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Cell-type-specific expression of rat steroid 5α-reductase isozymes

(sexual development/androgens/prostate/stroma/epithelium)

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ABSTRACT **The enzyme steroid 5α-reductase (EC 1.3.99.5) is a component of an intercellular signaling pathway that determines cell fate in the primordium of the mammalian reproductive tract. During male phenotypic sexual differentiation, the dihydrotestosterone product of this enzyme binds to the androgen receptor and initiates development of the external genitalia and prostate. Genes encoding two isozymes of steroid 5α-reductase with different biochemical properties and tissue distributions have recently been isolated. In the current study, we utilize *in situ* hybridization analysis to determine cell-type-specific expression patterns of the 5α-reductase isozyme mRNAs in two androgen target tissues (regenerating ventral prostate and epididymis) and a peripheral tissue (liver). In regenerating ventral prostate, the type 1 mRNA is expressed in basal epithelial cells whereas expression of the type 2 mRNA is largely confined to stromal cells. These results were confirmed by immunohistochemical analysis and are consistent with distinct roles played by the isozymes in the prostate. In the epididymis, both 5α-reductase isozyme mRNAs are expressed in epithelial cells. Only the type 1 mRNA is present in the liver. This mRNA is distributed in a striking spatial gradient extending from hepatocytes surrounding the portal triad (high expression) to those surrounding the central vein (low to absent expression). These findings demonstrate cell-type-specific expression of the steroid 5α-reductase isozymes and underscore their distinct and overlapping functions in androgen physiology.**

The morphogenesis of many mammalian organs during development depends on interactions between mesenchymal and epithelial cells (1). In certain glands, such as those of the male reproductive tract, the mesenchymal (stromal) cells can act as inducers of epithelial-cell fate by providing an environment conducive to the development of a particular glandular morphology (2, 3). In these and other tissues, the stromal signals that instruct a pluripotent epithelial cell to adopt an organ-specific role are currently under intense investigation. The signals so far identified include secreted growth factors and their receptors (4–8).

Steroid hormones represent a distinctive class of signaling molecules that influence the interactions of stromal and epithelial cells in the urogenital tract (9, 10). For example, the secretion of testosterone by the fetal testes is required in some species for the embryonic development of the seminal vesicles and epididymides, accessory sexual structures derived from the Wolffian duct (10). Similarly, the morphogenesis of the prostate requires the synthesis of dihydrotestosterone (DHT) by the enzyme steroid 5α-reductase (5α-reductase; EC 1.3.99.5) in the urogenital sinus anlage of this gland (10). Both testosterone and DHT bind to a transcription factor, the androgen receptor, which in turn activates gene networks that carry out the morphogenetic programs of a particular tissue (11).

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The distinct roles of testosterone and DHT in the development of the male reproductive tract were deduced from the analysis of individuals with phenotypic disorders of sexual differentiation (11). Males that fail to synthesize testosterone or the androgen receptor develop female internal and external genitalia (11). In contrast, males that lack one of two 5α-reductase genes develop normal internal male reproductive glands, including the epididymis and seminal vesicle, but have external genitalia resembling those of the female and no prostate (11, 12).

These observations were translated to the cellular level in elegant recombination studies employing stroma and epithelium from different regions of the murine embryonic reproductive tract (2, 3). For example, the recombination of urogenital sinus stroma with vaginal epithelium, followed by grafting into a male host, resulted in the formation of a prostate (13). The input of androgens and the inductive role of the stroma in this protocol were made clear by two types of experiments. (*i*) Grafting into a castrated male host did not lead to prostate development (14). (*ii*) The reconstitution of urogenital sinus stroma of a normal animal with urogenital epithelium from an androgen-receptor-deficient (*tfm*) animal yielded a prostate in male hosts. However, the reciprocal recombination of stroma from an androgen-receptor-deficient animal with normal epithelium did not (15, 16).

