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ANDROGEN REGULATION OF RNA SYNTHESIS IN TARGET TISSUES

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SUMMARY

The increased rate of protein synthesis induced in the prostate and seminal vesicle by the injection of dihydrotestosterone is preceded by an increased synthesis of RNA and is blocked by inhibitors such as actinomycin D. This increased protein synthesis can also be induced by administering into the lumen of the seminal vesicle RNA extracted from the seminal vesicle of an androgen-treated rat. When the RNA is placed on a Sepharose 4B polyuridylic acid column or a cellulose polythymidylic acid column, a small fraction of the RNA is tightly bound. The unbound RNA is eluted with buffer and subsequently the bound RNA is eluted with a more alkaline buffer. The injection of dihydrotestosterone *in vivo* greatly increases the incorporation of cytidine into this bound RNA. The bound poly (A)-rich RNA, presumably polyadenylic acid RNA, from the seminal vesicles of androgen treated males resulted in increased protein synthesis when instilled into the seminal vesicle of castrate males. In contrast, the unbound RNA from the seminal vesicle was ineffective when instilled into the seminal vesicles of castrate rats.

The increased rate of protein synthesis induced in the prostate and seminal vesicle by the injection of testosterone or dihydrotestosterone is preceded by an increased synthesis of RNA [1] and is blocked by inhibitors of RNA synthesis such as actinomycin D [2, 3]. The increased protein synthesis can also be induced by administration into the lumen of the seminal vesicle of RNA extracted from the seminal vesicle of an androgen-treated rat [4]. The hypothesis that steroid hormones are taken up and bound by specific receptor proteins in their target tissues and that the hormone–receptor complex enters the nucleus, interacting with chromatin by way of an acceptor protein, is widely accepted. These observations of increased protein synthesis are consistent with this hypothesis and support the inference that dihydrotestosterone acts on the genome to change the amount or the kind of RNA being synthesized. The prostate and seminal vesicle undergo rapid atrophy following the depletion of testosterone that ensues after castration [5]. During this atrophy induced by androgen deprivation there is decreased RNA synthesis and decreased protein synthesis.

Dr. Fujii and I reported in 1967 [4] that RNA prepared by cold phenol extraction from the seminal vesicles of adult rats or from testosterone treated immature rats and treated with deoxyribonuclease and pronase, will lead to a significant stimulation of protein synthesis and growth of the organ when the material is instilled into the seminal vesicle of an immature rat. Similar stimulatory effects were observed when seminal vesicle RNA was instilled into the seminal vesicles of seven-week-old or twelve-week-old rats. Treatment of the RNA preparation with ribonuclease rendered it ineffective. RNA from the seminal vesicle of an untreated immature rat or from a castrate adult rat was ineffective when instilled. An increased synthesis of RNA is one of the first metabolic alterations brought about by testosterone or dihydrotestosterone and precedes the increased activity of any of the enzymes [6]. Wicks and Kenney[7] observed a doubling of the incorporation of labeled phosphorus into RNA within 20 min after injection of testosterone in the rat. The administration of actinomycin D *in vivo* prevents the testosterone-induced stimulation of RNA synthesis in the prostate [8]. Ito and his colleagues [9] identified by methylated albumin Kieselguhr columns a fraction of prostatic RNA which becomes highly labeled three to six hours after injection of testosterone propionate. The base composition of this RNA, $A + U/G + C =$ 1·06, was intermediate between that of ribosomal RNA and of DNA-like RNA.

