

Tissue distribution and ontogeny of steroid 5 alpha-reductase isozyme expression.

A E Thigpen, ... , J D McConnell, D W Russell

J Clin Invest. 1993;**92**(2):903-910. <https://doi.org/10.1172/JCI116665>.

Research Article

The synthesis of dihydrotestosterone is catalyzed by steroid 5 alpha-reductase isozymes, designated types 1 and 2. Mutation of type 2 results in male pseudohermaphroditism, in which the external genitalia are phenotypically female at birth. Two striking and unexplained features of this disorder are that external genitalia of affected males undergo virilization during puberty and that these individuals have less temporal hair regression. The tissue-specific and developmental expression patterns of the 5 alpha-reductase isozymes were investigated by immunoblotting. The type 1 isozyme is not detectable in the fetus, is transiently expressed in newborn skin and scalp, and permanently expressed in skin from the time of puberty. There was no qualitative difference in 5 alpha-reductase type 1 expression between adult balding vs. nonbalding scalp. The type 2 isozyme is transiently expressed in skin and scalp of newborns. Type 2 is the predominant isozyme detectable in fetal genital skin, male accessory sex glands, and in the prostate, including benign prostatic hyperplasia and prostate adenocarcinoma tissues. Both isozymes are expressed in the liver, but only after birth. These results are consistent with 5 alpha-reductase type 1 being responsible for virilization in type 2-deficient subjects during puberty, and suggest that the type 2 isozyme may be an initiating factor in development of male pattern baldness.

Find the latest version:

<https://jci.me/116665/pdf>



Tissue Distribution and Ontogeny of Steroid 5 α -Reductase Isozyme Expression

Anice E. Thigpen,* Richard I. Silver,† Joseph M. Guileyardo,§ M. Linette Casey,|| John D. McConnell,‡ and David W. Russell*
Departments of *Molecular Genetics, †Urology, §Pathology, and ||Biochemistry,
University of Texas Southwestern Medical Center, Dallas, Texas 75235

Abstract

The synthesis of dihydrotestosterone is catalyzed by steroid 5 α -reductase isozymes, designated types 1 and 2. Mutation of type 2 results in male pseudohermaphroditism, in which the external genitalia are phenotypically female at birth. Two striking and unexplained features of this disorder are that external genitalia of affected males undergo virilization during puberty and that these individuals have less temporal hair regression. The tissue-specific and developmental expression patterns of the 5 α -reductase isozymes were investigated by immunoblotting. The type 1 isozyme is not detectable in the fetus, is transiently expressed in newborn skin and scalp, and permanently expressed in skin from the time of puberty. There was no qualitative difference in 5 α -reductase type 1 expression between adult balding vs. nonbalding scalp. The type 2 isozyme is transiently expressed in skin and scalp of newborns. Type 2 is the predominant isozyme detectable in fetal genital skin, male accessory sex glands, and in the prostate, including benign prostatic hyperplasia and prostate adenocarcinoma tissues. Both isozymes are expressed in the liver, but only after birth. These results are consistent with 5 α -reductase type 1 being responsible for virilization in type 2-deficient subjects during puberty, and suggest that the type 2 isozyme may be an initiating factor in development of male pattern baldness. (*J. Clin. Invest.* 1993. 92:903–910.) Key words: dihydrotestosterone • sexual differentiation • benign prostatic hyperplasia • prostate cancer • male pattern baldness

Introduction

Alterations in the conversion of testosterone into dihydrotestosterone by the enzyme steroid 5 α -reductase (5 α -reductase, E.C. 1.3.99.5) are associated with a number of human disorders. Decreased synthesis of dihydrotestosterone due to mutations in the 5 α -reductase gene results in affected 46,XY males being born with normal internal reproductive structures (epididymes, seminal vesicles, and vasa deferentia), but with external genitalia that resemble those of the female and a prostate that is hypoplastic (1–3). Strikingly, and for unknown reasons, virilization of the external genitalia occurs to varying extents in affected individuals during puberty (3, 4), although

secondary sexual hair remains sparse and they develop less male pattern baldness and acne (25).

The reduced temporal hair regression in 5 α -reductase-deficient individuals suggests that the synthesis of dihydrotestosterone may contribute to this frequent and characteristic male affliction (6). In support of this notion, earlier studies show that 5 α -reductase enzyme activity is increased in hair follicles of balding scalp but not in nonbalding scalp (7, 8). Interestingly, 5 α -reductase enzyme activity is normal in hair follicles of subjects with 5 α -reductase deficiency (9).

The recent isolation of two functional genes encoding different isozymes of 5 α -reductase in the human and rat provides an explanation for why these subjects have normal levels of enzyme activity in their hair follicles (10–13). The 5 α -reductase type 1 gene (*SRD5A1*) encodes an isozyme with an alkaline pH optimum (14, 15), whereas the 5 α -reductase type 2 gene (*SRD5A2*) encodes an isozyme with an acidic pH optimum (12, 16). The two 5 α -reductase isozymes can also be distinguished by their 4-azasteroid inhibitor pharmacologies (12, 17). Little is known about the relative tissue distribution and developmental expression patterns of the two 5 α -reductase isozymes. No genetic deficiencies of the type 1 enzyme have yet been reported. Mutations in the type 2 gene underlie classical 5 α -reductase deficiency (12, 16, 18), and the type 1 gene is normal in these subjects (14).

An increased synthesis of dihydrotestosterone may contribute to the development of benign and neoplastic growth of the prostate in aging males. In benign prostatic hyperplasia (BPH),¹ an increase in the organ content of dihydrotestosterone has been noted in some (19) but not all studies (20). Administration of a selective inhibitor of 5 α -reductase type 2 (the 4-azasteroid finasteride) has been shown to result in a decrease in prostate volume in some men with BPH (21, 22). The level of 5 α -reductase enzyme activity has been reported to be higher in prostate tumors in some (23), but not all studies (24, 25).

To gain insight into the physiological roles of each of the two 5 α -reductase isozymes in normal and pathological states, we investigated the tissue distribution and relative expression of each isozyme during human development.

Methods

Antibodies. Polyclonal anti-peptide antibodies were prepared following standard protocols in New Zealand White rabbits (26). A peptide representing amino acids 232 to 256 of the 5 α -reductase type 1 isozyme (11) was synthesized and coupled to keyhole limpet hemocyanin with *m*-maleimidobenzoic acid *N*-hydroxysuccinimide ester. A peptide representing amino acids 227 to 251 of the type 2 isozyme (12) was used as an antigen directly without coupling. Rabbits were injected subdermally with the conjugate or the free peptide and Freund's complete adjuvant as described (27). Antisera were collected after injection and

Address correspondence and requests for reprints to Dr. David W. Russell, Department of Molecular Genetics, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75235.

Received for publication 10 November 1992 and in revised form 18 March 1993.

J. Clin. Invest.

© The American Society for Clinical Investigation, Inc.

