Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 728–733, January 1996
Biochemistry

Targeted overexpression of androgen receptor with a liver-specific promoter in transgenic mice

(steroid receptor/hepatocarcinogen activation/dehydroepiandrosterone sulfotransferase/aging)

BANDANA CHATTERJEE*, CHUNG S. SONG, MYEONG H. JUNG, SHUO CHEN, CHRISTI A. WALTER, DAMON C. HERBERT, FRANK J. WEAKER, MICHAEL A. MANCINI, AND ARUN K. ROY

Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284

Communicated by Elwood V. Jensen, Institute for Hormone and Fertility Research, Hamburg, Germany, October 16, 1995

ABSTRACT **The rodent liver displays marked age- and sex-dependent changes in androgen sensitivity due to the sexually dimorphic and temporally programmed expression of the androgen receptor (AR) gene. We have altered this normal phenotype by constitutive overexpression of the rat AR transgene in the mouse liver by targeting it via the human phenylalanine hydroxylase (hPAH) gene promoter. These transgenic animals in their heterozygous state produce an ≈30-fold higher level of the AR in the liver as compared with the nontransgenic control. Androgen inactivation via sulfonation of the hormone by dehydroepiandrosterone sulfotransferase (DST), an androgen-repressible enzyme, also contributes to the age- and sex-dependent regulation of hepatic androgen sensitivity. DST has a broad range of substrate specificity and is responsible for the age- and sex-specific activation of certain polycyclic aromatic hepatocarcinogens as well, by converting them to electrophilic sulfonated derivatives. In the transgenic female, the hepatic expression of DST was ≈4-fold lower than in normal females, a level comparable to that in normal males. The hPAH-AR mice will serve as a valuable model for studying the sex- and age-invariant expression of liver-specific genes, particularly those involved in the activation of environmental hepatocarcinogens such as the aromatic hydrocarbons.**

The androgen receptor (AR) is a member of the ligand-activated steroid/thyroid hormone receptor superfamily of transcription factors. Similar to expression of most other members of this superfamily, expression of AR is regulated in a tissue-, sex- and age-dependent manner. The rodent liver is an important androgen target, and a number of genes for hepatic proteins are either induced or repressed by androgenic hormones (1). For example, androgens upregulate hepatic synthesis of the rat pheromone-binding protein α_{2u}-globulin, the mouse sex-limited protein (Slp), and carbonic anhydrase (CA) isozyme III, as well as various drug- and steroid-metabolizing enzymes, including specific cytochrome P450 isozymes (e.g., the steroid 2α-/16α-hydroxylase and fatty acid ω-hydroxylase) and estrogen and other aryl sulfotransferases (2–9). Dehydroepiandrosterone sulfotransferase (DST), on the other hand, is downregulated by androgenic hormones (10, 11). Because of the central metabolic role of the liver, sexual dimorphism of the sex steroid-metabolizing enzymes plays a critical role in maintaining sex-specific hormonal homeostasis. Thus, DST, which inactivates androgenic steroids by sulfonation, is overexpressed in the female liver and is repressed in the male liver during the androgen-sensitive period of young adult life (10, 11). This enzyme has a broad substrate specificity, and in addition to C-19 hydroxysteroids, it can use certain aromatic hydrocarbons such as benzo[*a*]pyrene, benz-[*a*]anthracene, pyrene, and their hydroxymethyl derivatives as substrates for sulfonation (12, 13). Unlike sulfonated androgens, which are hormonally inactive, sulfonated aromatic hydrocarbons are potent hepatocarcinogens, and the sulfonation reaction is considered to be an obligatory step in their activation process (13, 14). Sex- and age-specific expression of DST has specifically been implicated in the sexually dimorphic hepatocarcinogenic potential of the above-mentioned aromatic carcinogens, which are also commonly found environmental pollutants (11, 15, 16).