The role of cell-type-specific expression of 5α-reductase in the development of the male reproductive tract remains unexplored. Interest in this role increased with the recent finding that rats and humans have two 5α-reductase genes encoding isozymes (designated type 1 and type 2) with different biochemical properties and tissue distributions (17, 18). Furthermore, both 5α-reductase genes in the rat are regulated by DHT, the product of the encoded enzyme, via a form of feed-forward regulation. This regulatory mechanism is common among developmentally important genes (19). In the current study, we have used *in situ* mRNA hybridization and immunohistochemical analysis to determine the cell-type-specific expression patterns of the 5α-reductase isozymes in the regenerating rat ventral prostate, epididymis, and liver. The results suggest that the two isozymes play distinctive roles in androgen metabolism.

MATERIALS AND METHODS

***In Situ* Hybridization Probes.** [^{33}P]RNA probes were synthesized and purified as described (20). For the 5α-reductase type 1 mRNA, probes corresponded to nt 1–218, 215–500, and 495–741 of the cDNA (17). For the type 2 mRNA, probes corresponded to nt 1–283, 314–628, and 605–810 of the cDNA (18).

Tissues. Animals were anesthetized with halothane and sacrificed. Tissues were excised, fixed, paraffin-embedded, and sectioned at 5 μm onto poly(L-lysine)-coated slides. For *in situ* mRNA hybridization experiments, the fixative was 2% (wt/vol) paraformaldehyde/1% glutaraldehyde/1% satu-

Abbreviation: DHT, dihydrotestosterone.

rated picric acid/0.05 M sodium phosphate, pH 7.4. For immunohistochemistry experiments, Histochoice fixative (Amresco, Solon, OH) was used.

***In Situ* mRNA Hybridization.** Sections were pretreated as described (21). Briefly, sections were dewaxed, denatured, digested with proteinase K (10 μg/ml, 1 h, 37°C), refixed in paraformaldehyde, acetylated, and dehydrated. After air drying, sections were hybridized for 16 h under sealed coverslips at 50°C with 35 μl of ^{33}P-labeled probe mixture (5 × 10^6 cpm per template; 1.5 × 10^7 total cpm/ml) prepared as described (20). Sections were then rinsed, treated with RNase A (20 μg/ml, 30 min, 37°C), washed in 50% (vol/vol) formamide/0.1 M dithiothreitol/2× SSC (1× SSC = 150 mM NaCl/15 mM sodium citrate) at 65°C for 30 min, followed by a wash at 22°C for 15 min in 0.1× SSC. Air-dried sections were exposed to βmax Hyperfilm (Amersham) at 22°C for 3–4 days to estimate liquid emulsion exposure times. Sections were coated with Kodak NTB2 emulsion diluted 1:1 in water. After exposure, slides were treated with Kodak D-19 developer, fixed, and stained with hematoxylin and eosin.

Immunohistochemistry. Sections were digested for 1 min in 0.01% bacterial protease XXIV (Sigma) at 37°C and incubated with affinity-purified anti-5α-reductase antibodies. Immunocomplexes were detected by the avidin–biotin complex amplification technique using diaminobenzidine tetrahydrochloride as a chromogenic substrate. Sections were counterstained with hematoxylin.

Regenerating Prostate Experiments. Male Sprague–Dawley rats (275–300 g) (Sasco, Omaha, NE) were castrated (scrotal route, standard surgical procedures) on day 0. On day 7, experimental groups were injected s.c. for 3 consecutive days with 1 mg of testosterone acetate dissolved in oil vehicle [20% (vol/vol) ethanol in triolein] or oil vehicle alone. Ventral prostates were collected on day 10 and subjected to *in situ* mRNA hybridization or immunohistochemical analysis as described above.