0021-9738/93/08/0903/08 \$2.00

Volume 92, August 1993, 903–910

1. Abbreviations used in this paper: BPH, benign prostatic hyperplasia; CHO, Chinese hamster ovary.

tested by immunoblotting for their ability to recognize the 5α -reductase isozymes in cell lysates derived from permanent Chinese hamster ovary (CHO) cell lines expressing individual 5α -reductase cDNAs (CHO-1827, type 1 cDNA; CHO-1829, type 2 cDNA). Affinity purification of the antipeptide sera was done according to standard procedures (26).

Tissues. Samples were collected on ice into 5 mM Na_2HPO_4 , 2 mM KH_2PO_4 , pH 7.2, 140 mM NaCl, 3 mM KCl (PBS), less than 12 h postmortem, minced, quick-frozen in liquid nitrogen, and powdered on dry ice using a mortar and pestle. Tissue powder was homogenized with a polytron (Brinkmann Instruments, Inc. Westbury, NY) in 5 vols of 10 mM potassium phosphate, pH 7.0, 150 mM KCl, 1 mM EDTA, and 2 mM phenylmethylsulfonyl fluoride. Protein concentrations were measured (28) using gamma globulin as a standard. 5α -Reductase enzyme activity and steady state mRNA levels were measured as described previously (13, 14). BPH and prostate cancer specimens were obtained at the time of surgery with consent and Institutional Review Board approval and subjected to standard tissue preparation for routine pathologic study. For prostate cancer specimens obtained after radical prostatectomy, the Gleason's grade (29) of biopsy tissue and clinical stage were determined preoperatively and the final Gleason's grade and pathologic stage were determined postoperatively. The age (in years), race and type of baldness (according to the classification system of Hamilton [6]) of the male subjects analyzed in Fig. 9 were: AT16, 62, Caucasian, type II; AT17, 57, African-American, type I; AT6, 29, Caucasian, type I; AT14, 48, Caucasian, type II; AT18, 21, Caucasian, type II; AT2, 58, Caucasian, type VIII; AT8, 36, African-American, type VII; AT5, 39, Caucasian, type VII; AT9, 39, Caucasian, type IV; and AT15, 35, Caucasian, type VII. All scalp samples were collected at autopsy.

Immunoblotting. Aliquots (150 μg) of tissue lysates were electrophoresed on 7 to 15% (wt/vol) linear gradient polyacrylamide gels containing 0.1% (wt/vol) SDS, and transferred to Immobilon-P membranes by electroblotting at 400 mA for 30 min at 22°C in 25 mM Tris, 192 mM Gly, 20% (vol/vol) methanol, pH 8.4. Filters were incubated for 16 h at 4°C in 25 mM Tris, pH 7.5, 14 mM NaCl, 3 mM KCl (TBS) containing 10% (wt/vol) powdered milk and 0.2% (vol/vol) Tween-20. 5α -Reductase antisera were diluted 1 in 1,000 in TBS with powdered milk, Tween-20, and 0.5% (vol/vol) NP-40 and incubated with the filters at 22°C for 2–3 h. The filters were washed three times for 10 min in TBS containing 0.1% (wt/vol) SDS, 1% (vol/vol) NP-40, and 0.5% (wt/vol) deoxycholate prior to addition of a 1 in 2,000 dilution of a donkey anti-rabbit IgG serum coupled to horseradish peroxidase (Amersham Corp., Arlington Heights, IL). The second antibody was incubated with the filter for 20 min followed by three washes in the above buffer. Immune complexes were visualized by incubation with substrates for chemiluminescence detection (Amersham Corp.) for 1 min. Filters were exposed to x-ray film for 2 to 8 min.

Results

Antipeptide antibodies directed against the carboxy-termini of the type 1 and type 2 isozymes of 5α -reductase were generated in rabbits. Lysates prepared from CHO cell lines expressing either the type 1 or type 2 cDNAs were used to establish the optimum conditions for isozyme detection by immunoblotting. The anti-type 1 sera specifically recognized the type 1 isozyme, whereas the anti-type 2 sera recognized the type 2 isozyme and cross-reacted weakly in some but not all experiments with the type 1 isozyme (see below). The anti-type 1 sera could detect a level of enzyme equivalent to a specific activity of 0.8 pmol dihydrotestosterone $\text{min}^{-1} \text{mg}^{-1}$ cell lysate protein. The anti-type 2 sera was more sensitive and could detect a level of enzyme equivalent to a specific activity of 0.2 pmol dihydrotestosterone $\text{min}^{-1} \text{mg}^{-1}$ cell lysate protein. Dilution studies revealed that a twofold difference in the amount of

a particular 5α -reductase isozyme in a sample was visually detectable.

The distribution of the two isozymes in human tissues was initially determined by immunoblotting using the 5α -reductase polyclonal antisera. Preliminary studies were carried out with unpurified antisera and revealed a number of protein bands in addition to the 5α -reductase isozymes. To reduce background, antibodies that recognize the 5α -reductase isozymes were enriched by purification on peptide antigen-Sepharose columns. The results of a typical immunoblotting experiment using affinity-purified antisera and in which cell lysates prepared from nine tissues and five regions of the brain of an adult male (48-yr-old Caucasian) were analyzed are shown in Figs. 1 and 2.

As indicated by the asterisks in Fig. 1A, the type 1 isozyme was detected in the liver and chest skin. Several tissues contained proteins distinct from 5α -reductase that were recognized by the polyclonal antibodies (e.g., kidney, Fig. 1A). With the exception of the pituitary gland, the molecular weights of these other proteins were substantially larger than that of the type 1 enzyme ($M_r = 23,000$). The inclusion of antigenic peptide (7 $\mu\text{g}/\text{ml}$) in the immunoblotting reaction abolished recognition of only the type 1 isozyme (Fig. 1B). The recognition of the pituitary protein with a molecular weight slightly smaller than that of 5α -reductase type 1 was not affected by inclusion of peptide (Fig. 1B).

The 5α -reductase type 2 isozyme was detected in the epididymis, seminal vesicle, prostate, and liver (Fig. 2A, asterisks).

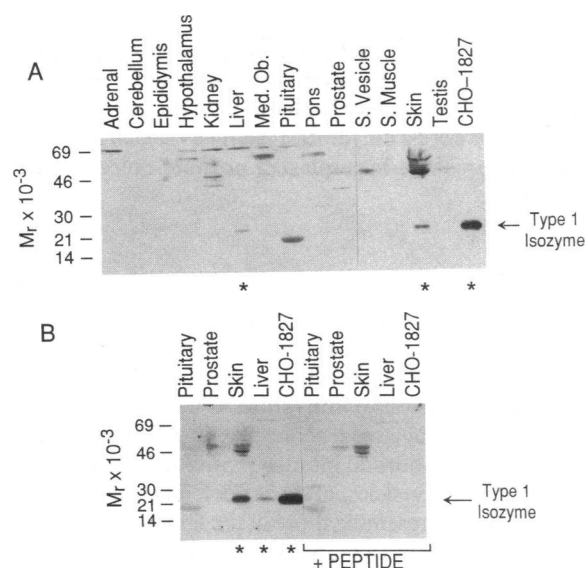


Figure 1. Expression of 5α -reductase type 1 isozyme in adult tissues. (A) Extracts were prepared from the indicated tissues (S. Muscle, skeletal muscle; S. Vesicle, seminal vesicle; Skin, chest skin; Med. Ob., medulla oblongata) and 150- μg aliquots were subjected to immunoblotting using affinity-purified anti-type 1 serum. Molecular weight standards are indicated on the left side of the autoradiograms. Tissues containing detectable 5α -reductase type 1 are indicated with an asterisk. The lane marked "CHO-1827" is a positive control and contained lysate (5 μg) from a CHO cell line transfected with the type 1 cDNA. Exposure time was 2 min. (B) 150- μg aliquots of the indicated tissues were immunoblotted with the type 1 antibody in the absence (left five lanes) or presence (right five lanes labeled "+ Peptide") of the antigenic peptide (7 $\mu\text{g}/\text{ml}$). Only recognition of the type 1 isozyme is abolished in the presence of antigenic peptide.