In addition to major sex-specific differences in the level of circulating androgens, the steady-state level of the AR mRNA in the liver of adult male rats is ≈20-fold higher than that in the adult female liver (unpublished observation). This sex difference in AR expression also correlates with the androgen responsiveness of the liver in young adult male and female rats (17). Furthermore, irrespective of sex, expression of the AR in the liver is regulated in an age-dependent manner; i.e., it is almost undetectable before puberty, rises rapidly in postpubertal life, and gradually declines during aging, reaching an almost nondetectable level after about 22–24 months of age (18). Sex- and age-dependent differences in the hepatic expression of the AR appear to be programmed through a number of cis regulatory elements within the AR promoter (19–21). Thus, sex- and age-specific expression of the AR gene plays a critical role in determining the hepatic expression of DST, with a corresponding increase in the risk of the carcinogenic potential of certain aromatic hydrocarbons. In order to understand the mechanism of androgen-dependent activation of these xenobiotic carcinogens and the consequent tumorigenesis in an age- and sex-invariant manner, we have explored the possible use of the transgenic technology with a targeted overexpression of the AR gene in the mouse liver via a constitutive liver-specific promoter. Earlier studies of Wang *et al.* (22) have shown that a fragment of the human phenylalanine hydroxylase (hPAH) gene promoter can direct the expression of the bacterial chloramphenicol acetyltransferase (CAT) reporter gene in transgenic mice in a predominantly liver-specific fashion. In this report, we describe successful overexpression of the rat AR in the mouse liver mediated through the hPAH promoter, and its downstream consequence on the androgen-repressible DST gene.

MATERIALS AND METHODS

Chimeric hPAH-AR Transgene Construct and Transgenesis. The transgene construct used in this study was prepared in the pTKβ vector (Clontech) by placing a promoter fragment (≈10 kb) of the hPAH gene (22) upstream of a 2.8-kb segment

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

Abbreviations: AR, androgen receptor; rAR, rat AR; hPAH, human phenylalanine hydroxylase; CAT, chloramphenicol acetyltransferase; DHEA, dehydroepiandrosterone; DST, dehydroepiandrosterone sulfotransferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT, reverse transcription; SV40, simian virus 40.

*To whom reprint requests should be addressed.

of the rat AR (rAR) cDNA, containing the entire AR protein coding sequence (Fig. 1). The transcription initiation site of the transgene is provided by the hPAH promoter, and the SV40 sequence-derived splice donor/acceptor site and polyadenylylation signal were used to ensure efficient synthesis of the transgene-specific mRNA. The rAR cDNA fragment was subcloned into pTKβ at the unique *Not* I site to produce pTKβ-rAR, following removal of the β-galactosidase cDNA fragment from the original pTKβ vector. The recombinant pUC19 plasmid containing the hPAH promoter (≈10 kb) was digested with *Sma* I, and the linearized plasmid DNA, following ligation to *Sal* I linker, was digested with the *Sal* I restriction enzyme to release the hPAH promoter fragment from the vector sequence. The hPAH promoter was finally subcloned into pTKβ-rAR at the unique *Xho* I site. The resulting hPAH-rAR fusion was excised as a *Cla* I–*Sal* I fragment and purified from vector sequences before its microinjection into the zygote.

Standard microinjection methods were used to introduce the transgene construct into male pronuclei of the zygotes derived from C57BL/6 mice (23). The resulting mice were screened for the presence of the transgene by PCR analysis of DNA prepared from tail biopsy tissue. The sense PCR primer (23-mer) corresponded to the SV40 sequence and included the junction sequence of the splice acceptor site. The antisense PCR primer (21-mer) corresponded to the rAR cDNA sequence from positions +1340 to +1320, +1 being the transcription start site of the rAR gene (24, 25). These two primers are expected to generate a 48-bp PCR product from the rAR template. The sense and antisense primers used for detection of the integrated transgene were 5′-AGGTCCCGGATCCG-GTGGTGGTG-3′ and 5′-GAGGCAGCCGCTCTCAGGG-TG-3′, respectively. The litters that were found positive by PCR screening were subsequently analyzed for the number of transgene copies by Southern blot analysis of tail DNA. Transgene-carrying founder mice were mated with C57BL/6J mice to establish the transgenic lines. Coamplification of the genomic template using β subunit of the thyroid-stimulating hormone (β-TSH)-specific sense and antisense primers provided the 386-bp invariant internal control PCR product to determine false positives and negatives during identification of transgenic mice (26). The amplimer sequences for amplification of a segment of the murine β-TSH genomic DNA were 5′-TCCTCAAAGATGCTCATTAG-3′ and 5′-GTAACTCA-CTCATGCAAAGT-3′. All animal protocols were approved by The University of Texas Health Science Center at San Antonio Animal Care Committee and are in accord with the National Institutes of Health Guidelines for the Care and Use of Experimental Animals.

