Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 5824–5828, August 1988
Biochemistry

Biosynthesis of catalytically active rat testosterone 5α-reductase in microinjected *Xenopus* oocytes: Evidence for tissue-specific differences in translatable mRNA

(female and male rat liver/prostate/size fractionation of mRNA/TLC analysis/3-oxo-5α-steroid Δ^4-dehydrogenase)

YIGAL FARKASH, HERMONA SOREQ, AND JOSEPH ORLY*

Department of Biological Chemistry, Institute of Life Sciences, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel

Communicated by Gordon H. Sato, April 25, 1988

ABSTRACT **The enzyme 4-ene-3-ketosteroid-5α-oxidoreductase [5α-reductase; 3-oxo-5α-steroid Δ^4-dehydrogenase, 3-oxo-5α-steroid: (acceptor) Δ^4-oxidoreductase, EC 1.3.99.5] plays a key role in androgen-dependent target tissues, where it catalyzes the conversion of testosterone to the biologically active dihydrotestosterone. The regulation of 5α-reductase expression has not been studied at the molecular level as the enzyme is a membrane protein that is labile in cell-free homogenates. We developed a sensitive bioassay of the enzyme activity expressed in *Xenopus* oocytes microinjected with rat liver and prostate mRNA. After microinjection, incubation of intact oocytes in the presence of [^{3}H]testosterone revealed the *in ovo* appearance of active 5α-reductase. Polyadenylylated RNA was fractionated by sucrose gradient centrifugation, and the enzymatic activity was shown to be encoded by a 1600- to 2000-base-pair fraction of hepatic poly(A)$^+$ RNA. 5α-Reductase mRNA was most efficiently translated when up to 80 ng of RNA was injected per oocyte. In the injected oocytes, 5α-reductase mRNA was found to be a short-lived molecule ($t_{1/2}$ = 2 hr), whereas its *in ovo* translatable 5α-reductase protein exhibited stable enzymatic activity for over 40 hr. Moreover, the levels of translatable tissue-specific 5α-reductase mRNAs as monitored in the *Xenopus* oocytes correlated with the variable 5α-reductase activities in female rat liver, male rat liver, and prostate homogenates; the ratio of their specific activities was of 2500:630:1, respectively. Altogether, these results provide supporting evidence in favor of the transcriptional control of 5α-reductase expression in rat tissues.**

The enzyme 4-ene-3-ketosteroid-5α-oxidoreductase [5α-reductase; 3-oxo-5α-steroid Δ^4-dehydrogenase, 3-oxo-5α-steroid: (acceptor) Δ^4-oxidoreductase, EC 1.3.99.5] catalyzes the reduction of the A ring of steroid hormones. This enzyme, located in the rough endoplasmic reticulum, is expressed in various androgen-dependent tissues, such as epididymis (1), prostate (2, 3), seminal vesicles (3), and brain (4). In these target tissues, 5α-reductase converts testosterone to its biologically potent derivative, 5α-dihydrotestosterone (DHT) (2, 5, 6). DHT is essential for normal male sex differentiation and has trophic effects on the growth of the male accessory glands (7, 8). Excessively high levels of DHT have been implicated in the pathogenesis of benign prostatic hyperplasia (9–11). Increased activity of 5α-reductase in skin is probably associated with the etiology of female hirsutism (12), acne (13), and male pattern baldness (14). It has also been suggested that modulation of 5α-reductase activity in the ovary is involved in controlling the onset of puberty (15). In addition to the androgen target tissues, high levels of 5α-reductase also exist in the liver (16, 17) and kidney (18), where the enzyme catalyzes the biological inactivation of 4-ene-3-ketosteroids, mainly adrenal corticosteroids (19).

Previous biochemical studies of 5α-reductase have revealed kinetic parameters, such as affinity constants to steroid hormones, as well as the enzyme subcellular distribution in the rough endoplasmic reticulum and/or nuclei of various tissues (1, 20–24). Because the activity of 5α-reductase is extremely unstable in cell-free preparations (1, 20), it has not been purified, and therefore the regulation of its expression could not be studied at the molecular level. To approach this issue, we pursued an experimental model in which 5α-reductase biosynthesis can be studied in intact living cells. Toward this aim, we designed a bioassay for the detection of newly synthesized 5α-reductase produced in mRNA-microinjected *Xenopus* oocytes. The oocytes efficiently translate microinjected heterologous mRNA, perform correct posttranslational processing and, most importantly for 5α-reductase, direct newly synthesized proteins into the correct subcellular compartments (for review, see ref. 25).