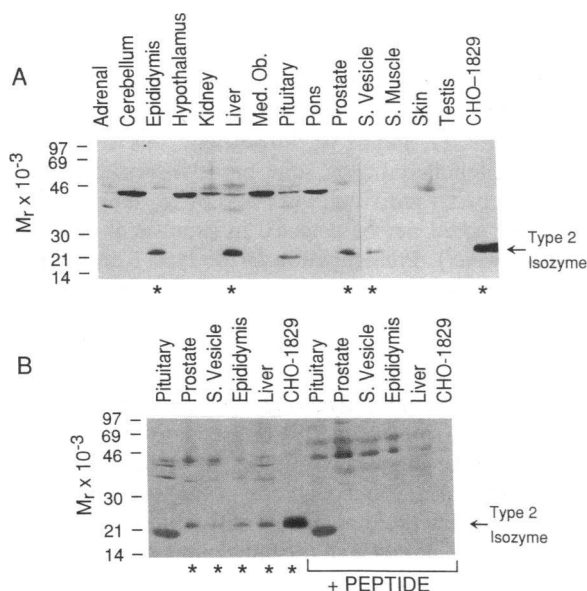


Figure 2. Expression of 5 α -reductase type 2 isozyme in adult tissues. (A) Extracts were prepared from the indicated tissues (S. Muscle, skeletal muscle; S. Vesicle, seminal vesicle; Skin, chest skin; Med. Ob., medulla oblongata) and 150- μ g aliquots were subjected to immunoblotting with affinity-purified anti-type 2 serum. Molecular weight standards are indicated on the left side of the autoradiograms. Tissues containing detectable 5 α -reductase type 2 are indicated with an asterisk. The lane marked CHO-1829 is a positive control and contained lysate (5 μ g) from a CHO cell line transfected with the type 2 cDNA. Exposure time was 2 min. (B) 150- μ g aliquots of the indicated tissues were immunoblotted with the type 2 antiserum in the absence (left six lanes) or presence (right six lanes labeled "+ Peptide") of the antigenic peptide (7 μ g/ml). Only recognition of the type 2 isozyme by the antiserum is abolished by inclusion of antigenic peptide in the immunoblotting reaction.

As with the type 1 antibodies, several high molecular weight proteins were detected in the different tissues, but the recognition of these proteins was not abolished by inclusion of the antigenic peptide (7 μ g/ml), whereas recognition of the type 2 isozyme was (Fig. 2 B). The results shown in Figs. 1 and 2 are representative of those obtained in surveys of tissues derived from five individuals.

To confirm and extend the results obtained in the immunoblotting experiments, the levels of 5 α -reductase isozyme mRNA (Fig. 3) and activity (Table I) were determined in these tissues. Type 1 mRNAs of 7.5 and 2.4 kilobases (kb) were detected in the cerebellum, hypothalamus, medulla oblongata, pons, and liver (Fig. 3 A). No type 1 mRNA was detected in the kidney (Fig. 3 A). Hybridization of the filter with a control cyclophilin cDNA probe revealed the presence of mRNA in all lanes of the blot (Fig. 3 A, lower autoradiogram).

Hybridization with 5 α -reductase type 2 cDNA probes revealed mRNAs of 2.4 and 4.4 kb in the epididymis, liver, prostate, and seminal vesicles (Fig. 3 B). A cyclophilin mRNA of 0.8 kb was detected in all tissues (Fig. 3 B, lower autoradiogram). In experiments not shown, no type 2 mRNA was detected in the medulla oblongata or pituitary. The overall RNA blotting results are representative of six different experiments.

5 α -reductase enzyme activity in tissue homogenates was determined at pH 7.0 (type 1 isozyme optimum) and at pH 5.0 (type 2 isozyme optimum). As indicated in Table I, very low to

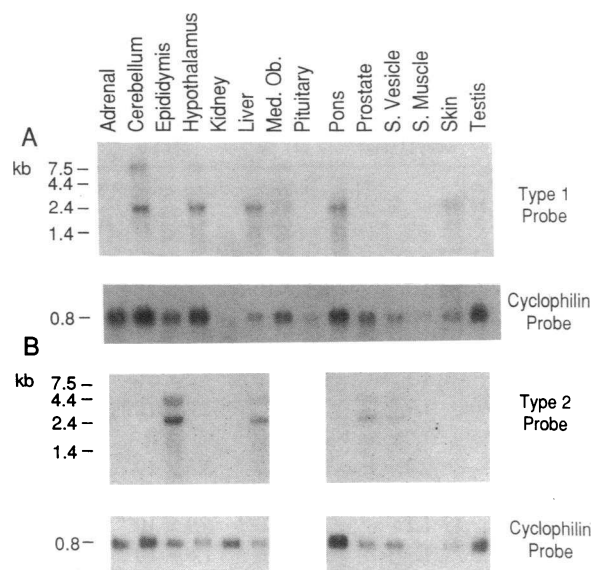


Figure 3. Detection of 5 α -reductase mRNAs in adult tissues by blot hybridization. 30- μ g aliquots of total RNA from the indicated tissues (see Fig. 2 for key) were separated electrophoretically, transferred to a nylon filter, and hybridized with cDNA probes derived from the indicated 5 α -reductase isozyme (A, Type 1; B, Type 2) as described previously (13). After washing and exposure to x-ray film (A, 96 h; B, 96 h) the filters were stripped of hybridized probe and rehybridized with a control probe derived from the ubiquitously expressed cyclophilin gene. After washing, the filters were exposed to x-ray film for 72 h. The positions to which RNAs of known size (in kilobases) migrated to are indicated on the left of the 5 α -reductase mRNA autoradiograms. The size of the cyclophilin mRNA was calculated by interpolation.

moderate enzyme activity was detected at pH 7.0 in all tissues except skeletal muscle and testis. Enzyme activity was detected at pH 5.0 in all tissues except skeletal muscle, adrenal, hypothalamus, and pons. Because the pH activity profiles of the two isozymes overlap considerably (12), the relative contributions of the two isozymes to these activities were estimated by calculating the ratio of pH 7.0/pH 5.0 activity (Table I). Tissues with a pH 7.0/pH 5.0 ratio of less than 1.0, such as the prostate, seminal vesicle, epididymis, and medulla oblongata appear to express predominantly 5 α -reductase type 2 (pH 5.0 optimum), whereas tissues that have a ratio greater than 1.0 express more type 1 isozyme (pH 7.0 optimum). A striking finding demonstrated by these enzyme activity measurements is the lack of correlation between the steady state mRNA levels encoding the type 1 isozyme and the level of enzyme activity. For example, although the level of type 1 mRNA in the hypothalamus is higher than that in skin (Fig. 3 A), the skin contains 1,400 times more enzyme activity at pH 7.0 than the hypothalamus (Table I). This result is confirmed by the immunoblotting results. In contrast, the levels of type 2 mRNA and pH 5.0 enzyme activity correlate quite well.