Transgene Expression: RNA Isolation and Reverse Transcription (RT)–PCR Analysis. Expression of transgene-specific transcripts and endogenous rAR mRNAs was analyzed by RT–PCR. Total RNAs from various mouse tissues were isolated by the guanidinium thiocyanate/phenol/chloroform/isoamyl alcohol procedure (27). The integrity of isolated RNA was determined from the ethidium bromide-stained pattern of RNA populations following their separation by agarose gel electrophoresis. The conditions for RT–PCR and the procedure for the competitive RT–PCR were the same as those reported earlier (19). To examine the extent of transgene expression in various tissues, the SV40 sequence-specific sense primer **a** (5′-TCTGCTCTAAAAGCTGCGGAATTG-3′) was used in conjunction with the rAR cDNA-specific antisense primer **b** (5′-GAGGCAGCCGCTCTCAGGGTG-3′). This primer pair yields a 450-bp RT–PCR product. The approximate locations of primers **a** and **b** within the transgene construct are shown in Fig. 1. The liver and prostate tissues of transgenic and normal animals were compared with regard to the extent of expression of the AR transgene and the endogenous AR gene. The sense primer used in this case spanned positions +3121 to +3140 of exon 5 of the rAR gene. The antisense primer spanned either positions +3571 to +3553 of rAR exon 8 (primer I in Fig. 3), for detection of total AR mRNAs derived from both the transgene and the endogenous gene, or positions +3834 to +3815 of exon 8 (primer II), for detection of the endogenous AR mRNAs alone. A 560-bp RT–PCR product of the constitutively expressed mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as the internal control. GAPDH-specific transcripts were detected by using the sense primer 5′-GGGTGATGCT-GGTGCTGAGTATAGT-3′ and the antisense primer 5′-GGATGCCCTTTAGTGGGCCCT-3′ (28).

Assay of DST mRNA and Enzyme Activity. Steady-state levels of DST mRNAs in the livers of transgenic and nontransgenic mice were examined by Northern blot analysis. Following standard Northern blotting procedures, the transferred RNAs on the nylon membrane were hybridized with the ^{32}P-labeled DST cDNA probe (10), and the hybridized RNAs were subsequently autoradiographed. After removal of the DST cDNA probe, the same RNA blot was rehybridized with a ^{32}P-labeled cDNA fragment of the mouse GAPDH mRNA. The radioactivities of the hybridized bands in the Northern blot were quantified with a Betascope blot analyzer. Conditions for cDNA hybridization and probe removal were as described (8).

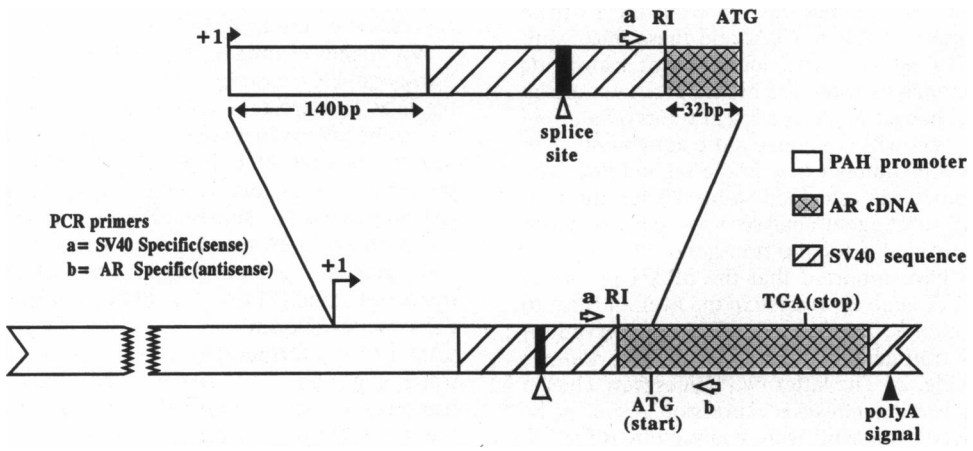

FIG. 1. The structure of the hPAH-rAR transgene construct. The cross-hatched box indicates the rAR cDNA fragment (2.8 kb) encoding the full-length rAR with the indicated translation initiation (ATG) and termination (TGA) sites. The hPAH promoter (≈10 kb, open box) provides the transgene transcription start site. The simian virus 40 (SV40) sequence contains the splice and polyadenylylation sites to ensure efficient transgene transcription. Use of primers **a** and **b** in the PCR can generate transgene-specific transcripts. The construct map is not drawn to the scale.

The enzymatic activity of DST in the liver cytosol was measured as described (29). In brief, the substrate dehydroepiandrosterone (DHEA) was incubated with ^{35}S-labeled 3′-phosphoadenosine 5′-phosphosulfate (NEN, DuPont) in the presence of the liver cytosol. Upon termination of the reaction, the reaction mixture was extracted with ethyl acetate and the radioactivity in the aqueous phase, containing the sulfated DHEA, was measured by scintillation spectrometry.

