Expression Cloning and Regulation of Steroid 5α-Reductase, an Enzyme Essential for Male Sexual Differentiation*

(Received for publication, April 24, 1989)

Stefan Andersson, Richard W. Bishop, and David W. Russell†‡
From the Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, Texas 75235

The conversion of testosterone into the more potent androgen, dihydrotestosterone, catalyzed by the enzyme steroid 5α-reductase, is required for differentiation of male external genitalia. Here, we report the isolation of cDNA clones encoding the rat steroid 5α-reductase using expression cloning in Xenopus oocytes. DNA sequence analysis demonstrates that the liver and ventral prostate forms of steroid 5α-reductase are identical hydrophobic proteins of 29 kDa. The amount of steroid 5α-reductase mRNA in liver increased in response to castration, but remained unchanged in the prostate. Testosterone administration to castrated induced expression of mRNA in the prostate but had no effect on liver. The data suggest that the steroid 5α-reductase gene is differentially regulated by testosterone in androgen-responsive versus non-responsive tissues.

A unique aspect of male sexual development is the requirement that the testis-derived hormone, testosterone, must be converted into dihydrotestosterone in target tissues that differentiate to form the male external genitalia (1). The conversion is catalyzed by a microsomal enzyme, steroid 5α-reductase, in the anlage of the external genitalia (2). The absence of steroid 5α-reductase activity underlies a rare form of male pseudohermaphroditism, originally termed pseudovaginal perineoscrotal hypospadias, in which the male external genitalia differentiate as female structures (3, 4). In addition to its location in androgen-responsive tissues, high levels of steroid 5α-reductase activity are found in female rat liver but not in male rat liver (5). Whether this liver enzyme is the same as that in male target tissues is both controversial and unknown. Moreover, the factors that regulate the expression of this enzyme and the reason for the required conversion of testosterone into dihydrotestosterone for normal male differentiation are poorly understood (2).

The action of steroid 5α-reductase is a late event in male sexual development, a complex process that requires the correct developmental interpretation of both genetic and hormonal signals (1, 6). The process is thought to begin after the establishment of chromosomal sex at fertilization with the expression of a master regulatory protein termed the testis determining factor. The gene for this protein has recently been cloned and shown to encode a protein with a structural motif (the zinc finger) commonly found in transcription factors (7, 8). This finding is consistent with a role for this protein in the induction of a differentiation program leading to development of the testis (7). The testis in turn produces two hormones, testosterone, and a protein called the Müllerian inhibiting substance (9). The latter hormone causes regression of the Müllerian ducts, which are the anlage of female internal genitalia (1, 9). Testosterone promotes development of the male internal genitalia (epididymides, vas deferentia, and seminal vesicles), and after conversion to dihydrotestosterone by steroid 5α-reductase, the differentiation of the external male structures (penis, scrotum, and prostate) (1).

The actions of both testosterone and dihydrotestosterone in male development are mediated through a single protein, the androgen receptor, a recently cloned member of the steroid hormone receptor family (10-13). Genetic defects in the androgen receptor prevent the differentiation of both internal and external male structures (14). Although dihydrotestosterone has been shown to bind to the androgen receptor with higher affinity than testosterone (15), it is presently not known why the action of the receptor in promoting the differentiation of the external male genitalia requires synthesis of the higher affinity ligand. This requirement must be attributable to the presence of other regulatory factors in the development of the external genitalia (2).

Although the role of steroid 5α-reductase in male sexual differentiation has been elucidated, molecular insights into the gene and protein have not yet been possible due to the lack of genetic and immunological tools. The enzyme has been partially purified from the rat and shown to be an integral membrane protein of the endoplasmic reticulum or nuclear membrane (16). Much controversy exists in the literature as to the number of steroid 5α-reductase isozymes present in the liver and prostate of the rat and their cofactor requirements (17, 18). Both the liver and prostate enzymes are inhibited in a competitive fashion by the steroid analogue 17β-N,N-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one (4-MA), suggesting that these proteins must at least share sequence homology in their substrate binding domains (19). Consistent with this prediction are the findings that the liver and prostate enzymes catalyze the reduction of similar steroid substrates, including testosterone, androstenedione, and progesterone (5, 20).