Here, we report that *Xenopus* oocytes are able to synthesize catalytically active 5α-reductase, directed by microinjected poly(A)$^+$ RNA prepared from female and male rat liver. For yet unknown reasons, there are remarkable differences between the specific activities of 5α-reductase in female liver, male liver (16, 17), and prostate (20). The *Xenopus* oocyte-mediated quantitative bioassay of 5α-reductase mRNA from these three tissues suggests that the expression of the enzyme is transcriptionally controlled.

METHODS

Wistar-derived 2- to 3-month-old rats were killed by cervical dislocation, and the dissected tissues were homogenized in a Waring Blendor in 10 mM potassium phosphate, pH 7.4/0.25 M sucrose/1 mM $MgCl_2$. Cell nuclei and debris were removed by a 10-min centrifugation at 1000 × *g*, and the supernatant was further centrifuged for 30 min at 20,000 × *g*. The resulting postmitochondrial supernatant was assayed for 5α-reductase activity. Incubation of the postmitochondrial supernatant was carried out in 0.2 ml of the above homogenizing medium supplemented with 10 mM glucose-6-phosphate, 1 mM NADPH, 1 unit of glucose-6-phosphate dehydrogenase, and 5 × 10^5 cpm of [^{3}H]testosterone (0.3 μM). After a 20-min incubation at 37°C, the reaction was terminated by the addition of 1.5 ml of ether, followed by vigorous mixing and a 4-min centrifugation at 2000 × *g*. The organic phase, which contained >90% of the initial radioactivity, was carefully removed and dried under a stream of nitrogen. Each sample was dissolved in 10 μl of ethanol and was analyzed by TLC as previously described (26). The TLC

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: DHT, dihydrotestosterone.

*To whom reprint requests should be addressed.

patterns were analyzed by using a personal computer and the Lotus 1-2-3 spread-sheet program.

Xenopus laevis oocytes were dissected and were microinjected (27) with guanidine thiocyanate-extracted poly(A)$^+$ RNA (28). The integrity of the various RNA preparations was examined by testing their ability to direct the synthesis of active cholinesterase in microinjected *Xenopus* oocytes (28, 29). To allow translation of 5α-reductase mRNA in the injected oocytes, groups of 10 oocytes were incubated up to 20 hr at 20°C in 150 μl of Barth medium (30). Activity of the *in ovo*-synthesized 5α-reductase was assayed by an additional incubation (5–20 hr) in the presence of [^{3}H]testosterone (0.3 μM). Thereafter, the oocytes were homogenized in a glass homogenizer with a Teflon pestle in 0.3 ml of Barth medium and 1.5 ml of ether. The ether-extracted steroids were analyzed by TLC.

RESULTS

The typical metabolism of testosterone as catalyzed by enzymes in the rat liver postmitochondrial supernatant is depicted in the TLC patterns presented in Fig. 1. Testosterone was first converted by 5α-reductase to DHT, which was further reduced by 3α-hydroxysteroid oxidoreductase to yield 5α-androstane-3α,17β-diol (androstanediol). Male liver postmitochondrial supernatant also exhibited a conversion of androstanediol to its glucoronate conjugate. Because 5α-reductase is the first enzyme in this metabolic pathway, summation of testosterone metabolites represents the total of 5α-reductase products.

The substantially different amounts of protein that were required to achieve comparable activities of 5α-reductase in the different tissue sources (Fig. 1) indicated a marked tissue variability of the enzyme activities. An additional quantitative comparison, shown in Table 1, revealed an exceedingly low activity of prostate 5α-reductase, which was 600 times lower than that observed in the male rat liver. Moreover, the activity of 5α-reductase in female rat liver was 2500-fold higher than the activity in the prostate.