Liao[10] proposed that the synthesis of ribosomal RNA may be a prerequisite for the synthesis or transport of messenger RNA. The protective removal of newly synthesized messenger RNA from its DNA template may be carried out by ribonucleoprotein particles [11] which prevent the degradation of the template RNA by nuclear ribonucleases [12]; this may also stimulate the synthesis of additional messenger RNA at that site. However, if the primary effect of testosterone were simply to facilitate in some way the transport of messenger RNA to the cytoplasm the specificity of androgen action would not be explained. The DNA-like RNA synthesized after testosterone treatment could differ qualitatively from that synthesized in the castrate animal. The ribosomes from the two types of tissue might differ in their half-lives or in their membrane binding properties [13]. Hormonal specificity in different tissues might be explained if the half lives of the various mRNAs

were different. If the synthesis of all kinds of RNA were reduced after castration, the absence of certain short-lived messages might change metabolism in the tissue as the longer-lived messages exerted greater influence on the pattern of metabolism.

The growth of accessory sex tissues in response to androgens suggests that there might be a concomitant increase in DNA synthesis and, indeed, the uptake of tritiated thymidine by seminal vesicles was increased 18 hours after androgen administration [14]. The synthesis of DNA in the prostate of three-week-old rats or mature castrate rats was stimulated but not until four days after administration of testosterone [1, 15]. Thus the increased synthesis of DNA following androgen administration is not an early action of the hormone, but rather a consequence of other cellular changes such as increased DNA polymerase activity.

Within a minute of the intravenous administration of testosterone the steroid is taken up by the prostate and largely converted to dihydrotestosterone and to lesser amounts of other 5α reduced androgens [16]. The cytoplasmic fraction of the rat ventral prostate contains two 3·5S proteins which selectively bind dihydrotestosterone [17]. Dihydrotestosterone is also bound to a 3S nuclear protein [18]. Fang and Liao[17] proposed that DHT is bound to the cytoplasmic 3·5S protein, transported to the nucleus and there the complex is converted to the 3S protein-dihydrotestosterone entity. Bruchovsky and Wilson[18] have shown that DHT is bound to an acidic protein in prostatic nuclei and Bashirelahi and Villee[19] showed that the incorporation of cytidine and guanosine into RNA by isolated prostatic nuclei was enhanced by dihydrotestosterone but not by testosterone.

Within 48 h of the administration of 1 mg of testosterone to rats castrated four days previously, the RNA content of prostate and seminal vesicle was restored to normal levels [20]. Injection of testosterone increased the incorporation of labeled cytidine into RNA by prostate and seminal vesicle during a 30-min incubation *in vitro*. The labeling of prostatic RNA *in vitro* was increased 84% over the control two hours after administration of androgen and 181% over control 48 h after injection of hormone.

Prostatic tissue from testosterone treated animals was incubated with ^{14}C cytidine and prostatic tissue from control animals was incubated with [^{3}H]-cytidine. The tissues were pooled and RNA was isolated by cold phenol extraction. The RNAs were subjected to electrophoresis on 2% polyacrylamide-0·5% agarose gels. The 30S, 18S, 5S and 4S RNAs were prominent and several minor RNAs were observed consistently. The administration of testosterone two hours before sacrifice increased the labeling of the 16 to 18S RNA and of the 4S RNA. Electrophoretic separation of prostatic nuclear RNA revealed that the high molecular weight RNA of the nucleus showed markedly increased labeling after testosterone administration. Analysis of the RNA by sucrose density gradient centrifugation confirmed the findings by polyacrylamide gel electrophoresis. The incorporation of cytidine into high molecular weight RNA in prostatic nuclei and into 16–18S RNA and 4S RNA in prostatic cytoplasm was increased two hours after the administration of testosterone.

Experiments using competitive DNA–RNA molecular hybridization indicated that the seminal vesicular RNA from animals injected with testosterone two hours before sacrifice and the seminal vesicular RNA from control castrate animals competed with equal affinity for binding to DNA. These findings suggest that the administration of androgen results in increased synthesis of RNA in target tissues without any detectable change in its composition. Analysis of the RNA by countercurrent distribution similarly indicated no change in the base composition of the rapidly labeled RNA from the prostate two hours after injection of androgen.