In this paper, we describe the cloning and sequence of...
Expression Cloning of Steroid 5α-Reductase
cDNAs encoding the rat liver and prostate steroid 5α-reductase enzymes. To circumvent the lack of probes for these cDNAs, we modified a Xenopus oocyte expression cloning strategy originally developed for the isolation of lymphokines (21), neurotransmitter receptors (22-24), and membrane transporters (25). The amino acid sequences deduced from the cDNAs reveal that the liver and prostate forms of steroid 5α-reductase are identical. However, RNA blotting experiments suggest that the expression of steroid 5α-reductase in these two tissues is differentially regulated by testosterone.

EXPERIMENTAL PROCEDURES
Steroid 5α-Reductase Enzyme Assay—Stage 5 and 6 oocytes were surgically removed from female Xenopus laevis (NASCO, Fort Atkinson, WI) and collagenase-treated as described by Julius et al. (24). Oocytes were injected with 50–100 nl of RNA (1 μg/ml) as described by Peacock et al. (26). After injection, the oocytes were incubated at 19°C for 24 h in modified Barth's saline solution (29) containing 1 mg/ml bovine serum albumin to allow expression of the injected RNA. Five to ten viable oocytes were then transferred to 1 ml of modified Barth’s saline solution containing 5 μM [14C]-labeled steroid (50 μCi/mmol, Du Pont-New England Nuclear), and incubated at 37°C for 15 h. This temperature-jump protocol is based on the observation that expression of mRNA in Xenopus is maximal at 19°C, whereas rat steroid 5α-reductase expressed in Xenopus has a temperature optima of 37°C (unpublished results). After the 37°C incubation, the oocytes were homogenized in the incubation medium and steroids were extracted with 10 ml of dichloromethane. The solvent was evaporated under air and the remainder of the residue was dissolved in 0.1 ml of chloroform/methanol (2:1, v/v) and subjected to thin-layer chromatography using Silica Gel 60 thin-layer chromatography plates (E. Merck, 5748-7, Darmstadt, West Germany). The mobile phase was chloroform/ethyl acetate (3:1, v/v). The chromatoplates were autoradiographed for 18 h at −70°C and the radioactive zones were identified by comparison with unknown steroid standards.

cDNA Cloning—Total RNA from female rat liver was extracted by a guanidinium isothiocyanate (Gibco) procedure (25). Poly(A)+-enriched RNA was isolated and size-fractionated by density gradient centrifugation on 10–25% (w/v) sucrose gradients containing methymercury hydroxide (28). After centrifugation at 4°C for 15 h at 76,800 × g, aliquots of RNA from each gradient fraction were assayed for steroid 5α-reductase mRNA by injection into Xenopus oocytes. Positive fractions from the sucrose gradients were combined and the RNA was concentrated by ethanol precipitation. First strand cDNA was synthesized using mRNA pretreated with 2.5 mM methylmercury hydroxide and AGCGGCCGC(T)20 as a primer. Second strand synthesis was performed according to standard procedures (27). The resulting cDNA was digested with NotI and EcoRI and size-fractionated on a 1% (w/v) agarose gel. Complementary DNAs greater than 1.3 kb were inserted into EcoRI and NotI sites of pBluescript (Stratagene, La Jolla, CA). Recombinant plasmids were propagated in Escherichia coli DH5α/F’IQ (GIBCO). A rat ventral prostate cDNA library was constructed as described above except that random hexamucleotides were used as primers and total poly(A)+ RNA was used as template. Single-strand cDNAs derived from prostate mRNA were inserted into the EcoRI site of ZapII (Stratagene). Recombinant baculovirus were propagated in F. coli XL1-Blue. Blueprints plasmids were subsequently rescued from Xap recombinants by superinfection with helper F1 baculovirus.

In the initial screening of the female rat liver cDNA library, plasmid minipreps were prepared from pools containing 150–200 cDNA clones/pool. Plasmid DNA was linearized with NotI and RNA was transcribed in vitro using bacteriophage T7 RNA polymerase (Pharmacia LKB Biotechnology Inc.) as described by Julius et al. (24). Xenopus oocyte injection was carried out as described above. Plasmid DNA from one positive pool was retranscribed and 960 colonies were randomly picked into individual 0.3-ml cultures maintained in 96-well microtiter plates. Plasmid DNAs were subsequently prepared from pools of 100–150 colonies from each well and assayed by microinjection. Sibling selection from the microrteter plate was carried out by matrix analysis as described in the text.