When injected with polyadenylylated RNA from female rat liver, *Xenopus* oocytes expressed a remarkable activity of 5α-reductase (Fig. 2*A*). Up to 35 pmol of testosterone was metabolized by intact oocytes during a 20-hr incubation with the substrate. Identification of androstanediol (Fig. 2*B*) indicated the additional expression of 3α-hydroxysteroid oxidoreductase in the injected oocytes. Control oocytes injected with water metabolized only 5 pmol of testosterone to DHT without further conversion to androstanediol (Fig. 2*C*). The linear accumulation of 5α-reductase products suggests that the newly synthesized enzyme is stable in the host oocyte and readily expresses its activity for at least 40 hr after microinjection.

To evaluate the stability of 5α-reductase mRNA in the injected oocytes, it was necessary to determine the enzyme activity in cell-free homogenates prepared at various times after microinjection. This experimental design excluded the possibility of concomitant enzyme synthesis during the assay period. By using this assay, it was found that the enzyme accumulates during the first 5 hr after microinjection (Fig. 3). After this period, the rate of 5α-reductase activity reaches a plateau, which suggests the cessation of any further enzyme synthesis. An alternative interpretation, which suggests a continuous synthesis and breakdown of the enzyme throughout the 40-hr incubation, is unlikely since the oocyte's ability to synthesize proteins gradually decreases *in vitro* by about 20% per day (H.S., unpublished observations). Therefore, it can be concluded that the apparent half-life of 5α-reductase mRNA in microinjected oocytes is ≈2 hr. This value is considerably shorter than that of mRNAs for several other

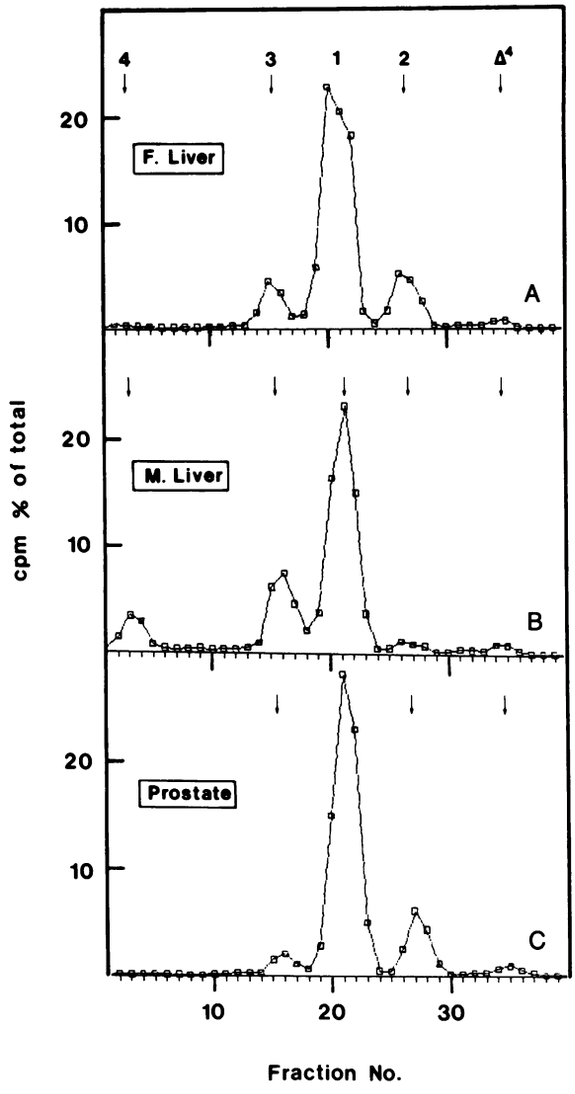

FIG. 1. Testosterone processing in rat liver and prostate. Freshly prepared postmitochondrial supernatants from female rat liver (*A*) (2 μg), male rat liver (*B*) (10 μg), and prostate (*C*) (5 mg) were incubated with [^{3}H]testosterone for 30 min (*A* and *B*) or 60 min (*C*). The products were analyzed by TLC. Chromatogram plates were cut, and radioactivity was determined with a Beckman scintillation counter. Steroid markers are 1, testosterone; 2, DHT; 3, androstanediol; 4, androstanediol glucoronate; and Δ^4, androstenedione.

inducible mammalian enzymes, such as acetylcholinesterase (≈10 hr, ref. 28) or plasminogen activator (≈3–4 hr, ref. 31).