Treating an animal with testosterone produces an alteration in the biological activity of the RNA in the prostate and seminal vesicle that becomes evident when the RNA is instilled into the lumen of the seminal vesicle [4, 21]. The instilled RNA led to increased protein synthesis and increased weight of the seminal vesicle. Thus, within two hours, testosterone administration increased the synthesis of all types of RNA [8, 22] and some of these, when instilled, mediate an increase in protein synthesis. The species of RNA effective when instilled in the seminal vesicle are in the 16 to 18S range [21].

The adminstration of estrogen, like castration, results in marked atrophy of the prostate [23] and has been used in treating prostatic carcinoma in man [24]. The hormonal control of prostatic growth is linked to changes in synthesis of RNA and protein, hence it seemed of interest to determine whether prostatic atrophy in estrogen treated animals might be linked to the synthesis of one or more kinds of RNA not present in control animals.

Samples of prostatic RNA from control and estrogen treated adult rats were compared by competitive DNA–RNA hybridization [25]. The results suggested that there are differences in the populations of hybridizable RNA from estradiol treated and control animals, that after estradiol treatment new species of RNA are present in the rat prostate that were absent from the prostates of control animals [26]. These new RNAs may be involved in the processes that bring about the regression of the tissue. They might code for certain enzymes involved in the metabolism of testosterone; the estrogen-induced atrophy of the prostate might result from an inhibition of the conversion of testosterone to its more active metabolites or from a greater rate of degradation of the active metabolites.

To explore this possibility, the prostates of untreated rats, of rats treated with a pharmacologic dose of estradiol-17β for three days, or of rats castrated three days previously were removed and incubated *in vitro* with [1,2-^{3}H]-testosterone. The

radioactive products were separated by thin layer chromatography and identified by gas–liquid chromatography and by crystallization to constant specific activity. The conversion of testosterone to dihydrotestosterone was not affected by treatment with estradiol but was reduced significantly by previous castration [27]. Estradiol treatment increased the formation of 17 keto metabolites of testosterone such as androsterone and androstenedione in the prostate. In contrast castration led to a reduction in the over-all metabolic transformation of testosterone in the prostate. Thus the estrogen-induced atrophy of the prostate is not due to decreased conversion of testosterone to DHT but is accompanied by a change in the ratio of 17 keto to 17 hydroxy metabolites of testosterone.

The synthetic steroid, cyproterone acetate, antagonizes the effects of androgen in both central and peripheral tissues involved in reproduction. It reduces the increase in weight of prostate and seminal vesicle induced by testosterone [28]. It inhibits the increased incorporation of leucine into rat prostatic ribosomes stimulated by testosterone [29] and inhibits the stimulation of RNA polymerase activity induced by testosterone [30].

To test the nature of the interaction between DHT and cyproterone acetate in regulating RNA synthesis in the prostate and seminal vesicle, adult castrate rats were given two daily doses of dihydrotestosterone with or without cyproterone acetate. DHT increased the weight and RNA content of the prostate and cyproterone inhibited these increases [31]. The incorporation of labeled cytidine into prostatic RNA was increased by DHT but depressed to control levels when cyproterone was injected along with the DHT. Similar effects were observed in the seminal vesicle. In other experiments the DHT and cyproterone were administered *in vivo* and the uptake of labeled cytidine and its incorporation into RNA was measured in seminal vesicles incubated *in vitro*. The synthesis of RNA measured in this way was increased by DHT administration and the increase was eliminated by cyproterone. The tissues incubated *in vitro* showed a greater incorporation of cytidine and a greater difference between treated and control groups than those in which the cytidine was administered *in vivo*. Cyproterone has been shown to compete with dihydrotestosterone for sites on the androgen receptors in prostate and seminal vesicle [32, 33]. Our experiments indicated that cyproterone did not simply inhibit the 5α reductase that converts testosterone to dihydrotestosterone, since it antagonized the effects of injected dihydrotestosterone. The experiments do not distinguish between the possibilities that cyproterone counteracts dihydrotestosterone only at the level of cytoplasmic receptors, only within the nucleus as the two interact with chromatin, or at both sites.