Nucleic Acid Sequencing and Primer Extension—Overlapping fragments from both DNA strands were subcloned into bacteriophage M13 vectors and sequenced by automated methods (29) using an Applied Biosystems model 370A DNA sequencer. For primer extension analysis, an antisense oligonucleotide complimentary to nucleotides 70–109 of Fig. 4A was annealed at 68°C to rat liver poly(A)+ RNA and extended with reverse transcriptase as described by Södhor et al. (30). Direct RNA sequencing of the steroid 5α-reductase mRNA was carried out as described by Geliebter et al. (31).

In Vitro Translation of DNA—Approximately 100 ng of RNA was translated in vitro using [35S]methionine (1100 Ci/mmol) and a rabbit reticulocyte lysate (Promega, Madison, WI) in the presence or absence of dog pancreas microsomes (32). After incubation for 1 h at 30°C, the reactions were terminated by adding cycloheximide to a final concentration of 0.2 mM or RNase A to 2 mg/ml. Experiments with products translated in vitro in the presence of 50 μg/ml trypsin (GIBCO) were performed with or without 2% (w/v) Triton X-100 (Boehringer Mannheim) for 30 min at 2°C. The protease reactions were terminated by adding soybean trypsin inhibitor (Cappel, Malvern, PA) to a final concentration of 1 mg/ml.

Physiology Experiments—Experiments were designed to allow comparison of mRNA levels in liver and prostate of normal rats, of 7-day castrated animals, of 10-day castrated animals, and of normal or 10-day castrated animals given testosterone on days 7–9 of the experiment. Sexually mature Sprague-Dawley male rats were castrated by standard surgical procedures on day 0. On day 7, experimental groups were subcutaneously injected for 3 consecutive days with 2 mg of testosterone acetate or testosterone propionate dissolved in 0.2 ml of sesame oil (5). Control animals were injected with sesame oil alone. On day 10 of the experiment, RNA was prepared from the livers and prostates of up to 15 animals in each experimental group, and analyzed by blotting as described in the legend to Fig. 8.

RESULTS AND DISCUSSION
Expression Cloning of the Rat Liver Steroid 5α-Reductase cDNA—Fig. 1 outlines the strategy used to obtain a full length cDNA for the rat liver steroid 5α-reductase. As a source of mRNA, we used female rat liver, which for physiologically unknown reasons expresses high levels of steroid 5α-reductase enzyme activity (16). Microinjection into Xenopus oocytes indicated that this mRNA could direct the synthesis of an enzyme that catalyzed the conversion of steroids into their 5α-reduced forms (see below). Sucrose gradient fractionation of rat liver mRNA indicated that this activity was encoded by an mRNA of about 2.5 kb (Fig. 1). Similar results have been obtained by Farkash et al. (33). The mRNA in this fraction was converted into cDNA, size-fractionated, and cloned into an RNA expression vector. To avoid problems with anti-sense inhibition, the cDNA library was constructed in an oriented manner (Fig. 1). Twenty pools, each containing 150–200 cDNA clones, were then used to synthesize mRNA that was in turn injected into oocytes to allow determination of steroid 5α-reductase activity by thin-layer chromatography analysis. From one active pool, a near full length cDNA encoding this enzyme was subsequently isolated by dilution cloning (Fig. 1).

Fig. 2 illustrates the results of thin-layer chromatography assays from the dilution cloning. In all experiments, assay of steroid 5α-reductase activity in injected oocytes was carried out using a temperature-jump protocol as detailed under “Experimental Procedures.” Microinjection of water into Xenopus oocytes revealed an endogenous activity capable of converting the testosterone substrate into androstenedione and little or no ability to convert these steroids into their 5α-reduced forms (lane 1). In contrast, when female rat liver mRNA was injected, the oocytes expressed an activity that generated both dihydrotestosterone and 5α-androstanedione, as well as at least two other steroid metabolites (lane 2). These latter unidentified steroids were derived from the 5α-reduced metabolites generated by the injected mRNA (see
Expression Cloning of Steroid 5α-Reductase

The sucrose gradient fractionation of rat liver RNA was used to construct an oriented cDNA library. In vitro transcription of plasmid pools with T7 RNA polymerase was followed by injection of Xenopus oocytes and assay of 5α-reductase activity by TLC. Subdivision of positive pools yielded unique 5α-reductase cDNA.