As little as 12.5 ng of poly(A)$^+$ RNA was sufficient to direct 5α-reductase activity at a 2.5-fold higher rate than that observed in control oocytes. The level of the newly expressed enzyme increased linearly with increasing amounts of injected mRNA, and no saturation was observed when up to 80 ng of RNA was injected per oocyte (Fig. 4).

To estimate the size of the mRNA molecules encoding 5α-reductase activity, polyadenylylated RNA was fractionated by sucrose gradient centrifugation, and various fractions were analyzed for their ability to direct the enzymatic activity in microinjected oocytes (Fig. 5). Greater than 80% of the 5α-reductase activity resided in fractions 18–20. The apparent size of the RNA molecules in these fractions was 1.6–2.0 kilobases, as determined by agarose gel electrophoresis (Fig. 5 *Inset*).

To assess whether the differences in the activities of 5α-reductase in prostate versus liver tissues are also reflected in their respective mRNA levels, the latter were expressed in

Table 1. 5α-Reductase activity in rat tissues and in *Xenopus* oocytes injected with poly(A)$^+$ RNA from those tissues

Tissue	Female rat liver	Male rat liver	Prostate
Specific activity in PMS,* nmol/hr per mg of protein	15 ± 5	3.8 ± 0.6	0.006 ± 0.002
Specific activity in washed microsomes,† nmol/hr per mg of protein	7.8 ± 0.4	—	—
RNA yield,‡ μg per g of tissue			
Total	22,500	20,000	22,200
Poly(A)$^+$	133	82.7	92
5α-Reductase mRNA activity *in ovo*,§ pmol per 20 hr per μg of injected RNA	30 ± 2	5.5 ± 0.8	ND

—, Not tested; ND, not detected.

*The specific activity of 5α-reductase (nmol of 5α-reduced steroids per hr per mg of protein) was determined in postmitochondrial supernatants (PMS).

†Female rat liver microsomes were prepared by a 90-min centrifugation at 75,000 × *g*. The microsomal pellet was resuspended in homogenizing buffer and was assayed for 5α-reductase activity as described in footnote *.

‡Poly(A)$^+$ RNA was purified by oligo(dT)-cellulose chromatography. Yields of RNA are presented as micrograms per gram of tissue, as determined from A_{260} values.

§Unfractionated polyadenylylated RNA from the various tissues was injected into groups of 10 oocytes (80 ng per oocyte), and 5α-reductase activity was determined. The mean activity (n = 4) induced in injected oocytes is presented as pmol of 5α-reduced steroids accumulated per 20 hr per μg of injected RNA. Background activity (5 ± 1 pmol per 20 hr per μg of injected RNA) in control (H_2O-injected) oocytes was subtracted. Activity in oocytes injected with prostate mRNA (6.4 ± 1 pmol per 20 hr per μg of injected RNA) was not significantly higher than background levels.

the oocytes. Poly(A)$^+$ RNA (80 ng) from male and female rat liver directed the synthesis of 5α-reductase activity capable of processing 5.5 ± 0.8 and 30 ± 2 pmol of testosterone per μg of injected RNA, respectively (Table 1). Conversely, the 5α-reductase activity expressed by oocytes injected with prostatic mRNA was not significantly higher than the background value. Similar results were obtained after injection of sucrose gradient fractions of poly(A)$^+$ RNA extracted from these three tissues.