RESULTS

In preliminary experiments, we were unable to detect the 5α-reductase type 1 mRNA by *in situ* hybridization in either normal or castrated ventral prostate sections. This apparent sensitivity problem was overcome by examining the expression of the type 1 mRNA in the regenerating ventral prostate. Previous studies have shown that the steady-state levels of the type 1 and type 2 mRNAs are markedly induced in the regenerating gland (18, 19). In a typical experiment of this type, animals were castrated and their prostates were allowed to regress for 7 days. Testosterone was then administered for 3 days, followed by sacrifice and analysis of the ventral prostate. This experimental regimen results in an increase in the number and size of the epithelial cells compared to the castrati and little overall change in the stromal cells of the gland (Fig. 1*A* and ref. 22). Hybridization with 5α-reductase type 1 sense-strand [^{33}P]RNA probes revealed a low background signal with no cell type specificity (Fig. 1*B*). In contrast, hybridization with type 1 antisense-strand [^{33}P]-RNA probes demonstrated intense labeling of some but not all epithelial cells examined under low (Fig. 1*D*) or high (Fig. 1*F*) magnification.

Hybridization of 5α-reductase type 2 sense-strand [^{33}P]RNA probes to regenerating ventral prostate sections

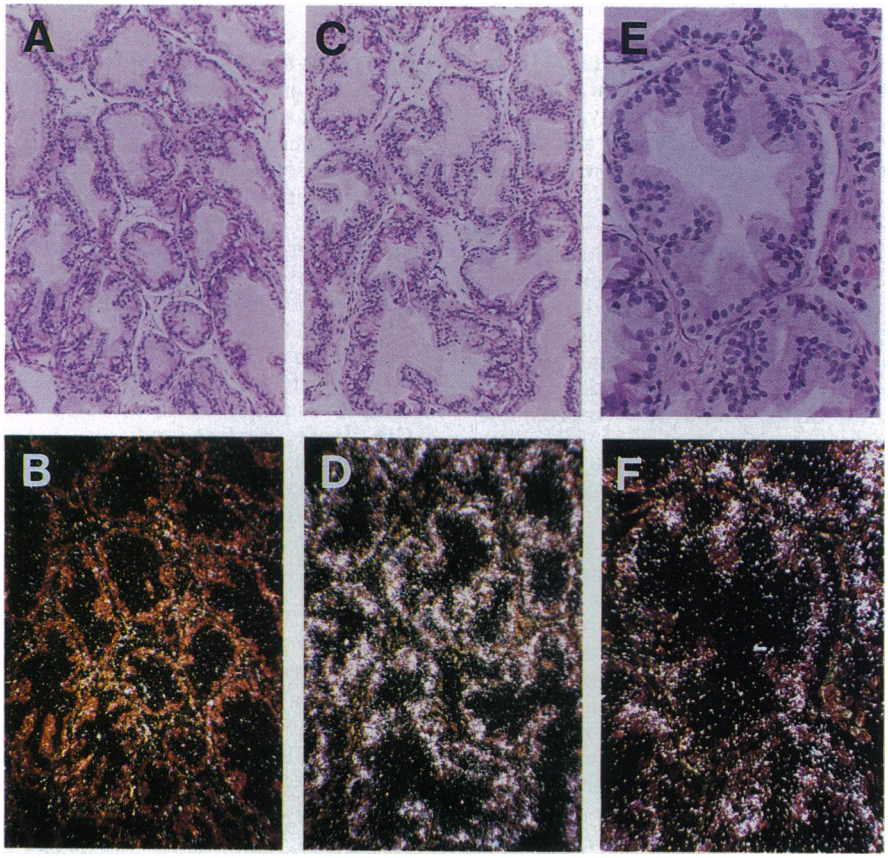

FIG. 1. Hybridization of 5α-reductase type 1 sense- and antisense-strand [^{33}P]RNA probes to regenerating rat ventral prostate. (*A*) Photomicrograph of a stained regenerating prostate section. (×150.) (*B*) Dark-field photomicrograph of a type 1 sense-strand probe hybridized to the prostate section in *A*. (×150.) Exposure time was 12 days. (*C*) Photomicrograph of a stained regenerating prostate section. (×150.) (*D*) Dark-field photomicrograph of a type 1 antisense-strand probe hybridized to the prostate section in *C*. (×150.) Exposure time was 12 days. (*E*) Photomicrograph of a stained regenerating prostate section. (×380.) (*F*) Dark-field photomicrograph of a type 1 antisense-strand probe hybridized to the prostate section in *E*. (×380.) Exposure time was 12 days.