**FIG. 1.** Expression cloning of steroid 5α-reductase. Female rat liver RNA was size-fractionated on 10-25% sucrose gradients and aliquots of RNA were assayed for steroid 5α-reductase activity in Xenopus oocytes. Peak activity fractions were used to construct an oriented cDNA library in a plasmid RNA expression vector. E. coli transformants from this library were pooled in groups of 150–200 colonies and assayed for enzyme expression. A thin layer chromatography assay was employed in which the substrate testosterone was synthesized from one of the initial 20 cDNA plasmid pools resulting in 5α-reduced metabolites from testosterone. Subsequent analysis of 5α-reduced metabolites from the various experiments were exposed to Kodak XAR-5 film for 16 h. In the chromatographic system employed, hydrophobic steroids migrate further than hydrophilic steroids. The positions of authentic steroid standards are shown on the left of the autoradiograms.

**FIG. 2.** Dilution cloning of a liver steroid 5α-reductase cDNA. Xenopus oocytes were injected with RNA from the indicated source and assayed for steroid 5α-reductase activity by thin-layer chromatography using [14C]testosterone as a substrate as described under “Experimental Procedures.” Lane 1, H2O-injected; lane 2, RNA from female rat liver; lane 3, RNA synthesized in vitro from a pool of 150-200 cDNA clones; lane 4, RNA synthesized from cDNAs inoculated in a 96-well microtiter plate; lane 5, RNA synthesized from a pool of 12 clones corresponding to a row from the microtiter plate; lane 6, RNA synthesized from eight clones corresponding to a column from this plate; and lane 7, RNA derived from a cDNA clone corresponding to the intersection of the row and column. Chromatograms from the various experiments were exposed to Kodak XAR-5 films for 16 h. In the chromatographic system employed, hydrophobic steroids migrate further than hydrophilic steroids. The positions of authentic steroid standards are shown on the left of the autoradiograms. T, testosterone; A, androstenedione; DHT, 5α-dihydrotestosterone, 5αA, 5α-androstenedione. An endogenous Xenopus enzyme in the oocytes converts testosterone into androstenedione. Steroids marked with an asterisk are uncharacterized metabolites derived from the 5α-reduced compounds by endogenous Xenopus enzymes (see Fig. 3). The amount of 5α-reduced metabolites in a given experiment varied depending on the batch of oocytes injected and is thus not calculated here.

**FIG. 3.** Substrate specificity of the cloned steroid 5α-reductase. Xenopus oocytes obtained from a single animal were injected with in vitro synthesized RNA derived from the steroid 5α-reductase cDNA clone and then assayed for enzyme activity using the indicated [14C]-labeled steroid substrates (5 μM) in the absence (—) or presence (+) of the competitive inhibitor 4-MA (6 μM). The various steroids and metabolites are identified on the left and right of the autoradiograms: P, progesterone; 5αP, 5α-dihydroprogesterone; others are as indicated in the legend to Fig. 2. The amount of 5α-reduced metabolites for each substrate is indicated at the bottom of the figure and was determined by liquid scintillation counting after cutting out appropriate zones from the chromatograms. In lanes 5 and 6, all radioactive derivatives of dihydrotestosterone were counted. In experiments not shown, the pattern of metabolites obtained when dihydrotestosterone was employed as a substrate was identical in both H2O-injected and steroid 5α-reductase RNA-injected oocytes. Not inhibited by 4-MA in this experiment (lane 2). The data in lanes 3 and 7 indicate that both androstenedione and progesterone were substrates for the cloned enzyme. As with testosterone, 4-MA efficiently blocked the reduction of these steroids (lanes 4 and 8, respectively). When radiolabeled dihydrotestosterone was used as a substrate (lane 5), the inhibitor had no effect on the conversion of this compound.
Expression Cloning of Steroid 5α-Reductase