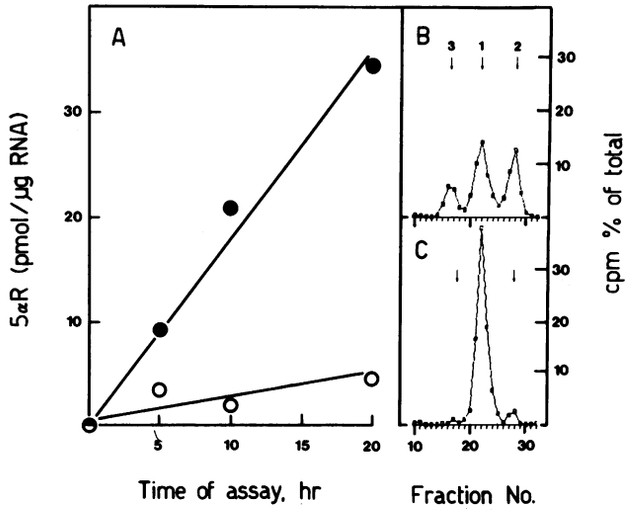

FIG. 2. (*A*) Time-dependent 5α-reductase activity in microinjected *Xenopus* oocytes. After injection with either 80 ng of female rat liver poly(A)$^+$ RNA (●) or 50 nl of water (○), oocytes were incubated in Barth medium for 20 hr at 20°C. 5α-Reductase activities were determined by further incubation with [^{3}H]testosterone. At each time point, 10 oocytes were homogenized and processed for extraction of steroids. Two examples of TLC analysis of the steroids extracted from the RNA- (*B*) and H_2O- (*C*) injected oocytes after 20 hr of incubation with [^{3}H]testosterone are presented. Steroid markers are as in Fig. 1. 5αR, 5α-reductase.

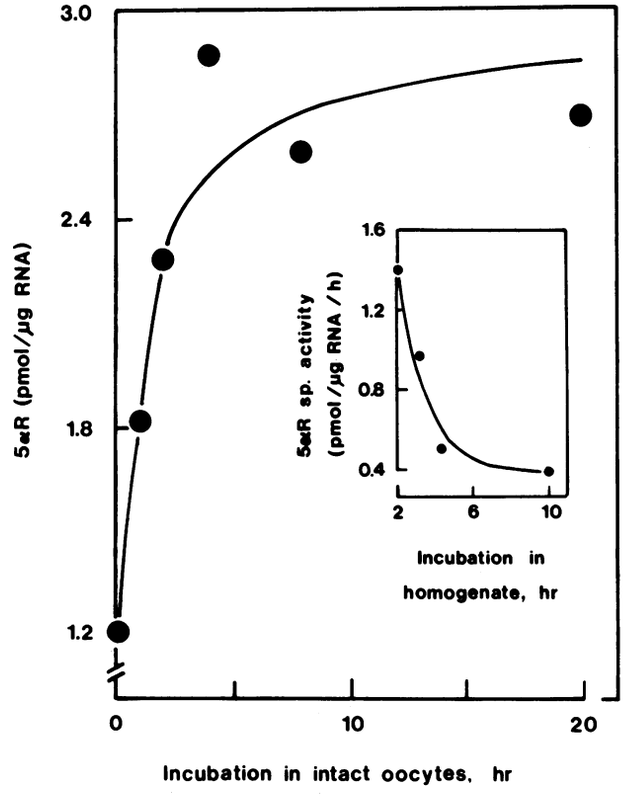

FIG. 3. Kinetics of 5α-reductase synthesis in *Xenopus* oocytes microinjected with female rat liver poly(A)$^+$ RNA. After incubation in Barth medium for the indicated times, groups of 30 oocytes were homogenized in homogenizing buffer, and 5α-reductase activity was determined during a 2-hr incubation, as described for postmitochondrial supernatants. The relatively low 5α-reductase activity in homogenates of RNA-injected oocytes resulted from the short time of assay, which was necessary to avoid a substantial inactivation of the enzyme in the cell-free homogenates. (*Inset*) Time-dependent decay of 5α-reductase activity in homogenized oocytes. 5αR, 5α-reductase.

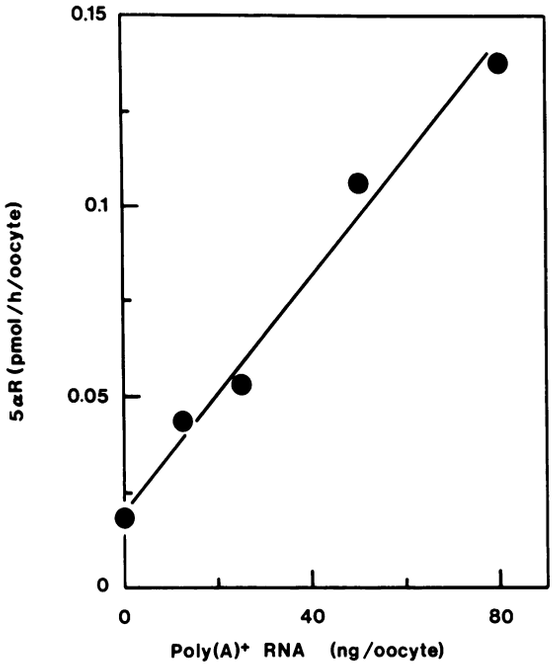

FIG. 4. 5α-Reductase activity expressed in oocytes microinjected with various amounts of female rat liver poly(A)$^+$ RNA. The indicated amounts of RNA were injected in 50 nl of H_2O per oocyte. After a 20-hr incubation, [^{3}H]testosterone was added for an additional period of 20 hr, and TLC analysis of the steroids was conducted. 5αR, 5α-reductase.