gave no specific signal (Fig. 2 *A* and *B*). However, hybridization with type 2 antisense-strand [^{33}P]RNA probes revealed large numbers of silver grains over the stromal cells (Fig. 2 *C* and *D*). Analysis under high magnification emphasized the near exclusive stromal cell hybridization pattern (Fig. 2 *E* and *F*). Similar results for the type 2 mRNA were obtained in the ventral prostates of normal and castrate rats (data not shown).

To confirm the *in situ* mRNA hybridization results, antipeptide antibodies directed against the 5α-reductase isozymes were used in immunohistochemical experiments. Incubation of regenerating ventral prostate sections with a preimmune antibody revealed no specific cellular staining pattern (Fig. 3*A*). In marked contrast, incubation with a 5α-reductase type 1 antiserum resulted in intense perinuclear staining of the basal epithelial cells (Fig. 3*B*). Similar experiments with a 5α-reductase type 2 antiserum showed intense perinuclear staining of the stromal cells of the regenerating gland (data not shown).

To determine whether the epithelial and stromal cell-type-specific expression patterns of the ventral prostate were common to all male reproductive glands, we next examined the distribution of 5α-reductase isozyme mRNAs in the caput epididymis of a normal animal (Fig. 4). Hybridization of a type 1 antisense-strand [^{33}P]RNA probe revealed a distribution of silver grains predominantly localized over the epithelium of the gland (Fig. 4 *A* and *B*). Hybridization of a type 2 antisense-strand [^{33}P]RNA probe revealed a similar, but more intense distribution of grains over these same cells (Fig. 4 *C* and *D*). Analysis under high magnification revealed that the silver grains were localized over the principal cells (23) of the gland. Control incubations with epididymal sections and sense-strand RNA probes did not reveal any cell-type-specific hybridization patterns (data not shown).

In a final series of experiments, we determined the distribution of the 5α-reductase type 1 mRNA in the normal male liver. In contrast to the ventral prostate and epididymis, RNA blotting experiments have shown that only the type 1 mRNA is present in the rat liver (18). Control hybridizations to liver sections with type 1 sense-strand [^{33}P]RNA probes revealed a dispersed pattern of silver grains localized throughout the organ (Fig. 5 *A* and *B*). Hybridization of antisense-strand probes showed a striking spatial gradient of expression in hepatocytes. Very few silver grains were localized over hepatocytes surrounding the central veins, whereas enormous numbers were present over hepatocytes encircling the portal triads (Fig. 5 *C* and *D*). No hybridization was detected in endothelial cells lining the blood vessels or in Kupffer cells (data not shown).

DISCUSSION

In the current paper, we have used *in situ* mRNA hybridization and immunohistochemical analyses to determine the cell-type-specific expression patterns of the 5α-reductase type 1 and type 2 mRNAs and their encoded isozymes. In the regenerating ventral prostate, the data indicate that expression of the type 1 mRNA is largely confined to the basal epithelial cells of the gland, whereas expression of the type 2 mRNA occurs predominantly in the stromal cells. The latter cell-type-specific expression pattern was not altered by the presence or absence of testicular androgens. In the epi-