Sequence of Liver Steroid 5α-Reductase—Fig. 4A shows the nucleotide sequence of the liver steroid 5α-reductase cDNA and the deduced amino acid sequence of the protein. The cDNA insert in the expressing clone was 2,465 base pairs in length and included a long 3'-untranslated region of 1,691 base pairs and an extended translation reading frame of 765 base pairs. A potential polyadenylation signal is present at position 2,446, upstream of a tract of A residues, suggesting that the 3' end of this cDNA is authentic. In the predicted amino acid sequence, there are three methionine residues in the first 19 amino acids. The context of the first ATG is identical in six out of nine nucleotides with the ideal Kozak consensus sequence (38), suggesting that this codon may specify the amino-terminal methionine of steroid 5α-reductase. With this assumption, the open reading frame would encode a hydrophobic protein of 255 amino acids with a predicted M, of 29,343. Over 50% of the amino acids in the protein sequence have hydrophobic side chains. Consistent with this amino acid composition, a hydropathy plot (Fig. 4B) suggests a protein with many hydrophobic regions. A comparison of the sequence shown in Fig. 4A to others in the National Biomedical Research Foundation protein data bank and the GenBank DNA sequence collection did not reveal any sequences that were homologous to steroid 5α-reductase.

Characterization of Steroid 5α-Reductase Protein and mRNA—Several reports in the literature have identified a rat liver protein of M, 50,000 that either has steroid 5α-reductase activity or can be cross-linked to a photoactivatable derivative of 4-MA (36, 37). To ensure that the sequence shown in Fig. 4A represented the complete coding region of steroid 5α-reductase, we carried out three kinds of experiments. First, as shown in Fig. 5, in vitro translation in a rabbit reticulocyte lysate of RNA generated from the steroid 5α-reductase cDNA yielded a protein product with an apparent M, of 26,000 (lane 3). When the translation reactions were carried out in the presence of dog pancreas microsomes, a protein product of identical size was observed (lane 4), suggesting the absence of a cleavable signal sequence in this protein. That the steroid 5α-reductase translated in vitro was incorporated into microsomes was demonstrated by protease protection experiments. If the vesicular structure of the microsomes was maintained, the translated product was largely resistant to digestion by trypsin (lane 5). However, if the microsomes were disrupted with the detergent Triton X-100 prior to protease treatment, then the steroid 5α-reductase protein was susceptible to digestion (lane 6).

We next determined the approximate location of the carboxyl terminus of the protein by analyzing the expression of

![Fig. 4. Nucleotide sequence of the cDNA corresponding to the rat steroid 5α-reductase mRNA, predicted amino acid sequence, and hydropathy profile of the protein. A, nucleotides are numbered on the right-hand side. The amino acids are numbered above the sequence with position 1 arbitrarily assigned to the first methionine codon in the nucleotide sequence. Two polyadenylation signals are overlined. B, the sequence of the steroid 5α-reductase protein was subjected to a hydropathy analysis using the algorithm of Kyte and Doolittle (43). Sequences above the central dividing line are hydrophilic, and those below the line are hydrophobic.](image-url)
RNA derived from a series of 3'-truncated derivatives of the cDNA. The steroid 5α-reductase cDNA plasmid was linearized by cleavage with four restriction enzymes that left intact or removed progressively larger portions of the predicted 3'-untranslated region and/or carboxyl terminus of the protein. RNA was transcribed in vitro from these templates, microinjected into oocytes, and the oocytes were assayed for steroid 5α-reductase activity using testosterone as a substrate. As summarized in Fig. 6A, expression of the intact steroid 5α-reductase mRNA is indicated on the right of the autoradiogram.

The amino-terminal region of steroid 5α-reductase was examined by carrying out primer extension experiments on liver mRNA. An oligonucleotide primer 40 bases in length was radiolabeled, annealed to mRNA from female and male rat liver, and extended with reverse transcriptase. As shown in Fig. 6B, a single product of 125 nucleotides was detected when RNA from female or male liver was used as a template. These results are consistent with a single 5' end for the steroid 5α-reductase mRNA in this tissue and suggest that the cDNA sequence shown in Fig. 4A represents a near full length clone. In additional experiments not shown, the steroid 5α-reductase mRNA in female rat liver was sequenced directly using the above primer. The results indicated that the mRNA extends only 17 nucleotides upstream of the 5' end of the cDNA sequence shown in Fig. 4A. There were no inframe translation stop codons in this 5' sequence.