We have continued our efforts to identify the specific RNA which is effective when instilled into the seminal vesicle. In the earlier experiments we had used either an entire RNA fraction extracted from the tissue with cold phenol (Table 1), or subfractions of this separated by sucrose density gradient centrifugation. The instillation of polyuridylic acid into the seminal vesicle led to increased incorporation of phenylalanine into protein without any significant increase in over-all protein synthesis [34]. Analogous experiments in the uterine system showed that each of the synthetic polynucleotides, poly A, poly G, poly C and poly U led to increased incorporation of the amino acid coded for but had no effect on the incorporation of other amino acids [35]. This suggested that the RNA instilled in seminal vesicle or uterus was acting as a template rather than in some other capacity.

A covalently linked region of 100 to 200 nucleotides rich in polyriboadenylic acid has been identified in rapidly labeled polyribosome-associated RNA and in the heterogeneous nuclear RNA of a number of eukaryotic cells [36–38]. It has been inferred that most, if not all, of the messenger RNAs in eukaryotic cells, with the exception of the histone messenger RNA, contain a polyadenylic acid-rich region at the 3′ hydroxy end. Poly (A) rich RNA can be separated from the mass of RNA extracted from the cell by binding it to polyuridylic acid or to polythymidylic acid bound, in turn, to sepharose or to nitrocellulose.

RNA prepared by cold phenol extraction, purified by deoxyribonuclease and by treatment with ethanol and ether, is placed on a sepharose 4B-polyuridylic acid column. The unbound RNA is eluted by passing a pH 5·1 acetate buffer through the column. The eluting solution is replaced with 100 mM Tris buffer pH 9 and the bound RNA is eluted. The material absorbing at 260 nm is combined, desalted, and concentrated on biofiber or minicon B 15.

We had shown previously that the injection of estradiol increased within 30 min the incorporation of cytidine or adenosine into the bound (poly

Table 1. Effect of instilled RNA on protein synthesis in rat seminal vesicle

	Saline control	RNA instilled
Protein content, mg/g tissue	84·0	82·7
Specific activity, c.p.m./100 μg protein	302·0	668·0

Adult male rats, castrated 7 days previously, were injected with 1 mg dihydrotestosterone. Six hours later they were killed and RNA was prepared from the seminal vesicles. The RNA (200 μg/rat) was instilled into one seminal vesicle and saline into the contralateral seminal vesicle of six adult castrate rats. 5 μCi of [^{3}H]-leucine was instilled simultaneously in each seminal vesicle. The rats were killed 4 hours later and the amount and specific activity of the vesicular proteins were measured.

Table 2. Effect of dihydrotestosterone on the incorporation of [^{3}H]-uridine into poly (A)-rich RNA of the seminal vesicle of adult castrate rats

	Saline control	DHT treated
Total RNA, c.p.m./100 μg	940	1680
Poly (A) RNA, c.p.m./100 μg	1650	2430

Adult male rats, castrated 7 days previously, were injected sc with 1 mg dihydrotestosterone. Six hours later they were killed, the seminal vesicles were removed, rinsed and incubated one hour in 20 mM phosphate buffer, pH 7·4. After the incubation RNA was extracted with cold phenol, placed on a polythymidylic acid cellulose column, and eluted.

A rich) RNA from the uterus of either immature or adult castrate rats [39]. In analogous experiments with the seminal vesicle we found that the fraction of RNA bound to polythymidylic acid columns is eluted with 10 mM Tris, pH 7·6. The incorporation of labeled uridine into the bound, presumably poly (A) rich, RNA of the seminal vesicle is greatly increased by the injection of androgen, dihydrotestosterone, into adult castrate males (Table 2). We were interested to find that the injection of FSH into immature rats markedly increased within two hours the incorporation of [^{3}H]-uridine into the bound, poly (A) rich RNA of the testis (Table 3). This suggests that the poly (A) rich RNA plays some role in the response of the cell to gonadotropins as well as in the response to steroids.