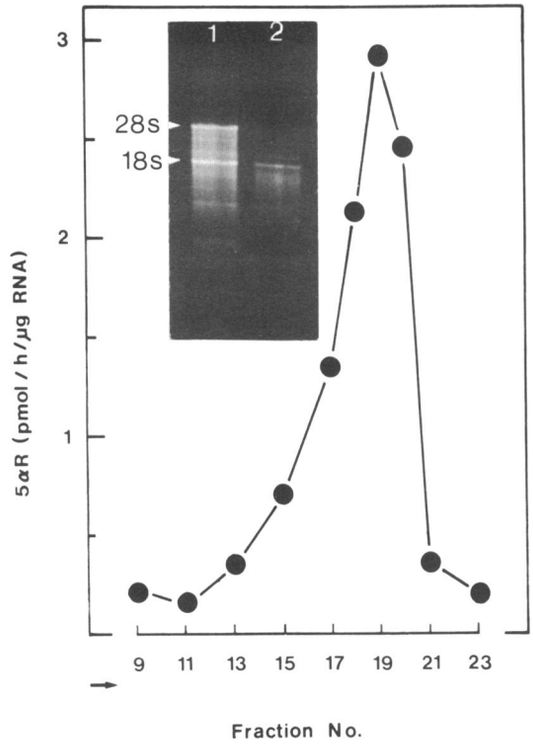

FIG. 5. Expression of 5α-reductase in *Xenopus* oocytes microinjected with size-fractionated female rat liver polyadenylylated RNA. Poly(A)$^+$ RNA (500 μg) was denatured and was size-fractionated by sucrose gradient centrifugation, as previously described (32), and RNA from each fraction was precipitated and injected into five oocytes. 5α-Reductase activity was monitored, as described in Fig. 2. (*Inset*) Agarose minigel electrophoresis. Lanes: 1, female rat liver poly(A)$^+$ RNA; 2, pooled RNA from fractions 18–20 of the sucrose gradient. Arrowheads depict the migration of 28S (4900 base pairs) and 18S (2000 base pairs) ribosomal RNA markers. 5αR, 5α-reductase.

DISCUSSION

The results presented in this report demonstrate that *Xenopus* oocytes translate and successfully express catalytically active 5α-reductase after microinjection of rat hepatic polyadenylylated RNA. So far, due to its lability, 5α-reductase has not been purified, and thus neither antibodies nor cDNA clones are available. Consequently, the molecular nature of the enzyme and the regulation of its biosynthesis have not yet been studied. The mRNA-microinjected *Xenopus* oocytes therefore provide a sensitive experimental tool to investigate the mode of 5α-reductase expression. After injection of size-fractionated poly(A)$^+$ RNA, the *Xenopus* oocyte bioassay revealed that a 5α-reductase is encoded by a 1600- to 2000-base-pair mRNA, hence predicting the expected molecular mass of the enzyme to be ≤50,000 Da.

We have previously shown that steroid-metabolizing activities can be readily determined by adding radioactive steroid precursors to intact cultured cells and following their time-dependent metabolism by sampling the culture medium (26). Such an experiment is feasible due to the fast transport and the practically immediate equilibrium (15–30 sec) of added steroids with the inner cytoplasmic environment of the intact cells (33, 34). Based on these observations, we monitored the activity of newly synthesized 5α-reductase in mRNA-injected oocytes by measuring the metabolism of [^{3}H]testosterone added to intact eggs. By using this methodology, we avoided the loss of enzyme activity that would have occurred if the activity had been measured in cell-free preparations.