**The Liver and Ventral Prostate Forms of Steroid 5α-Reductase Are Identical**—To determine if the steroid 5α-reductase activities in the liver and prostate were due to the expression of a single mRNA, we screened a randomly primed cDNA library derived from ventral prostate mRNA with the insert derived from the liver cDNA clone. A single prostate cDNA clone indicated that it began at nucleotide 1 and terminated at nucleotide 1955 of the liver cDNA sequence shown in Fig. 4A. The sequences were identical between the two clones in these regions. The complete coding region of the prostate-derived cDNA was further subjected to DNA sequence analysis and compared to that of the liver cDNA again revealed no differences. These results suggested that the enzyme activities in these two tissues were the consequence of expression of the same mRNA. It remained possible, however, that the liver form of steroid 5α-reductase was also expressed in the prostate and that we had in fact isolated a second cDNA encoding the liver enzyme.

We next carried out a series of blot hybridization experiments (Fig. 7). RNA was isolated from female and male rat liver and prostate, electrophoresed on an agarose gel, transferred to a nylon membrane, and probed with the full length liver cDNA insert. An identically sized mRNA of approximately 2.4 kb was detected in all three tissues (Fig. 7A). The relative abundance of this mRNA (female liver > male liver > prostate) closely paralleled steroid 5α-reductase enzyme activity in these three tissues (16). In agreement with the identical-sized mRNAs detected in these two tissues, primer extension analysis of prostate mRNA indicated that the 5' end of the steroid 5α-reductase mRNA in this tissue was identical to that in the liver (data not shown).

Hybridization of the steroid 5α-reductase cDNA to rat
genomic DNA provided additional evidence that the liver and prostate forms of this enzyme are encoded by the same gene. As shown in Fig. 7B, a small number of hybridizing bands were detected after digestion of genomic DNA with four different restriction enzymes. In some lanes (e.g. BamHI-digested DNA) there were differences in the intensities of the hybridization signals. We believe this result is due to the presence of a larger or smaller number of exons on a given DNA fragment rather than to the existence of multiple steroid 5α-reductase genes. This conclusion is substantiated by the results obtained with the enzyme EcoRI that cleaves the cDNA roughly in half (nucleotide position 1334, Fig. 4A), and thus would be expected to, and does, generate at least two hybridizing bands of equal intensity on the autoradiogram (Fig. 7B).

Regulation of Steroid 5α-Reductase Expression in the Ventral Prostate—The expression of steroid 5α-reductase enzyme activity in the liver and prostate has been shown to be under hormonal control (5, 38, 39). To examine the possibility that this regulation might be exerted at the transcriptional level, and to provide further physiological evidence that our cDNA encoded steroid 5α-reductase, we determined mRNA levels in the liver and prostate after administration of androgens to castrated animals (Fig. 8). In the liver of untreated animals, the steroid 5α-reductase cDNA probe hybridized to an mRNA of 2.4 kb (lane 1). Subcutaneous administration of testosterone to normal animals did not affect the expression of this mRNA (lane 5). In contrast, the levels of steroid 5α-reductase mRNA in the liver increased approximately 5-fold in the 7-day (lane 2) and 10-day (lane 4) castrated animals. In this experiment, testosterone did not decrease the enhanced expression of the steroid 5α-reductase mRNA in the castrated animals (lane 3), suggesting that this hormone may work in concert with other regulatory influences to control expression of steroid 5α-reductase in the liver.

In the ventral prostate of normal animals, a much lower level of an identically sized mRNA was detected (Fig. 8, lane 6). Pharmacological levels of testosterone did not increase this level of expression (lane 10), nor did castration for a 7-day or 10-day period (lanes 7 and 9, respectively). However, a dramatic 9-fold increase in steroid 5α-reductase mRNA levels was detected in castrated animals given testosterone for a 3-day period (lane 8). Subsequent hybridization of this filter with actin and cyclophilin cDNA probes indicated that near identical amounts of RNA were electrophoresed in each lane (data not shown). The results obtained with the prostate RNA provide strong evidence that the cDNA we have isolated does in fact encode steroid 5α-reductase.