Immunohistochemical Analysis of AR. Immunohistochemical staining of frozen tissue sections was carried out by the avidin–biotin-peroxidase technique following the experimental conditions described earlier (30). Liver was perfused through the portal vein, first with Hanks' balanced salt solution and then with 4% formaldehyde in 0.1 M potassium phosphate (pH 7.4). Small pieces of the liver and prostate (about 2–3 mm^3) were immersion-fixed in 4% paraformaldehyde in 0.1 M potassium phosphate (pH 7.2) for 2 hr at 4°C and then cryoprotected in graded sucrose solutions (7% to final 30% in potassium phosphate at pH 7.4). The immunoglobulin G (IgG) fraction of the rabbit antiserum to the peptide fragment of the first 21 aa from the N terminus of the rat AR was used as the primary antibody (ref. 31; a gift from Gail S. Prins). Five-micrometer-thick tissue sections were incubated with the primary antibody (diluted in 3% normal goat serum) for 2.5 hr at room temperature. Biotinylated goat anti-rabbit IgG (Vector Laboratories) was used as the secondary antibody and, after color development with diaminobenzidine, sections were mounted for microscopic examination. No staining was observed when sections were treated with the preimmune rabbit serum (instead of the anti-AR antiserum), which served as the negative control for the immunostaining procedure.

RESULTS

In this study, we have used a hybrid transgene containing the hPAH promoter (≈10 kb) fused to the complete coding region of rAR cDNA. This construct also contains the sequences necessary for splicing (derived from the SV40 T-antigen gene), placed upstream of the rAR start codon, and the transcription termination signal (also derived from the SV40 sequence), placed at the 3′ end of the rAR cDNA (Fig. 1). The SV40 T-antigen splicing and polyadenylylation sites are known to allow enhanced expression of transgenes in the mouse (32). With the hPAH-rAR transgene construct, we were able to generate three founder mice, of which one failed in germline transmission of the transgene to the progeny and therefore was not analyzed any further. Several F_1 progenies were evaluated for transgene expression in the liver biopsy samples collected following partial hepatectomy of the animals at around 8 weeks of age. All of the biopsy samples derived from the F_1 mice showed high expression of AR mRNA, and these mice were subsequently bred to establish two independent transgenic lines. Southern blot analysis using the hPAH promoter as the hybridization probe showed single-site integrations of multiple copies of the transgene, with a heterozygote copy number of 5 in one line and a copy number of 2 in the second line. The transgene copy number has remained stable during the next five generations. All subsequent analyses were performed on mice that were heterozygous for the transgene.

Wang *et al.* (22) have reported that the hPAH promoter selectively directs CAT gene expression in the liver and also to some extent in the kidney. On the basis of these observations we investigated AR transgene expression in the liver, kidney, lung, and prostate (Fig. 2). The latter two tissues were chosen for their low and high endogenous AR expression, respectively. The transgene-derived transcripts were analyzed by RT–PCR using the SV40-specific sense primer **a** and the AR sequence-specific antisense primer **b** as described under *Materials and Methods.* These two primers only amplify the transgene-derived AR transcript. Constitutive expression of GAPDH-

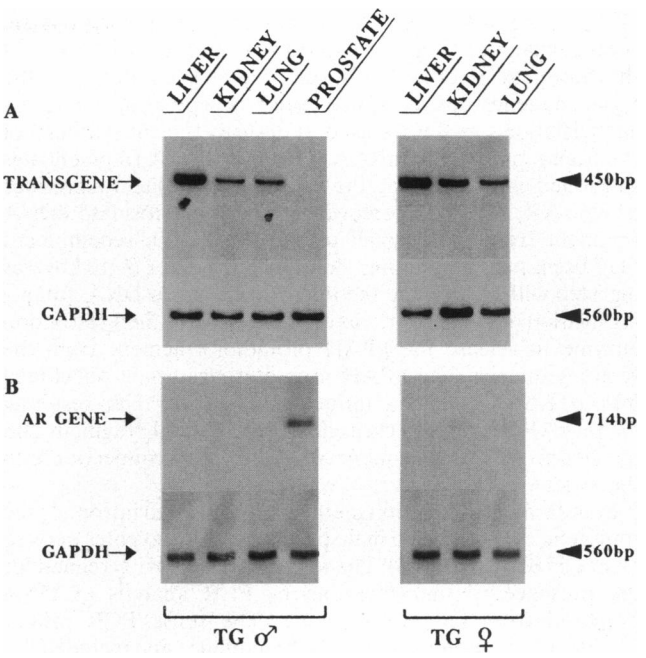

FIG. 2. AR gene expression in multiple tissues of transgenic male (TG ♂) and transgenic female (TG ♀) animals. (*A*) The transgene transcript yields a 450-bp RT–PCR product with primers **a** and **b** (the primers are shown in Fig. 1). (*B*) The endogenous AR transcripts with the sense primer at exon 5 and antisense primer II at exon 8 yield a 714-bp product (see Fig. 3). Constitutive expression of GAPDH mRNA was followed from the 560-bp RT–PCR product generated with GAPDH-specific sense and antisense primers. The results shown are representative of multiple animals from two different transgenic lines.