In addition to the tissue distribution studies, the ontogeny of 5 α -reductase isozyme expression was examined by immunoblotting. A preliminary survey of seven fetal tissues (liver, adrenal, testis, ovary, brain, scalp, chest skin, and genital skin) derived from males and females of gestational ages of 12.8, 14.7, 17.2, 18, and 19 wk, revealed the presence of only the type 2 isozyme in genital skin (data not shown).

Table 1. Measurement of 5 α -Reductase Enzyme Activity in Human Tissues

Tissue/cell line	Protein assayed (mg) [‡]	<i>V</i> _{max}		Ratio pH 7.0/5.0 activity
		pH 7.0	pH 5.0	
(nmol/min/mg/protein)*				
Skeletal muscle	1.1	ND [§]	ND [§]	—
Adrenal	0.81	6.4 × 10 ⁻⁵	ND	+∞
Kidney	1.7	2.3 × 10 ⁻⁴	1 × 10 ⁻⁵	2.3
Prostate	1.5	7.3 × 10 ⁻⁴	1.6 × 10 ⁻²	0.05
Seminal vesicle	1.1	3.7 × 10 ⁻⁴	1.4 × 10 ⁻²	0.03
Epididymis	1.1	1.4 × 10 ⁻³	7.2 × 10 ⁻³	0.2
Testis	1.1	ND [§]	2.2 × 10 ⁻⁵	-∞
Hypothalamus	1.06	2.0 × 10 ⁻⁵	ND [§]	+∞
Skin	0.46	2.8 × 10 ⁻²	3.3 × 10 ⁻³	8.5
Pons	1.0	2.2 × 10 ⁻⁴	ND [§]	+∞
Medulla oblongata	1.1	4 × 10 ⁻⁵	6.9 × 10 ⁻⁴	0.06
Cerebellum	1.3	1.5 × 10 ⁻⁴	1.6 × 10 ⁻⁴	0.9
Liver	1.0	—	—	—
CHO-1827 [†]	0.005	4.9	5.9 × 10 ⁻¹	8.3
CHO-1829 [‡]	0.005	2.5 × 10 ⁻¹	4.2	0.06

* Values are the averages from two different experiments. Assay conditions were: [¹⁴C-labeled testosterone] = 10 μ M, [NADPH] = 5 mM, 60 min incubation at 37°C. [‡] Protein concentrations were determined as described in Methods. [§] ND, Not detected. ^{||} 5 α -Reductase enzyme activity could not be measured accurately in liver extracts due to substrate metabolism by other steroid-modifying enzymes. [†] CHO-1827 cells express a transfected type 1 cDNA. CHO-1829 cells express a transfected type 2 cDNA.

The expression patterns of the two 5 α -reductase isozymes in the tissues of newborns were surveyed next. As shown in the upper panel of Fig. 4, the type 1 isozyme was detected in the liver, and in the skin and scalp of newborns. The type 2 isozyme was detected in the liver, prostate, seminal vesicle, and epididymis, and was also present in the skin and scalp (Fig. 4, lower panel). The recognition of both isozymes by their respective antisera was abolished by inclusion of antigenic peptide in the immunoblotting reactions (Fig. 4, panels labeled “+ Peptide”).

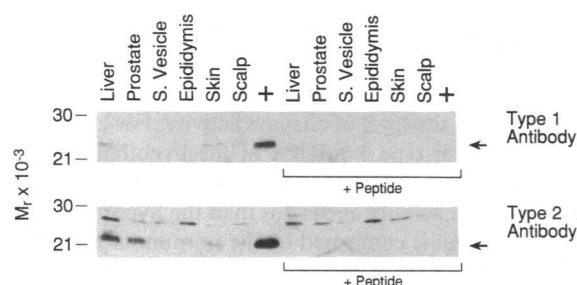


Figure 4. Expression of 5 α -reductase isozymes in newborn tissues. 150- μ g aliquots of cell lysates prepared from the indicated tissues of a 5-mo-old African-American male were immunoblotted as described in Methods with a 5 α -reductase type 1 antiserum (upper panel) or a type 2 antiserum (lower panel). Lanes labeled “+ Peptide” were immunoblotted in the presence of the antigenic peptides (7 μ g/ml). Molecular weight markers are indicated on the left. Exposure time was 3 min.

Tissue extracts from the liver, skin, and prostate of adolescents and adults were next prepared and subjected to immunoblotting. The data in the upper panel of Fig. 5 indicate that 5 α -reductase type 1 is absent from fetal liver but is detectable in this organ at age 7, 21, and 62 yr. Surprisingly, this isozyme is not present in prepubertal chest skin, but is expressed in this organ in postpubertal individuals (21 and 62 yr of age). The type 1 isozyme could not be detected in prostate at any age (Fig. 5, upper panel). Preincubation of the type 1 antiserum with the peptide antigen completely abolished recognition of this isozyme in liver, skin, and transfected CHO cell lysates (Fig. 5, upper panel labeled “+ Peptide”).

The type 2 isozyme showed a similar expression pattern in the liver, being absent in the fetal organ but present at ages 7, 21, and 62 yr (Fig. 5, lower panel). This isozyme could not be detected in any of the chest skin samples, but was present in all of the prostate samples. The recognition of the type 2 isozyme (but not cross-reacting proteins) by the antiserum was blocked by incubation in the presence of the antigenic peptide (Fig. 5, lower panel lanes labeled “+ Peptide”).

The qualitative data of Fig. 6 summarize the developmental expression patterns of the two 5 α -reductase isozymes in the liver, skin, and scalp. In all, 27 liver samples and 24 skin and scalp samples were analyzed from individuals ranging in age from 12.7-wk gestation to 81 yr. In the liver, the two isozymes had an identical temporal pattern of expression (Fig. 6, upper panel), whereas in the skin the two isozymes differed in their expression patterns (Fig. 6, lower panel). Both were detected in the neonate, but only the type 2 isozyme was detected in the skin and scalp of a 1.6- and a 3.0-yr-old individual. However, the level of type 2 expression in these two individuals was less than that in younger newborns. From age 15 through adulthood, only the type 1 isozyme was detected in the skin and scalp samples (Fig. 6, lower panel).