Conclusions—The data presented here provide evidence that a single mRNA encodes the enzyme steroid 5α-reductase in both the liver and prostate of the rat. DNA sequence analysis indicates that this mRNA encodes a hydrophobic protein with a M, of 29,000. This size was confirmed by in vitro translation, mRNA truncation experiments, and primer extension analysis of liver mRNA. Blot hybridization analyses of RNA and genomic DNA support the existence of a single mRNA and gene for steroid 5α-reductase. Experiments in a castrated animal model suggest that the expression of steroid 5α-reductase may be under transcriptional control in both the liver and ventral prostate. Additional experiments are currently in progress to determine the mechanism of the apparent differential regulation by testosterone in these two tissues.

The contributions of steroid 5α-reductase and its product dihydrotestosterone to male sexual development are clearly illustrated by the clinical syndrome of pseudovaginal perineoscrotal hypospadias (14). In addition to this developmental role, dihydrotestosterone in the mature organism is involved in the normal maintenance of many different cellular and organ processes. In fact, the suggestion has been made that it is dihydrotestosterone and not testosterone that is the more...
Expression of steroid 5α-reductase and subsequent dihydrotestosterone synthesis may contribute to a large number of human diseases and endocrine abnormalities. Localized overproduction of dihydrotestosterone in the prostate is postulated to be a factor in benign prostate hypertrophy, a condition that affects a majority of elderly men (40). Similarly, dihydrotestosterone has been implicated in the formation of acne and in the manifestation of male pattern baldness (41). Finally, a role for this hormone in the development and or susceptibility to cancer of the prostate, the second most prevalent form of cancer in the United States, has been hypothesized (42). The precise contribution of steroid 5α-reductase to these disease states has so far remained uncertain due to the absence of biochemical and genetic tools. The results presented here clarify many controversies that have existed in the literature for years concerning this enzyme and they may provide some necessary tools.

Acknowledgments—We thank Gloria Brunschede, Daphne Davis, and Daphne Norsworthy for excellent technical assistance, David Julius for helpful advice, J. Ian Mason for steroids and advice, Jim Metherall for microsomes, Richard Gibson for veterinary assistance, and Mike Brown, Joe Goldstein, and Jean Wilson for advice and critical reading of the manuscript.