specific transcripts was used to normalize transgene expression in all four tissues (Fig. 2*A*). The PCR assays were performed under conditions where the extent of amplification from both the AR and GAPDH templates remained within the linear range. The results showed a robust expression of the transgene in the liver, kidney, and lung but no detectable expression in the prostate. On the other hand, when samples were analyzed for endogenous AR transcripts with primers specific to exon 5 and exon 8, a strong PCR band was noted only in the prostate, as expected (Fig. 2*B*). Results presented in Fig. 2 also show a similar extent of transgene expression in the liver, kidney, and lung of both male and female animals. The relatively high AR transgene expression in the lung is somewhat unexpected, since the original report of Wang *et al.* (22) showed almost negligible expression of the hPAH-CAT gene in the lung.

We further examined the relative steady-state levels of the transgene AR transcript and the endogenous AR transcript in the liver and prostate—the two tissues with highest levels of transgene and endogenous AR expression, respectively. These results are presented in Fig. 3. Amplification of total AR transcripts (endogenous plus transgene-specific, yielding a 451-bp product) was directed by the sense primer spanning a 20-bp region in exon 5 and the antisense primer I spanning a 19-bp region of exon 8. Amplification of the endogenous AR transcript alone (714-bp RT–PCR product) was achieved with the same sense primer at exon 5, but a second 20-bp antisense primer from a further downstream region of exon 8 (primer II) which is not part of the rAR cDNA sequence present in the transgene construct. Quantification of the AR mRNA within the total RNA derived from liver and prostate was also performed by competitive RT–PCR (19), and the results are presented in Table 1. These results (Fig. 3 and Table 1) make the following points: (*i*) the transgenic male and female livers contained similar amounts of transgene AR transcripts; (*ii*)

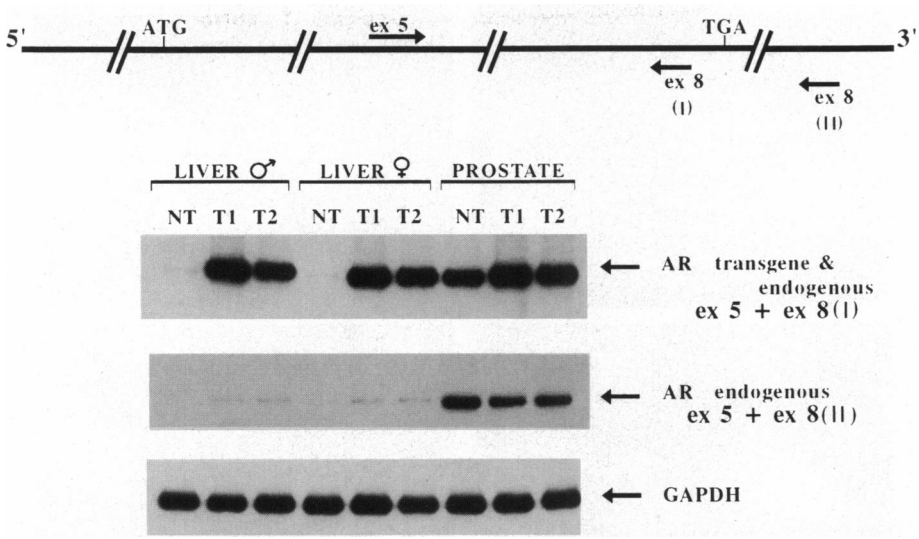

FIG. 3. Endogenous and transgene-derived AR expression in the liver and prostate. Lanes T_1 and T_2 represent two different transgenic animals; lanes NT represent a nontransgenic animal. The prostate and liver samples were derived from same transgenic males. The line drawing at the top depicts the full-length rAR mRNA (not drawn to scale). The general positions for the sense primer at exon 5 (ex 5) and the antisense primers I and II at exon 8 (ex 8) used to detect the endogenous and the transgene rAR transcripts are also shown in the line drawing. GAPDH expression was used as internal control.

transgene-derived AR expression in the liver occurred to approximately the same extent as endogenous AR expression in the prostate of the same animals; (*iii*) both transgenic and nontransgenic males exhibited roughly the same level of AR expression in the prostate; (*iv*) in normal mice, as expected, endogenous AR expression in the prostate was about 30-fold higher than that in the liver.