The expression patterns of the 5 α -reductase isozymes were next analyzed in three common pathophysiological states: hy-

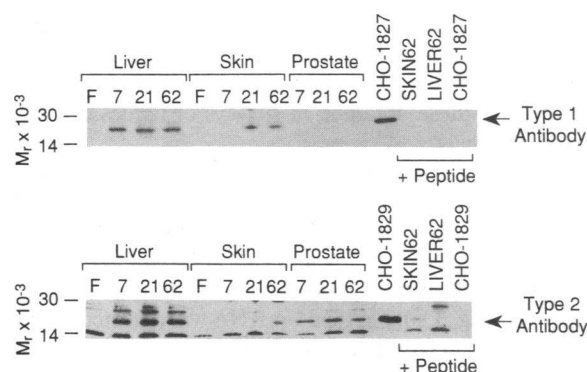


Figure 5. Expression of 5 α -reductase isozymes in adolescents and adults. 150- μ g aliquots of cell lysates prepared from the indicated tissues (F, fetal; 7, 21, 62, age in years; CHO-1827 and CHO-1829, CHO cells transfected with the type 1 and 2 cDNAs, respectively) were subjected to SDS-PAGE, transferred to nylon membranes and immunoblotted with a type 1 (upper panel) or a type 2 (lower panel) 5 α -reductase antisera. Molecular weight standards are indicated on the left. Arrows on right indicate positions of the 5 α -reductase isozymes. In the three rightmost lanes of both panels (labeled “+ Peptide”), filters were incubated with the 5 α -reductase antisera in the presence of 7.0 μ g/ml of the antigenic peptide before challenge with the second antibody. Exposure time was 2 min.

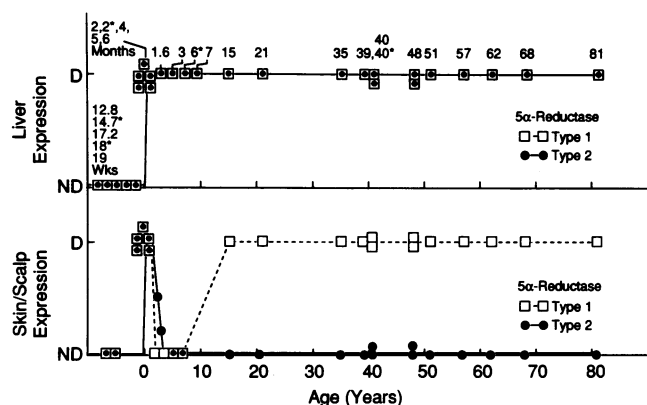


Figure 6. Temporal expression of 5 α -reductase isozymes in the liver and skin/scalp as determined qualitatively by immunoblotting. Arbitrary (qualitative) expression is indicated on the ordinate (D, detected; ND, not detected), and age (in years unless otherwise designated) is indicated on the abscissa. Subjects were male unless marked by an asterisk.

perplastic prostate, neoplastic prostate, and male pattern baldness. The data of Fig. 7 demonstrate that, as in the normal prostate, the type 2 isozyme was the only 5 α -reductase detectable in hyperplastic tissue or neoplastic tumors. A spectrum of tumor progression states was represented in the six adenocarcinoma samples analyzed. The Gleason pattern scores ranged from 5 to 9 and the pathologic stage ranged from B-2 to D-1. This experiment also demonstrates the weak cross-reactivity of the type 2 antiserum with the type 1 isozyme (Fig. 7, *rightmost two lanes*). The different molecular weights of the two isozymes however (type 1 > type 2), allows for differentiation of reaction from cross-reaction.

To examine 5 α -reductase isozyme expression in male pattern baldness, seven segments ($\sim 1 \text{ cm}^2$ each) were taken from a 51 yr-old Caucasian male with Hamilton type VIII baldness (6). The seven segments formed an arbitrary gradient from the crown (-4) to the hairline (0) to the peripheral region (+2) of the scalp (Fig. 8). Extracts were prepared from each segment

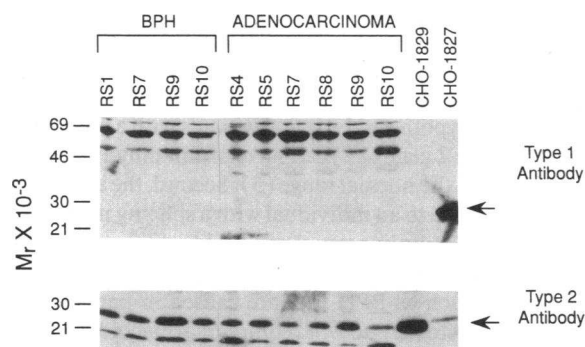


Figure 7. Detection of 5 α -reductase type 2 in BPH and prostate cancer. Lysates (150 μg) prepared from the indicated prostate tissue were subjected to SDS-PAGE and immunoblotting with the indicated 5 α -reductase isozyme antiserum. Arrows on the right of the autoradiograms indicate the positions of the 5 α -reductase isozymes. Exposure time was 8 min for the upper panel and 2 min for the lower panel. The filter in the upper panel was intentionally overexposed to demonstrate that 5 α -reductase type 1 could not be detected in the prostate.

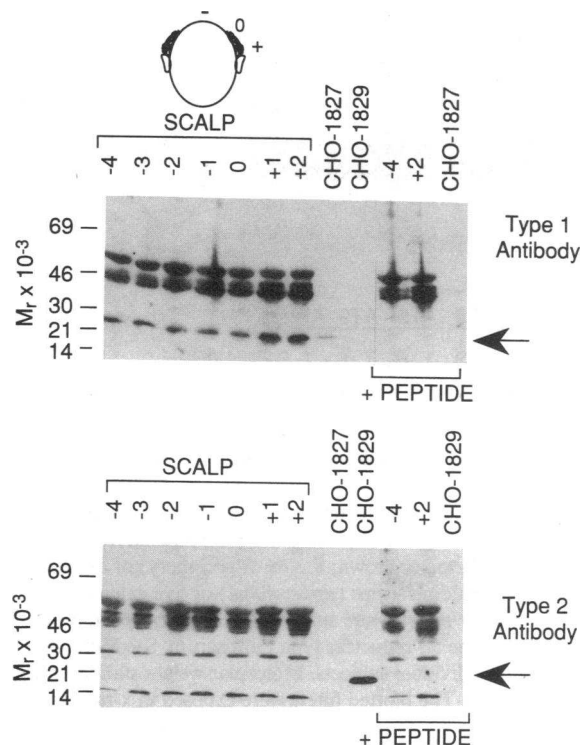


Figure 8. 5 α -Reductase expression in balding scalp. Protein extracts were prepared from the indicated portions (crown [-4], hair line [0], periphery [+2]) of a balding scalp and subjected to SDS-PAGE and immunoblotting with the indicated antiserum. The subject was a 51-yr-old Caucasian male with Hamilton type VIII baldness (6). Molecular weight standards are indicated on the left. The positions to which the 5 α -reductase isozymes migrated are indicated by arrows on the right. Lanes marked "+ Peptide" contained antigenic peptide (7.0 $\mu\text{g}/\text{ml}$) in the immunoblotting reaction. Exposure time was 3 min.

and blotted with the type-selective 5 α -reductase antibodies. The type 1 isozyme is present in all segments, regardless of the presence or absence of follicles containing terminal hairs (Fig. 8, *upper panel*). In contrast, the type 2 isozyme could not be detected in any region of the balding adult scalp (Fig. 8, *lower panel*). Both antibodies cross-reacted with a group of proteins in the mol wt 50,000 range. These proteins serve as loading controls to illustrate that essentially equal amounts of protein were electrophoresed in each lane and subsequently transferred to the filters. Coincubation of the antisera with the antigenic peptides specifically blocked the recognition of 5 α -reductase but did not diminish the signal obtained with the other proteins (Fig. 8, *lanes labeled "+ Peptide"*).