In our experiments with poly (A) rich RNA from the uterus we had found that this fraction contained all of the biological activity when instilled into the uteri of immature rats. It led to increased protein synthesis and increased the activities of glucose-6-phosphate dehydrogenase and ornithine decarboxylase, enzymes that are also increased by the injection of estradiol *in vivo*. Comparable experiments with the seminal vesicle revealed that the bound, poly (A) rich RNA from the seminal vesicle of androgen treated males resulted in increased protein synthesis when instilled into the seminal vesicle of castrate males (Table 4). In contrast, the unbound RNA from the seminal vesicle was ineffective when instilled into the seminal vesicles of castrate rats.

Polyadenylic acid, first discovered in mammalian cells [40, 41] originates in the cell nucleus as part of the metabolically unstable nuclear RNA and appears in cytoplasmic mRNA [42, 43]. The polyadenylic acid segment in cytoplasmic mRNA becomes progressively shorter with age; however, the poly (A) RNA isolated from the polysomes is not different from the poly (A) RNA in other cytoplasmic fractions [44].

It seems well established that an early effect of androgen administration to immature or castrate adult rats is a stimulation of RNA metabolism in the prostate and seminal vesicle. Androgen stimulates the synthesis of all major classes of RNA in these target tissues [22].

The experiments reported here showed that the administration of dihydrotestosterone to castrate adult rats led to a prompt increase in the incorporation of [^{3}H]-cytidine into poly (A) rich (polythymi-

Table 3. Effects of FSH on the incorporation of [^{3}H]-uridine into poly (A)-rich RNA of immature rat testis

	Saline control	FSH treated
Total RNA, c.p.m./100 μg	1140	1415
Poly (A) RNA, c.p.m./100 μg	3370	8615

Immature rats were injected with 50 μg FSH or with saline and with 2 μCi [^{3}H]-uridine. Two hours later the rats were killed, testicular RNA was extracted with phenol and poly (A)-rich RNA was prepared using a cellulose-polythymidylic acid column.

Table 4. Effect of instilled poly(A)RNA on protein synthesis in rat seminal vesicles

	Saline treated	Unbound RNA	Poly(A)RNA
Protein content, mg/g tissue	78·4	79·6	78·8
Specific activity, c.p.m./100 μg protein	286·0	304·0	416·0

Adult male rats, castrated 7 days previously, were injected with 1 mg dihydrotestosterone. Six hours later they were killed and RNA was prepared from the seminal vesicles. Poly(A)RNA separated using polythymidylic acid cellulose column. The unbound RNA or poly(A)RNA (15 μg) was instilled simultaneously with 5 μc of [^{3}H]-leucine in each seminal vesicle. The rats were killed 4 h later and the specific activity of the vesicular proteins were measured.

dylic acid bound) RNA. The mechanism by which the steroid–receptor complex interacts with the nuclear chromatin and increases the transcription of specific portions of the genome is not yet clear. However, one of the products of the transcription process is a poly (A) rich RNA. The poly (A) rich RNA produced in the seminal vesicle in response to administered dihydrotestosterone, when separated from the mass of RNA by Sepharose-poly T chromatography and instilled into the lumen of the seminal vesicle increased the synthesis of protein. The result is parallel to the effect of uterine poly (A) rich RNA instilled into the uterus, which increased protein synthesis and the activity of specific estrogen-sensitive uterine enzymes. As in the uterus, instilling an aliquot of the unbound RNA did not result in increased protein synthesis. Thus the biological activity of the RNA in both systems resides in a very small fraction of the total cellular RNA, the fraction that is bound to polythymidylic or polyuridylic acid-Sepharose columns. The inference that this is a messenger RNA, though very tempting, remains to be proven.

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