Cellular distribution of transgene expression in the liver of male and female mice was examined by immunohistochemical staining of the AR protein (Fig. 4). In the case of transgenic males, AR was distributed in both the cytoplasmic and nuclear compartments of hepatocytes, with selective enrichment in the nuclei (Fig. 4*A*). However, in the case of transgenic female livers, immunoreactivity was almost exclusively detected in the cytoplasmic compartment (Fig. 4*B*). Both the male and female transgenic mice express about the same level of AR mRNAs (Fig. 3). Because of the low level of immunoreactivity of the nontransgenic males, such a clear delineation of the nucleocytoplasmic differences in the distribution of AR in the liver of these animals was not readily apparent (Fig. 4*C*). The section of the prostate tissue which showed a high level of immunoreactivity in the nuclei of epithelial cells serves as a positive control (Fig. 4*D*).

Since hepatic expression of the AR in transgenic mice is ≈30-fold higher than nontransgenic males, we have examined possible virilization of the female liver at this unusually high receptor concentration. Such a downstream consequence of AR overexpression in the female liver, even in the absence of exogenously administered androgens, was reflected by a 3- to 4-fold repression of DST. In the Northern blot shown in Fig. 5*A*, high DST mRNA expression is evident in nontransgenic female livers; the RNAs from all three nontransgenic (NT) female mice show strong hybridizable bands with the DST cDNA as the radiolabeled probe (lanes N_1, N_2, and N_3). However, in contrast to normal females, the liver of transgenic females shows a marked repression of DST mRNAs (lanes T_1, T_2, and T_3). Comparison of the radioactivities in the DST cross-hybridizing bands for lanes N_1, N_2, and N_3 with those for lanes T_1, T_2, and T_3 showed that AR overexpression resulted in an ≈5-fold decrease in the steady-state DST mRNA level in the transgenic females. A similar degree of repression of the DST enzymatic activity in the liver of the same three transgenic females was observed (Fig. 5*B*). Thus, targeted overexpression of the AR alone in the liver, in the absence of any androgen supplementation, causes suppression of both DST mRNAs and the enzyme activity.

Table 1. AR mRNA in normal and transgenic mice, quantitated by competitive RT–PCR

Mouse	AR mRNA, ng/μg of total RNA	
	Liver	Prostate
Normal male	0.05	1.35
Transgenic male	1.53	1.50
Transgenic female	1.47	–

The competitor template constituted a truncated AR mRNA with an internal deletion of nucleotide positions 2662–2822 as described (19). The values are normalized to equal synthesis of cDNAs from total RNAs.

DISCUSSION

Because of their important role in reproduction and development, altered expression of the androgen and estrogen receptor genes in transgenic animals has generally been a challenging undertaking. However, successful disruption of the estrogen receptor gene in the mouse has been achieved with no apparent developmental abnormalities in the null animals of either sex (33). For AR transgene expression, Bingham *et al.* (34) have reported limited success in expressing the human AR in the mouse directed by the constitutive neuron-specific enolase (NSE) promoter and an interferon-inducible antiviral protein (M_x) promoter. Although the constitutive NSE promoter failed to produce immunodetectable human AR, the inducible M_x promoter-AR transgene produced human AR in some tissues including kidney and lung, albeit at a low level similar to the expression of endogenous mouse AR in these tissues. In contrast, our results show successful targeted overexpression of the AR via a constitutive tissue-specific promoter.

Both our transgenic lines show tissue-specific expression of the transgene, and so far no developmental or gross anatomical abnormalities have been noticed. Thus, we can cautiously conclude that overexpression of the AR in tissues targeted by the hPAH promoter does not interfere with the development of either the male or female fetuses. It may be of significance