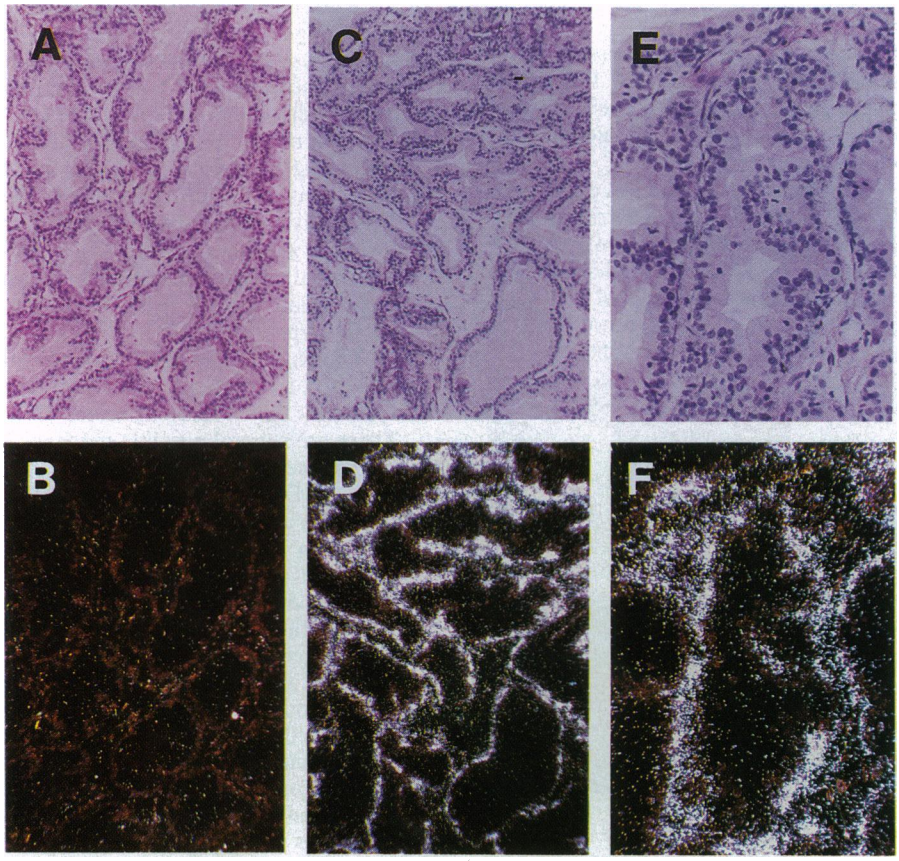

FIG. 2. Hybridization of 5α-reductase type 2 sense- and antisense-strand [^{33}P]RNA probes to regenerating rat ventral prostate. (*A*) Photomicrograph of a stained regenerating prostate section. (×150.) (*B*) Dark-field photomicrograph of a type 2 sense-strand probe hybridized to the prostate section in *A*. (×150.) Exposure time was 12 days. (*C*) Photomicrograph of a stained regenerating prostate section. (×150.) (*D*) Dark-field photomicrograph of a type 2 antisense-strand probe hybridized to the prostate section in *C*. (×150.) Exposure time was 12 days. (*E*) Photomicrograph of a stained regenerating prostate section. (×380.) (*F*) Dark-field photomicrograph of a type 2 antisense-strand probe hybridized to the prostate section in *E*. (×380.) Exposure time was 12 days.

