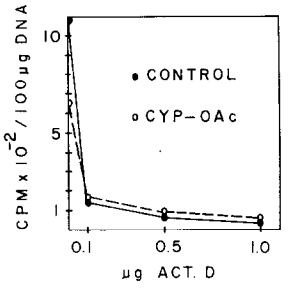


Fig. 1. Effect of *in vivo* cyproterone acetate on the incorporation of [$^3$H] UTP into rRNA catalyzed by isolated prostate nuclei. Two hours before sacrifice, 3 rats each received subcutaneously 4 mg cyproterone acetate in sesame oil; 3 control rats received the vehicle alone. rRNA synthesis was estimated from the inhibition of labelling by low concentrations of actinomycin D, in the incubation medium described in Methods.

The effect of acute "chemical" castration due to cyproterone acetate on ventral prostate nuclear rRNA synthesis, compared with nuclei from liver, was examined (table 1). Two hours after the antiandrogen, a consistent reduction in ventral prostate rRNA synthesis was present. This inhibition averaged −26 and −39%, depending upon whether the lesser or greater amount of CypOAc had been administered. No consistent pattern was observed with liver nuclei.

## 4. Discussion

The dose-dependent fall in rRNA synthesis 2 hr after cyproterone acetate is comparable to that observed 12–18 hr after surgical castration [15]. Twenty-four hours after castration, *in vitro* nuclear rRNA synthesis was reduced by half and diminished another 25% during the next 24 hr. During the initial period

of rapid decline, the apparent half life of the loss in activity was about 12 hr. This decrease is consistent with the gradual utilization or loss of a pool of factors necessary for rRNA synthesis. The complex nature of the decay curve, involving 2, if not 3 distinct changes in rate, suggests that these interactions involve more than 1 critical component. On the contrary, inhibition by CypOAc is abrupt. The presence of residual rRNA synthesis 2 hr after the antiandrogen could be due to its continuing dilution by testicular androgens. Alternately, initially only 1 component of the reactions that underwrite rRNA synthesis may be acutely sensitive to the agent.

It is also of interest to compare these rates of decrease in rRNA synthesis with the pattern of [$^3$H] androgen-binding exhibited by isolated prostate nuclei. Following *in vivo* administration of labelled testosterone, the specific radioactivity of purified nuclei was determined. In normal rats, 80% of the retained radioactive steroid, of which over 90% was DHT, was lost within 2–2½ hr [16]. Most of the remainder was retained for at least 12 more hr. The pattern in 3-day castrates, uncomplicated by large pools of endogenous androgens, was similar. Baulieu has observed non-histone uterine nuclear binding proteins with high affinity for estradiol [17]: the prostate nuclear proteins responsible for the prolonged binding of [$^3$H] DHT may represent a comparable class of proteins. The steroid that initially is bound and is rapidly lost exhibits an apparent half life of about 1 hr.

These results suggest that in the rat ventral prostate, the maintenance of most ongoing rRNA synthesis is continuously dependent upon a supply of cytosol–DHT complex. If its provision to nuclei is interrupted, by CypOAc, or surgical castration, rRNA synthesis progressively declines. If the CypOAc-induced decline in rRNA synthesis is subsequently shown to continue at the same rate, a fall of 40% in 2 hr would provide an indirect estimate of the average apparent half life of a steroid-dependent moiety(ies) believed to be required to underwrite rRNA synthesis–about 2½ hr. Due to competition by endogenous androgens, this value would likely be greater than the true half life.

Uterine cytosol –estradiol complex has been shown by 3 groups to enhance uterine RNA synthesis [18–20]. In liver, nucleolar RNA synthesis was reported to be sensitive to α-amanitin, which implies that an RNA product of non-nucleolar origin is necessary for continuing rRNA synthesis [21]. Within 1½ hr, hydrocortisone stimulated hepatic nucleolar RNA polymerases Ia and Ib, probably by increasing the activity of these enzymes rather than "unmasking" more DNA template [22]. Inhibition by CypOAc of both DHT–cytosol receptor binding to chromatin and rRNA synthesis provides further evidence for the important role that the transport of DHT–cytosol complexes to, and their binding by, chromatin plays in the function of androgen-sensitive target organs. It would not be surprising to find that CypOAc reduces the activity of nucleolar enzymes Ia and Ib isolated from the prostate, and assayed with exogenous DNA template.

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