REFERENCES

1. Wilson, J. D. (1978) Annu. Rev. Physiol. 40, 279-306
2. Wilson, J. D. (1975) Handb. Physiol. 5, 491-508
3. Walsh, P. C., Madden, J. D., Harrod, M. J., Goldstein, J. L., MacDonald, P. C., and Wilson, J. D. (1974) Nature 248, 944-949
4. Imperato-McGinley, J., Guerrero, L., and Peterson, T. (1978) Science 205, 949-951
5. Moore, R. J., and Wilson, J. D. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 1991-1995
6. Jost, A. (1960) Harvey Lect. 55, 201-226
7. Page, D. C., Mosher, R., Simpson, E. M., Fisher, E. M. C., Mardon, G., Pollack, J., McGillivray, B., de la Chapelle, A., and Brown, L. G. (1987) Cell 51, 1091-1104
8. Evans, R. M., and Hollenberg, S. M. (1988) Cell 52, 1-3
9. Cate, R. L.,Mattaliano, R. J., Hess, C., Tizard, R., Farber, N. M., Cheung, A., Ninfa, E. G., Frey, A. Z., Gash, D. J., Chow, E. P., Fisher, R. A., Bertonis, J. M., Torres, G., Wallner, B. P., Ramachandran, K. L., Rabin, R. C., Manganaro, T. F., MacLaughlin, D. R., and Donahoe, P. K. (1986) Cell 45, 685-698
10. Chang, C., Kokontis, J., and Liao, S. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7211-7215
11. Lubahn, D. B., Joseph, D. R., Sar, M., Tan, J., Higgs, H. N., Larson, R. E., French, F. S., and Wilson, E. M. (1988) Mol Endocrinol. 2, 1265-1275
12. Tilley, W. D., Marcelli, M., Wilson, J. D., and McPhaul, M. J. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 327-331
13. Evans, R. M. (1988) Science 240, 889-895
14. Griffin, J. E., and Wilson, J. D. (1989) in The Metabolic Basis of Inherited Disease (Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) pp. 1919-1944, McGraw-Hill, New York
15. Wilbert, D. M., Griffin, J. E., and Wilson, J. D. (1983) J. Clin. Endocrinol. Metab 56, 113-120
16. Moore, R. J., and Wilson, J. D. (1972) J. Biol. Chem. 247, 958-967
17. McGuire, J. S., Jr., and Tomkins, G. M. (1960) J. Biol. Chem. 235, 1634-1635
18. Ichihara, K., and Tanaka, C. (1987) Biochem. Int. 15, 1005-1011
19. Liang, T., Heiss, C. E., Ostrove, S., Rasmussen, G. H., and Cheung, A. (1983) Endocrinology 112, 1460-1468
20. McGuire, J. S., Jr., Hollis, V. W., Jr., and Tomkins, G. M. (1960) J. Biol. Chem. 235, 3112-3117
21. Nomiy, T., Sideras, P., Naito, T., Bergstedt-Lindquist, S., Azuma, C., Severinson, E., Tanabe, T., Kinashi, T., Matsuda, F., Yachida, Y., and Horjo, T. (1986) Nature 321, 640-646
22. Mae, Y., Nakayama, K., Tamaki, H., Harada, Y., Kuno, M., and Nakajima, S. (1987) Nature 329, 836-838
23. Lubbert, H., Hoffmann, B. J., Snutch, T. P., Van Dyke, T., Levine, A. J., Hartig, F. R., Lester, H. A., and Davidson, N. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 4392-4396
24. Julius, D., Mac Dermott, A. B., Axel, R., and Jessell, T. M. (1988) Science 241, 558-564
25. Hediger, M. A., Coady, M. J., Ikeda, T. S., and Wright, E. M. (1987) Nature 330, 378-381
26. Peacock, S. L., Bates, M. P., Russell, D. W., Brown, M. S., and Goldstein, J. L. (1988) J. Biol. Chem. 263, 7383-7385
27. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, pp. 1-454, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
28. Schweinfest, C. W., Kwiatkowski, R. W., and Dottin, R. P. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 4977-5000
29. Smith, L. M., Sanders, J. Z., Kaiser, R. J., Hughes, P., Dodd, C., Connell, C. W., Heiser, C., Kent, S. B. H., and Hood, L. E. (1986) Nature 321, 674-679
30. Sudhof, T. C., Russell, D. W., Brown, M. S., and Goldstein, J. L. (1987) Cell 48, 1061-1069
31. Geliebter, J., Zeff, R. A., Melvold, R. W., and Nathanson, S. G. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 3371-3375
32. Walter, P., Ibrahim, I., and Blobel, G. (1981) J. Cell Biol. 91, 545-550
33. Farkash, Y., Soreq, H., and Orly, J. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 5824-5828
34. Miller, W. L. (1988) Endocr. Rev. 9, 295-318
35. Kozak, M. (1986) Cell 44, 283-292
36. Liang, T., Cheung, A. H., Reynolds, G. F., and Rasmussen, G. H. (1985) J. Biol. Chem. 260, 4890-4896
37. Cheng, K.-C. (1988) FASEB J. 2, 356 (abstr.)
38. McGuire, J. S., Jr., and Tomkins, G. M. (1959) J. Biol. Chem. 234, 791-794
39. Gustafsson, J. Å., Mode, A., Norstedt, G., and Skett, P. (1983) Annu. Rev. Physiol. 45, 51-60
40. Cunha, G. R., Donjacour, A. A., Cooke, P. S., Mee, S., Bigby, R. M., Higgins, S. J., and Sugimura, Y. (1987) Endocr. Rev. 8, 398-362
41. Mooradian, A. D., Morley, J. E., and Korenman, S. G. (1987) Endocr. Rev. 8, 1-28
42. Lippman, M. E. (1981) in William’s Textbook of Endocrinology (Wilson, J. D., and Foster, D. W., eds) pp. 1309-1326, 7th Ed., W. B. Saunders Company, Philadelphia
43. Kyle, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105-132
44. Lehrman, M. A., Russell, D. W., Goldstein, J. L., and Brown, M. S. (1987) J. Biol. Chem. 262, 3354-3361
45. Lehrman, M. A., Schneider, W. J., Sudo, T. C., Brown, M. S., Goldstein, J. L., and Russell, D. W. (1985) Science 227, 140-145
46. Church, G. M., and Gilbert, W. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 1991-1995