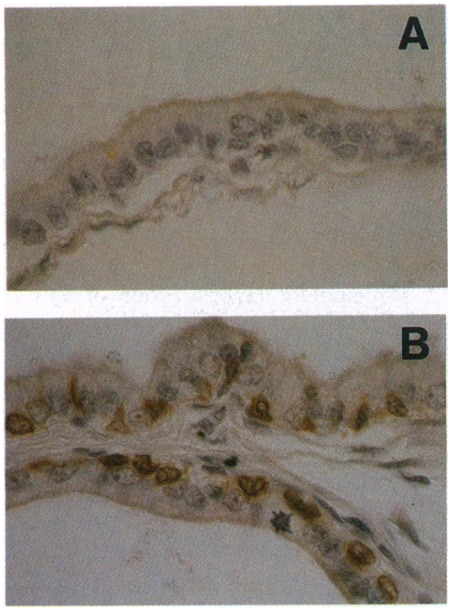

FIG. 3. Immunohistochemical localization of 5α-reductase type 1 in regenerating rat ventral prostate. An affinity-purified anti-peptide antibody raised in rabbits against residues 228–252 of the type 1 isozyme was used at a concentration of 2.5 μg/ml. Immunoblot experiments showed that this antiserum did not cross-react with the type 2 isozyme (data not shown). (*A*) Photomicrograph of a stained prostate section incubated with preimmune antiserum. (×1200.) (*B*) Photomicrograph of the stained prostate section incubated with immune antiserum. (×1200.) A perinuclear stain is detected only in basal epithelial cells.

didymis, both 5α-reductase mRNAs are expressed in the epithelial cells of the gland. In the liver, the type 1 mRNA is expressed in a positional gradient in which the level is very high in hepatocytes proximal to the portal triad and very low in hepatocytes surrounding the central vein.

The finding that the 5α-reductase type 1 mRNA expression is limited to the basal epithelial cells of the ventral prostate suggests that this isozyme plays an important role in glandular growth and maintenance. The basal epithelial cells are thought to be precursors of the lumenal epithelial cells (24). In contrast to the lumenal cells, basal cells do not undergo apoptosis in response to androgen deprivation and they retain

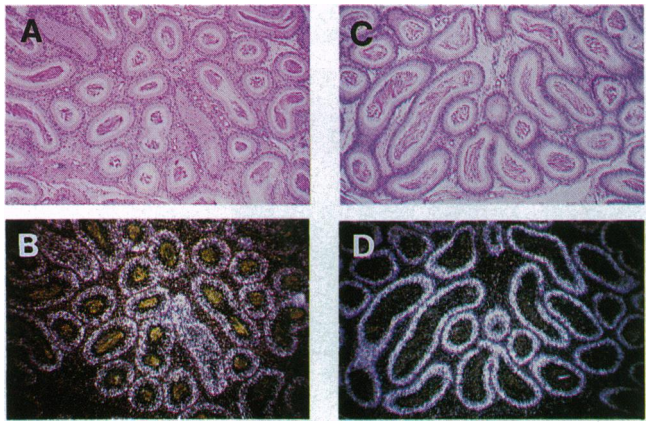

FIG. 4. Hybridization of 5α-reductase type 1 and type 2 antisense-strand [^{33}P]RNA probes to epididymis. (*A*) Photomicrograph of a stained epididymis section. (×110.) (*B*) Dark-field photomicrograph of a type 1 antisense-strand probe hybridized to the section in *A*. (×110.) Exposure time was 9 days. (*C*) Photomicrograph of a stained epididymis section. (×110.) (*D*) Dark-field photomicrograph of a type 2 antisense-strand probe hybridized to the section in *C*. (×110.) Exposure time was 9 days.

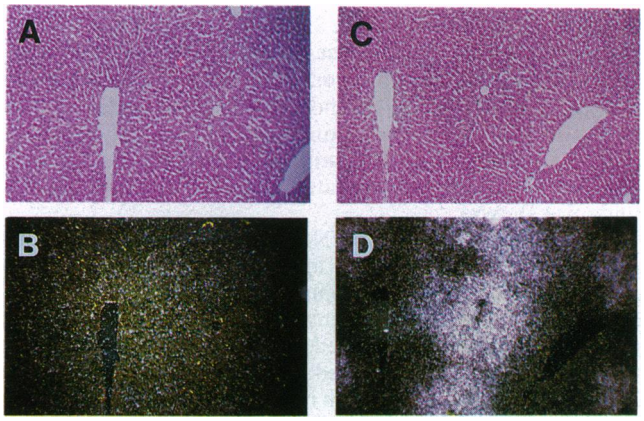

FIG. 5. Hybridization of 5α-reductase type 1 sense- and antisense-strand [^{33}P]RNA probes to liver. (*A*) Photomicrograph of a stained liver section. (×70.) (*B*) Dark-field photomicrograph of a type 1 sense-strand probe hybridized to the section in *A*. (×70.) Exposure time was 9 days. (*C*) Photomicrograph of a stained liver section. (×70.) (*D*) Dark-field photomicrograph of type 1 antisense-strand probe hybridized to the section in *C*. (×70.) Exposure time was 9 days.