To determine if the expression of 5 α -reductase type 1 in the scalp varied between balding and nonbalding men, scalp samples corresponding to the top and side of five unrelated nonbalding or slightly balding individuals (Fig. 9, *upper panel*) and five unrelated extensively balding individuals (Fig. 9, *lower panel*) were subjected to immunoblotting with the anti-type 1 sera. With one exception, the levels of 5 α -reductase type 1 did not differ dramatically between these individuals, or between regions of the scalp with or without hair.

The exception was a slightly balding individual (AT14, Fig. 9, *upper panel*) who had Hamilton type II balding (6) and a marked decrease in the expression of 5 α -reductase type 1. This

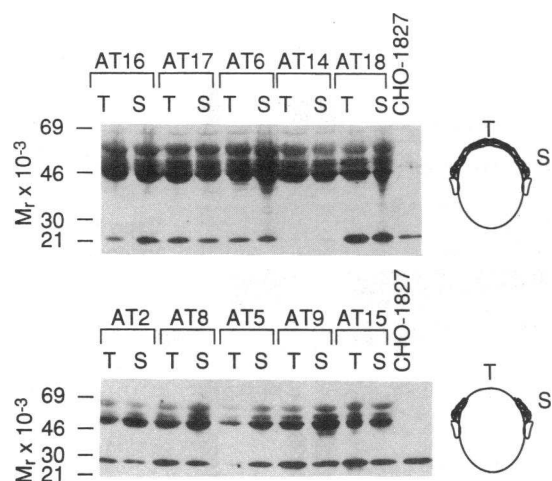


Figure 9. Expression of 5 α -reductase type 1 in the balding and non-balding scalp. 150- μ g aliquots of protein extracts prepared from the indicated regions (T, top or crown; S, side or periphery) of five non-balding or slightly balding scalps (*upper panel*) or five extensively balding scalps (*lower panel*) were subjected to SDS-PAGE and immunoblotting with the 5 α -reductase type 1 antiserum. See Methods for description of individual subjects. Molecular weight standards are indicated on the left. The blotted filters were exposed to x-ray film for 2 min.

reduction appeared to be specific for the 5 α -reductase isozyme, as the levels of cross-reacting proteins were not diminished relative to other individuals. The level of the type 1 isozyme was similarly decreased in the liver of subject AT14 (data not shown). The basis for the decreased type 1 expression in these organs is not known, however unlike the other individuals, subject AT14 was very obese (> 400 lbs) and it is conceivable that this condition contributed to the finding. In other experiments, the expression of 5 α -reductase type 1 in female scalp was qualitatively equivalent to that of male scalp (summarized in Fig. 7), a result that agrees with previous enzyme activity measurements (8).

Discussion

In this paper we describe the tissue specific and ontological expression patterns of two 5 α -reductase isozymes. The results of immunoblotting experiments indicate that expression of the type 1 isozyme begins at birth in the liver and that there are two waves of expression in the scalp and skin: the first beginning at or just before birth and ending around age 2–3 yr, and the second beginning during puberty and continuing throughout life. No qualitative differences in the expression of the type 1 isozyme were detectable in balding and nonbalding scalp of adults. The type 2 isozyme is the only form detected in the normal prostate at all ages examined, and in adenomatous and malignant neoplastic tissue. Similarly, the type 2 isozyme is the sole 5 α -reductase in two other adult male reproductive organs (seminal vesicle and epididymis) and in fetal genital skin. A single wave of expression of the type 2 isozyme occurs in the skin and scalp, beginning at or just before birth and ending around age 2–3 yr.

Mutations in the gene (*SRD5A2*) encoding 5 α -reductase type 2 underlie a form of male pseudohermaphroditism char-

acterized by normal Wolffian duct-derived glands but abnormal external genitalia and prostate (3). There are apparently no other symptoms associated with this genetic disease, suggesting that the chief role of 5 α -reductase type 2 in the embryo is virilization of the external genitalia and urogenital sinus (3). Consistent with this hypothesis, the type 2 isozyme was detected by immunoblotting in fetal genital skin but not in any of six other extra-genital tissues. These results confirm and extend a previous study in which 5 α -reductase enzyme activity measured in tissue slices was reported to be highest in the urogenital tract, and absent or very low in other tissues (30).

Among postnatal tissues, the type 2 isozyme was detected by immunoblotting in the liver, prostate, seminal vesicle, and epididymis (Figs. 2 and 3). Expression in the liver appears to commence at birth (Fig. 6). (The actual window of this induction period is between 19 weeks of gestational age and 2 months [Fig. 6]). In contrast, induction in the genital tract occurs in the embryo (30). The physiologic role of 5 α -reductase type 2 in tissues other than the prostate and anlagen of the external genitalia remains unknown, since these organs do not appear to be affected by the genetic absence of this isozyme or by its pharmacologic inhibition (21, 22). Furthermore, women who inherit two mutations in the type 2 gene have normal sexual development and are fertile (4).

An intriguing feature of 5 α -reductase type 2 deficiency has been the observation that affected individuals virilize to different extents during puberty (3–5). Masculinization occurs regardless of the type of mutation present in the *SRD5A2* gene, i.e., both in subjects with a complete deletion of the gene, and hence no residual type 2 activity, and in subjects with only qualitative defects in the enzyme (16, 18). Two findings reported here are consistent with a role for the 5 α -reductase type 1 isozyme in the observed virilization. First, the expression of this isozyme begins at or near birth in the liver and continues throughout life in this organ (Fig. 6). Second, the synthesis of the type 1 isozyme is induced at or during puberty in the skin and scalp and continues thereafter (Figs. 6, 8, 9). Given the large contribution of the skin and liver to relative body mass (~ 25%), it is reasonable to assume that the production of dihydrotestosterone by these organs at puberty drives virilization of the external genitalia and influences the pattern of hair distribution in type 2-deficient subjects. If this hypothesis is correct, then dihydrotestosterone can act in a true endocrine fashion as well as by the autocrine or paracrine mechanisms typically ascribed to this hormone (3). Two clinical observations support this hypothesis. First, individuals with a complete deletion of the type 2 gene (12) can have serum dihydrotestosterone levels within the normal range (5). Second, the administration of testosterone to an individual with a splicing mutation in the type 2 gene results in normal serum dihydrotestosterone levels (31). These results also suggest that the effective treatment of diseases such as BPH that are caused in part by the action of dihydrotestosterone will require pharmacological inhibition of both 5 α -reductase isozymes.