We first used poly(A)$^+$ RNA extracted from female rat liver, where this activity is markedly high. When injected oocytes were allowed to synthesize 5α-reductase and 20 hr later the enzyme activity was measured, the levels of the accumulating 5α-reduced testosterone metabolites were 7 times higher as compared with H_2O-injected oocytes. The linear time-dependent 5α-reductase activity indicated that the amount of active enzyme protein remained constant during the period of the assay. This suggested that 5α-reductase mRNA was no longer translated shortly after microinjection, as was directly corroborated by using a cell-free assay of the active enzyme. This analysis predicts a relatively short half-life of about 2 hr for 5α-reductase mRNA, although more conclusive data regarding this issue require the use of cDNA probes. When combined with the observed extended stability of the catalytic activity, which remained constant for at least 40 hr after microinjection, these findings imply that the content of 5α-reductase in the female rat liver is probably regulated at the level of mRNA.

The linear increase of 5α-reductase activity observed when up to 80 ng of poly(A)$^+$ RNA was injected per oocyte depends on the ability of the mRNA coding for this nonabundant membrane protein to compete with the bulk of other hepatic and oocyte mRNAs (reviewed in ref. 25). This reflects both the abundance of 5α-reductase mRNA and the efficiency with which it forms expressible initiation complexes with rough endoplasmic polysomes. The efficient translation of 5α-reductase mRNA is additional supporting evidence in favor of the putative transcriptional control of 5α-reductase expression.

The present study demonstrates the range of 5α-reductase activity among the organs tested; female rat liver, male rat liver, and prostate homogenates exhibited a ratio of specific activities of 2500:630:1, respectively. This result confirms previous reports describing similar tissue-specific and sex-dependent differences of 5α-reductase activities (16, 17, 35–38). Two possible mechanisms that could determine this variability are allosteric modulation of enzyme activity or

regulation of the mRNA levels of the enzyme. The *Xenopus* oocyte-mediated bioassay of 5α-reductase mRNA levels is an appropriate experimental approach to discriminate between these two alternative possibilities, in the absence of antibodies or cDNA probes. The ratio of 5α-reductase activities determined *in ovo* from the female rat liver, male rat liver, and prostate poly(A)$^+$ RNAs was found to be similar to that determined in tissue homogenates. Furthermore, similar results were obtained after injection of size-fractionated poly(A)$^+$ RNA extracted from these three tissues. The above experiments strongly suggest that the 5α-reductase activities in these tissues are directly determined by their respective mRNA levels. Therefore, our results are in good agreement with previous biochemical studies, which failed to identify cytoplasmic activators or inhibitors as responsible for the sex differences in hepatic 5α-reductase activities (16).

Finally, the successful expression of 5α-reductase in *Xenopus* oocytes suggests a straightforward strategy for the molecular cloning and selection of 5α-reductase cDNA clones by exploiting this bioassay for screening liver cDNA libraries and their *in vitro* prepared mRNA transcripts. A recent example for this strategy is the substance K receptor, which is a scarce membrane protein (39). A cDNA probe will provide the means to portray the detailed mechanisms by which the genetic elements coding for 5α-reductase direct the expression of such differently active tissue-specific enzymes. In addition, molecular probing of 5α-reductase expression will assist the study of enzyme involvement in brain and skin responses to androgens and most importantly in the etiology of prostatic neoplasia.

We are grateful to Dr. Patrick Dreyfus of this department for helpful advice. This work was supported by Grant No. 3255/84 from the United States–Israel Binational Sciences Foundation (BSF), Jerusalem, Israel (J.O.) and by Fund for Basic Research, administered by the Israel Academy of Sciences and Humanities (J.O.).