their proliferative capacity for long periods of time in a castrated animal (25). Expression of the type 1 isozyme may thus protect the basal cells from apoptotic death by producing low levels of DHT from testosterone synthesized by peripheral organs. In addition, the induction of 5α-reductase type 1 that occurs with readministration of testosterone would be expected to stimulate cell division and repopulation of the acini with lumenal epithelial cells.

The finding that the 5α-reductase type 2 mRNA is largely if not exclusively confined to the stromal cells of the ventral prostate sheds light on two previous observations. (*i*) Individuals with a genetic deficiency of 5α-reductase type 2 fail to develop normal prostates (26), and (*ii*) stromal cells of the murine urogenital sinus can direct the formation of a prostate when reconstituted with vaginal or bladder epithelium (3). It seems likely that the absence of the type 2 enzyme from the embryonic stromal cells of the genetically deficient individuals prevents the synthesis of an inductive signal required by the epithelial cells to form the prostate. In support of this idea, the type 2 isozyme has a high affinity for testosterone (apparent K_m = 75 nM, ref. 18) and would thus be particularly well suited for reducing the small amounts of testosterone secreted by the developing testes (27).

There are several possible mechanisms through which the 5α-reductase type 2 isozyme could induce prostate development. The DHT synthesized by this isozyme in the stroma could act in a paracrine manner to stimulate gene expression and pattern formation in the adjacent epithelium. Alternatively, DHT could act in an autocrine manner to stimulate the expression of stromal cell genes that in turn drive morphogenesis of the epithelial cells. This latter possibility is more likely, since the expression of the androgen receptor (the recipient of the DHT signal) in the fetal prostate is confined to the stroma (ref. 9 and references therein). Furthermore, epithelial cells with a defective androgen receptor respond to normal stromal cells (15, 16), and DHT has been shown to act as a direct mitogenic stimulus on stromal cells (31).

In contrast to the prostate, the epididymis does not require DHT for its embryonic development (11). The observation that both 5α-reductase isozyme mRNAs are expressed in epithelial cells of the epididymis (Fig. 4) is consistent with this finding and further suggests that they play distinct roles in this organ versus those in the prostate. These epididymal functions are currently unknown but could involve an action of DHT in facilitating sperm maturation that is yet to be

defined. Thus, both isozymes are expressed in a positional gradient along the gland, with activity being highest in the segment of the epididymis immediately next to the testis and decreasing toward the tail of the gland (18, 28). The 5α-reductase expression gradient thus parallels the maturation of sperm as they travel along the epididymis (23). The importance of the type 2 isozyme is further reflected by the finding that expression of this isozyme and mRNA is higher in the epididymis than in any of 17 other rat tissues surveyed (18).

The spatial gradient of 5α-reductase type 1 mRNA expression in hepatocytes of the liver (Fig. 5) may reflect two inputs. (*i*) The concentration of the enzyme in hepatocytes surrounding the portal triad would facilitate catabolism of circulating testosterone and other steroids since the direction of blood flow in the organ is from portal triad to central vein. We note, however, that the distributions of some drug-inactivating cytochrome P-450 enzymes are exactly opposite (i.e., central vein ≫ portal triad hepatocytes) from that seen for the 5α-reductase type 1 isozyme (29). (*ii*) The type 1 isozyme distribution may reflect the developmental lineage of hepatocytes, since precursor stem cells cluster around the portal triad and more terminally differentiated cells around the central vein (30). If the latter hypothesis is true, then 5α-reductase may play an as yet unknown role in hepatocyte differentiation.

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