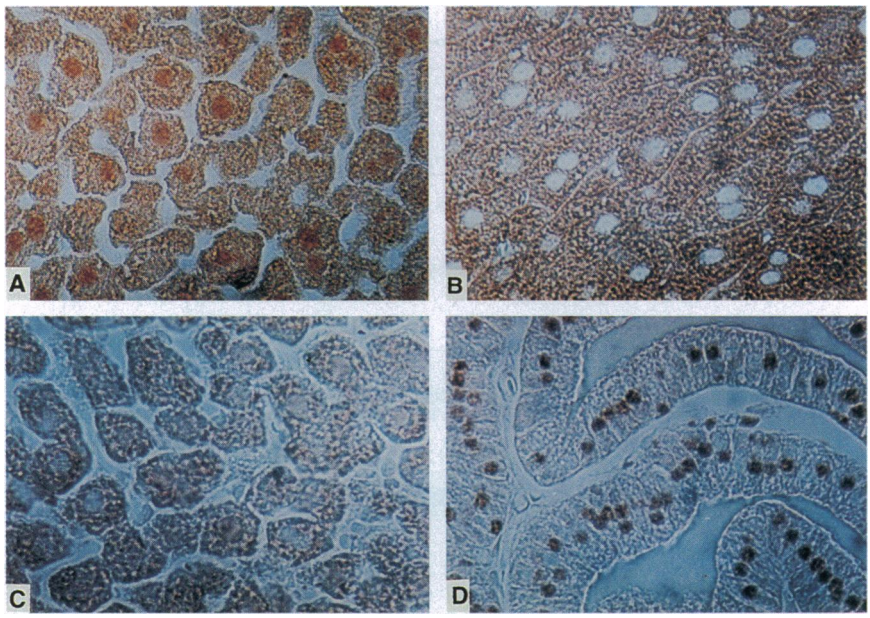

FIG. 4. Immunohistochemical localization of the AR in the liver of a transgenic male (*A*), liver of a transgenic female (*B*), liver of a nontransgenic male (*C*), and prostate of a nontransgenic male (*D*). In the transgenic male (*A*), both cytoplasmic and nuclear distribution of the AR is evident. For the transgenic female (*B*), the AR is almost exclusively localized in the cytoplasm. No immunostaining either in the liver or in the prostate was observed when preimmune rabbit serum was used in place of the anti-AR antiserum.

that despite the fact that both the male and female transgenic animals were reproductively competent, brother–sister mating has only rarely produced homozygous progenies. In fact, out of 50 batches of different litters born through matings of heterozygous transgenic mice, litters from only 2 batches are, at this point, candidate homozygotes. This finding may indicate that AR overexpression in the hPAH-targeted tissues can be tolerated only to a limited extent, and doubling this level of expression in homozygous progenies may be deleterious during embryogensis or pregnancy.

Binding studies with recombinant AR have shown that estradiol-17β can compete for the labeled methyltrienolone (a synthetic androgen) with 60–80% inhibition at a 100-fold molar excess of the unlabeled estrogen (35). Furthermore, at a high concentration, estradiol-17β is also capable of activating the AR to mediate transactivation of a mouse mammary tumor virus promoter (MMTV)-CAT reporter plasmid in transfected cells (35). We have confirmed these results and found that at a 100- to 1000-fold molar excess, estradiol-17β is almost equipotent to 5α-dihydrotestosterone in the AR-mediated transactivation of the MMTV-CAT construct in cultured PC3 cells (human prostatic carcinoma-derived) (36). These observations indicate that at least under *in vitro* conditions, the AR not only can crossreact with estradiol-17β but also can evoke the functional response. Our observation of partial virilization of the transgenic mouse liver with respect to the androgenic repression of DST, even without any androgen supplementation, may be considered as a physiological extension of these *in vitro* observations (37). However, at this point other possible mechanisms such as ligand-independent activation of a small subset of the receptor population (38) or activation by the low level of adrenal-derived androgens in the female cannot be discounted.

Initial studies with certain polycyclic aromatic hydrocarbons, such as benzo[*a*]pyrene, benz[*a*]anthracene, chrysene, and their proximate hydroxymethyl metabolites have indicated that DST-mediated sulfonation of these compounds converts them to electrophilic metabolites (13, 14). These metabolites can readily form DNA adducts due to the dissociation of the sulfate ion and generation of highly reactive benzylic carbonium ions. Although a number of sulfotransferases, including phenol sulfotransferase, estrogen sulfotransferase, and DST, are expressed in the liver, it appears that only the androgen-repressible DST is involved in the activation of these procarcinogens. Sulfonation of hydroxymethyl polycyclic aromatic hydrocarbons is not inhibited to any significant extent by pentachlorophenol, a potent inhibitor of the phenol sulfotrans-