Two recent studies also support the expression of 5 α -reductase in adult skin. Harris et al. characterized a 5 α -reductase activity in scalp with the biochemical and pharmacological properties of the type 1 isozyme (32). Imperato-McGinley and colleagues further showed that sebum production, an androgen-dependent process, in subjects with complete androgen insensitivity was undetectable, whereas production in 5 α -reductase type 2 deficient subjects was normal (33). They hypothe-

sized that expression of the type 1 isozyme in the skin might drive sebum production.

A surprising finding in the current studies is the oscillating expression of both 5 α -reductase isozymes in the skin and scalp during development (Fig. 6). Analysis of 24 individuals ranging from 12.7 wk of gestational age to 81 yr, revealed that both 5 α -reductase genes are induced in this organ at birth and continue to be expressed until ~ 2–3 yr of age. Thereafter, the expression of both is extinguished until puberty, at which time the type 1 isozyme, but not the type 2 isozyme, is induced in the skin and scalp (Fig. 6). The physiological reasons and the factors that mediate this temporal pattern of expression are not known; however, given the role of androgens in 5 α -reductase regulation (10, 34), it is intriguing to speculate that the expression of one gene may influence the timing or expression level of the other. Since 5 α -reductase type 2-deficient subjects have less temporal hair regression (1–5), the pulse of type 2 expression in the scalp may influence the development of baldness later in life.

In support of this speculation regarding type 2 expression, we could find no qualitative differences in the steady state levels of 5 α -reductase type 1 in the scalps of balding and nonbalding men (Fig. 9), nor were any regional expression differences detected in the balding scalp (Fig. 8). Thus, at the level of resolution afforded by these studies, no evidence for abnormal expression of the type 1 isozyme as a feature of male pattern baldness was found. The interpretation of these results must be tempered by the qualitative nature of the findings. We cannot rule out low levels of expression that are below the sensitivity of detection by our antibodies (as clearly demonstrated by the mRNA and enzyme activity studies (Fig. 3, Table I)), nor can we assess changes in cell type specific expression. It remains to be seen whether similar results will be obtained in hirsutism (35, 36) and acne (37), two disorders that are manifest together with the reappearance of 5 α -reductase type 1 expression in the skin at puberty.

In the prostate, cell division and glandular growth are dependent on the synthesis of dihydrotestosterone (3). In both the normal prostate and in the two most common pathological conditions of this gland, BPH and prostate cancer, the type 2 isozyme appears to be responsible for this dihydrotestosterone synthesis (Figs. 2, 4, 5, 7). This result was somewhat surprising because mRNA for the type 1 isozyme is readily detected in the prostate (reference 11, Fig. 3). However, very little type 1 enzyme activity is present in prostate extracts, suggesting that the mRNA may be poorly translated (14), a hypothesis amply confirmed by major discrepancies between type 1 enzyme activity and mRNA levels in other tissues (Fig. 3, Table I). In BPH, the proliferation of both epithelial and stromal cells typically underlies the benign growth of the gland that may eventually lead to obstruction of the urethra (38). In contrast, most prostate tumors (including those analyzed here) involve neoplastic growth of epithelial cells in the gland (39). The observation that a relatively constant amount of the 5 α -reductase type 2 isozyme is detectable in both BPH and adenocarcinoma specimens suggests that either this protein may be expressed in both epithelial and stromal cells or that expression in a particular cell type may be altered in these disease states. Future immunohistochemical studies in our laboratory will attempt to distinguish these possibilities and to further determine the role of 5 α -reductase expression in prostate disease.

Finally, the tissue distributions of the human 5 α -reductase

isozymes are quite different from those reported for the rat proteins (13). For example, in the rat, only the type 1 isozyme is expressed in the liver and the ventral prostate expresses both isozymes. Furthermore, there is a marked sexual dimorphism in the type 1 expression pattern in the rat liver: the levels of enzyme activity and mRNA are 20 times higher in the female compared to the male (10), a result not found in the human organ (Fig. 6, and Thigpen, A. E., Guileyardo, J. M., and D. W. Russell, unpublished observations). These disparate expression patterns suggest that the two isozymes may be differentially regulated and therefore serve different physiological functions in the human and the rat.

Acknowledgments

We thank Kristi Cala, John Dawson, and Edith Womack for technical assistance or advice, Drs. J. J. Barnard, M. S. Brown, J. L. Goldstein, H. H. Hobbs, E. P. Jenkins, P. C. MacDonald, and J. D. Wilson for advice and critical reading of the manuscript, and members of the UT Southwestern Institute of Forensic Sciences for tissue samples.

This work was supported by research grants from the National Institutes of Health (GM43753), the Robert A. Welch Foundation (I-0971), and Glaxo, Inc. A. E. Thigpen is the recipient of a postdoctoral fellowship (HD-07504) from the National Institutes of Health.

References

1. Walsh, P. C., J. D. Madden, M. J. Harrod, J. L. Goldstein, P. C. MacDonald, and J. D. Wilson. 1974. Familial incomplete male pseudohermaphroditism, Type 2. Decreased dihydrotestosterone formation in pseudovaginal perineoscrotal hypospadias. *N. Engl. J. Med.* 291:944–949.
2. Imperato-McGinley, J., L. Guerrero, T. Gautier, and R. E. Peterson. 1974. Steroid 5 α -reductase deficiency in man: An inherited form of male pseudohermaphroditism. *Science (Wash. DC)*. 186:1213–1215.
3. Griffin, J. E., and J. D. Wilson. 1989. The androgen resistance syndromes: 5 α -reductase deficiency, testicular feminization, and related disorders. In *The Metabolic Basis of Inherited Disease*. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York. 1919–1944.
4. Peterson, R. E., J. Imperato-McGinley, T. Gautier, and E. Sturla. 1977. Male pseudohermaphroditism due to steroid 5 α -reductase deficiency. *Am. J. Med.* 62:170–191.
5. Imperato-McGinley, J., M. Miller, J. W. Wilson, R. E. Peterson, C. Shackleton, and C. Gajdusek. 1991. A cluster of male pseudohermaphrodites with 5 α -reductase deficiency in Papua New Guinea. *Clin. Endocrinol.* 34:293–298.
6. Hamilton, J. B. 1951. Patterned loss of hair in man: Types and incidence. *Ann. NY Acad. Sci.* 53:708–728.
7. Bingham, K. D., and D. A. Shaw. 1973. The metabolism of testosterone by human male scalp skin. *J. Endocrinol.* 57:111–121.
8. Schweikert, H. U., and J. D. Wilson. 1974. Regulation of human hair growth by steroid hormones. I. Testosterone metabolism in isolated hairs. *J. Clin. Endocrinol. Metab.* 38:811–819.
9. Schmidt, J. A., and H.-U. Schweikert. 1986. Testosterone and epitestosterone metabolism of single hairs in 5 patients with 5 α -reductase-deficiency. *Acta Endocrinol.* 113:588–592.
10. Andersson, S., R. W. Bishop, and D. W. Russell. 1989. Expression cloning and regulation of steroid 5 α -reductase, and enzyme essential for male sexual differentiation. *J. Biol. Chem.* 264:16249–16255.
11. Andersson, S., and D. W. Russell. 1990. Structure and biochemical properties of cloned and expressed human and rat steroid 5 α -reductases. *Proc. Natl. Acad. Sci. USA*. 87:3640–3644.
12. Andersson, S., D. M. Berman, E. P. Jenkins, and D. W. Russell. 1991. Deletion of steroid 5 α -reductase 2 gene in male pseudohermaphroditism. *Nature (Lond.)*. 354:159–161.
13. Normington, K., and D. W. Russell. 1992. Tissue distribution and kinetic characteristics of rat steroid 5 α -reductase isozymes. *J. Biol. Chem.* 267:19548–19554.
14. Jenkins, E. P., S. Andersson, J. Imperato-McGinley, J. D. Wilson, and D. W. Russell. 1992. Genetic and pharmacological evidence for more than one human steroid 5 α -reductase. *J. Clin. Invest.* 89:293–300.
15. Jenkins, E. P., C.-L. Hsieh, A. Milatovich, K. Normington, D. M. Berman, U. Francke, and D. W. Russell. 1991. Characterization and chromosomal mapping of a human steroid 5 α -reductase gene and pseudogene and mapping of the mouse homologue. *Genomics*. 11:1102–1112.