1. Scheer, H. & Robaire, B. (1983) *Biochem. J.* **211,** 65–74.
2. Bruchovsky, N. & Wilson, J. D. (1968) *J. Biol. Chem.* **243,** 2012–2021.
3. Schmidt, H., Noack, I. & Voigt, K. D. (1972) *Acta Endocrinol. (Copenhagen)* **69,** 165–172.
4. Martini, L. (1982) *Endocr. Rev.* **3,** 1–25.
5. Anderson, K. M. & Liao, S. (1968) *Nature (London)* **219,** 277–279.
6. Baulieu, E. E., Lasnitzki, I. & Robel, P. (1968) *Nature (London)* **219,** 1155–1156.
7. Imperato-McGinley, J., Peterson, R. E., Gautier, T. & Strula, E. (1979) *J. Steroid Biochem.* **11,** 637–645.
8. Wilson, J. D., Griffin, J. E. & George, F. E. (1979) *Arthritis Rheum.* **22,** 1275–1283.
9. Meike, A. W., Collier, E. S., Middleton, R. G. & Fang, S.-M. (1980) *J. Clin. Endocrinol. Metab.* **51,** 945–947.
10. Geller, J., Albert, J., Lopez, D., Geller, S. & Niwayama, G. (1976) *J. Clin. Endocrinol. Metab.* **43,** 686–688.
11. Wilson, J. D. (1980) *Am. J. Med.* **68,** 745–756.
12. Kuttenn, F., Mowszowicz, I., Schaison, G. & Mauvais-Jarvis, P. (1977) *J. Endocrinol.* **75,** 83–91.
13. Sansone, G. & Reisner, R. M. (1971) *J. Invest. Dermatol.* **56,** 366–372.
14. Bingham, K. D. & Shaw, D. A. (1973) *J. Endocrinol.* **57,** 111–121.
15. Eckstein, B. (1985) in *Female Adolescence*, eds. Flamming, C., Venturoli, S. & Givens, J. R. (Year Book Medical, Chicago), pp. 95–116.
16. Yates, F. E., Herbst, A. L. & Urqhart, J. (1958) *Biochem. J.* **63,** 887–902.
17. Liang, T., Heiss, C. E., Ostrove, S., Rasmusson, G. H. & Cheung, A. (1983) *Endocrinology* **112,** 1460–1468.
18. Massa, R. & Martini, L. (1974) *J. Steroid Biochem.* **5,** 941–947.
19. Schulster, D., Burstein, S. & Cooke, B. A. (1976) *Molecular Endocrinology of the Steroid Hormones* (Wiley, New York), pp. 156–162.
20. Moore, R. J. & Wilson, J. D. (1974) *Biochemistry* **13,** 450–456.
21. Gustafsson, J.-A. & Pousette, A. (1973) *Biochemistry* **13,** 875–881.
22. Bertics, P. J. & Karavolas, H. J. (1985) *J. Steroid Biochem.* **22,** 795–802.
23. Campbell, J. S., Bertics, P. J. & Karavolas, H. J. (1986) *J. Steroid Biochem.* **24,** 801–806.
24. MacIndoe, J. H. & Woods, G. R. (1981) *Endocrinology* **108,** 1407–1413.
25. Soreq, H. (1985) *CRC Crit. Rev. Biochem.* **18,** 199–238.
26. Goldring, N. B. & Orly, J. (1985) *J. Biol. Chem.* **260,** 913–921.
27. Soreq, H., Parvari, R. & Silman, I. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 830–834.
28. Prody, C., Zevin-Sonkin, D., Gnatt, A., Koch, R., Zisling, R., Goldberg, O. & Soreq, H. (1986) *J. Neurosci. Res.* **16,** 25–35.
29. Soreq, H., Zevin-Sonkin, D. & Razon, N. (1984) *EMBO J.* **3,** 1371–1375.
30. Gurdon, J. B., Lane, C. D., Woodland, H. R. & Marbaix, G. (1971) *Nature (London)* **233,** 177–182.
31. Miskin, R. & Soreq, H. (1981) *Nucleic Acids Res.* **9,** 3355–3363.
32. Burmeister, M., Avivi, A., Schlessinger, J. & Soreq, H. (1984) *EMBO J.* **3,** 1499–1505.
33. Giorgi, E. P. & Stein, W. D. (1981) *Endocrinology* **108,** 688–697.
34. Giorgi, E. P. (1980) *Int. Rev. Cytol.* **65,** 49–115.
35. Hanukoglu, I., Karavolas, H. J. & Goy, R. W. (1977) *Brain Res.* **125,** 313–324.
36. Denef, C., Magnus, C. & McEwen, B. S. (1974) *Endocrinology* **94,** 1265–1274.
37. Massa, R., Justo, S. & Martini, L. (1975) *J. Steroid Biochem.* **6,** 567–571.
38. Verhoeven, G., Heyns, W. & De Moor, P. (1976) *Eur. J. Biochem.* **65,** 565–576.
39. Masu, Y., Nakayama, K., Tamaki, H., Harada, Y., Kuno, M. & Nakanishi, S. (1987) *Nature (London)* **329,** 836–838.