16. Thigpen, A. E., D. L. Davis, A. Milatovich, B. B. Mendonca, J. Imperato-McGinley, J. E. Griffin, U. Francke, J. D. Wilson, and D. W. Russell. 1992. Molecular genetics of steroid 5 α -reductase 2 deficiency. *J. Clin. Invest.* 90:799–809.
17. Thigpen, A. E., and D. W. Russell. 1992. Four-amino acid segment in steroid 5 α -reductase 1 confers sensitivity to finasteride, a competitive inhibitor. *J. Biol. Chem.* 267:8577–8583.
18. Thigpen, A. E., D. L. Davis, T. Gautier, J. Imperato-McGinley, and D. W. Russell. 1992. The molecular basis of steroid 5 α -reductase deficiency in a large Dominican kindred. *N. Engl. J. Med.* 327:1216–1219.
19. Siiteri, P. K., and J. D. Wilson. 1970. Dihydrotestosterone in prostatic hypertrophy. I. The formation and content of dihydrotestosterone in the hypertrophic prostate of man. *J. Clin. Invest.* 49:1737–1745.
20. Walsh, P. C., G. M. Hutchins, and L. L. Ewing. 1983. Tissue content of dihydrotestosterone in human prostatic hyperplasia is not supranormal. *J. Clin. Invest.* 72:1772–1777.
21. Stoner, E., and The Finasteride Study Group. 1992. The clinical effects of a 5 α -reductase inhibitor, finasteride, on benign prostatic hyperplasia. *J. Urol.* 147:1298–1302.
22. Gormley, G. J., E. Stoner, R. C. Bruskewitz, J. Imperato-McGinley, P. C. Walsh, J. D. McConnell, G. L. Andriole, J. Geller, B. R. Bracken, J. S. Tenover et al. 1992. The effect of finasteride in men with benign prostatic hyperplasia. *N. Engl. J. Med.* 327:1185–1191.
23. Bruchovsky, N., P. S. Rennie, F. H. Batzold, S. L. Goldenberg, T. Fletcher, and M. G. McLoughlin. 1988. Kinetic parameters of 5 α -reductase activity in stroma and epithelium of normal, hyperplastic, and carcinomatous human prostates. *J. Clin. Endocrinol. Metab.* 67:806–816.
24. Brendler, C. B., A. L. Follansbee, and J. T. Isaacs. 1985. Discrimination between normal, hyperplastic and malignant human prostatic tissues by enzymatic profiles. *J. Urol.* 133:495–501.
25. Hudson, R. W., and D. Wherrett. 1990. Comparison of the nuclear 5 α -reduction of testosterone and androstenedione in human prostatic carcinoma and benign prostatic hyperplasia. *J. Steroid Biochem.* 35:231–236.
26. Harlow, E., and D. Lane. 1988. *Antibodies: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 726 pp.
27. Russell, D. W., W. J. Schneider, T. Yamamoto, K. L. Luskey, M. S. Brown, and J. L. Goldstein. 1984. Domain map of the LDL receptor: Sequence homology with the epidermal growth factor precursor. *Cell*. 37:577–585.
28. Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.
29. Gleason, D. F. 1977. Histologic grading and clinical staging of prostatic carcinoma. In *Urologic Pathology: The Prostate*. M. Tannenbaum, editor. Lea and Febiger, Philadelphia. 171–197.
30. Siiteri, P. K., and J. D. Wilson. 1974. Testosterone formation and metabolism during male sexual differentiation in the human embryo. *J. Clin. Endocrinol. Metab.* 38:113–125.
31. Price, P., A. H. Wass, J. E. Griffin, M. Leshin, M. O. Savage, D. M. Large, D. E. Bu'Lock, D. C. Anderson, J. D. Wilson, and G. M. Besser. 1984. High dose androgen therapy in male pseudohermaphroditism due to 5 α -reductase deficiency and disorders of the androgen receptor. *J. Clin. Invest.* 74:1496–1508.
32. Harris, G., B. Azzolina, W. Baginsky, G. Cimis, G. H. Rasmussen, R. L. Tolman, C. R. H. Raetz, and K. Ellsworth. 1992. Identification and selective inhibition of an isozyme of steroid 5 α -reductase in human scalp. *Proc. Natl. Acad. Sci. USA*. 89:10787–10791.
33. Imperato-McGinley, J., T. Gautier, L.-Q. Cai, B. Yee, J. Epstein, J., and P. Pochi. 1992. The androgen control of sebum production. Studies of subjects with dihydrotestosterone deficiency and complete androgen insensitivity. *J. Clin. Endocrinol. Metab.* 76:524–528.
34. George, F. W., D. W. Russell, and J. D. Wilson. 1991. Feed-forward control of prostate growth: dihydrotestosterone induces expression of its own biosynthetic enzyme, steroid 5 α -reductase. *Proc. Natl. Acad. Sci. USA*. 88:8044–8047.
35. Jenkins, J. S., and S. Ash. 1973. The metabolism of testosterone by human skin in disorders of hair growth. *J. Endocrinol.* 59:345–351.
36. Thomas, J. P., and R. J. Oake. 1974. Androgen metabolism in the skin of hirsute women. *J. Clin. Endocrinol. Metab.* 38:19–22.
37. Sansone, G., and R. M. Reisner. 1971. Differential rates of conversion of testosterone to dihydrotestosterone in acne and in normal human skin—A possible pathogenic factor in acne. *J. Invest. Dermatol.* 56:366–372.
38. Walsh, P. C. 1992. Benign prostatic hyperplasia. In *Campbell's Urology*. P. C. Walsh, A. B. Retik, T. A. Stamey, and E. D. Vaughan, editors. W. B. Saunders, Philadelphia. 1007–1027.
39. Stamey, T. A., and J. E. McNeal. 1992. Adenocarcinoma of the prostate. In *Campbell's Urology*. P. C. Walsh, A. B. Retik, T. A. Stamey, and E. D. Vaughan, editors. W. B. Saunders, Philadelphia. 1159–1221.