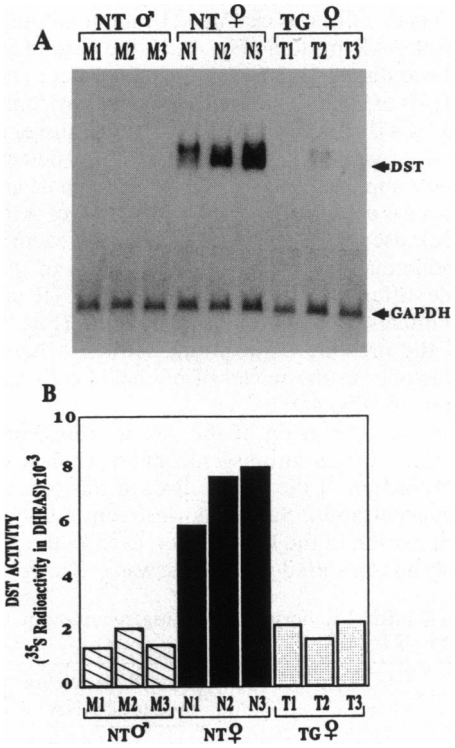

FIG. 5. DST mRNA levels (*A*) and enzyme activity (*B*) in nontransgenic and transgenic animals. N_1, N_2, and N_3 are three nontransgenic female mice. T_1, T_2, and T_3 are three transgenic female mice. M_1, M_2, and M_3 are nontransgenic males. The same Northern blot was hybridized initially with the DST cDNA probe and subsequently with the GAPDH cDNA probe. All animals are from the same line. The transgenic females from the second line also showed similar down-regulation of DST expression.

ferase, but is strongly inhibited by DHEA, a typical substrate for DST (14). Such a specific effect of DHEA may explain the molecular basis of a number of animal studies claiming that pharmacological doses of DHEA can serve as a prophylactic agent for certain specific types of cancer (39). The hPAH-AR transgenic mice and the wild-type counterpart will be of great value in elucidating the anticancer effect of DHEA and the age- and sex-specific differences in the hepatocarcinogenic potential of hydroxymethyl polycyclic aromatic hydrocarbons.

Finally, there has been a continuing controversy on the role of the steroid ligands in the translocation of steroid receptors from the cytoplasmic to the nuclear compartment. The initial proposal that binding of the steroid ligand activates the receptor (40) has been questioned by a number of investigators (41, 42). Those authors argue that steroid receptors either are intrinsically nuclear proteins or may exist in a dynamic equilibrium between the nuclear and cytoplasmic compartments irrespective of their interactions with steroid ligands (41–43). The issue of ligand-mediated nuclear translocation of the AR has specifically been the center of this controversy. Despite the results of cultured cells transfected with the AR expression vector, a lack of *in vivo* data on this subject has been a bone of contention for the critics of the two-step model. In the case of the hPAH-AR transgenic mice, immunohistochemical localization of the AR in the liver clearly shows that in contrast to the male, the overexpressed AR in the female is almost exclusively localized in the cytoplasm. These results provide further credence to the idea that, at least for the androgenic steroids, ligand binding may play an important role in AR translocation to the nucleus.

We thank Ms. Nyra White for secretarial assistance. The generous supply of the AR antibody from Dr. Gail Prins and the hPAH promoter from Dr. Savio Woo are gratefully appreciated. This work was supported by National Institutes of Health Grants PO1-AG06872, RO1-AG03527, and R37-DK14744. B.C. is a career scientist of the Department of Veterans Affairs.

1. Roy, A. K. & Chatterjee, B. (1983) *Annu. Rev. Physiol.* **45,** 37–50.
2. Roy, A. K., Chatterjee, B., Demyan, W. F., Milin, B. S., Motwani, N. M., Nath, T. S. & Schiop, M. J. (1983) *Rec. Prog. Horm. Res.* **39,** 425–461.
3. Mancini, M. A., Majumdar, D., Chatterjee, B. & Roy, A. K. (1989) *J. Histochem. Cytochem.* **37,** 149–157.
4. Scarlett, C. O. & Robins, D. M. (1995) *Mol. Endocrinol.* **9,** 413–423.
5. Jeffery, S., Carter, N. D., Clark, R. G. & Robinson, I. C. (1990) *Biochem. J.* **266,** 69–74.
6. Colby, H. D. (1980) in *Advances in Sex Hormones*, eds. Thomas, J. A. & Singhal, R. (Urban and Schwarzenger, Baltimore, MD), Vol. 4, pp. 27–73.
7. Waxman, D. J. (1988) *Biochem. Pharmacol.* **37,** 71–84.
8. Demyan, W. F., Song, C. S., Kim, D. S., Her, S., Gallwitz, W., Rao, T. R., Slomczynska, M. A., Chatterjee, B. & Roy, A. K. (1992) *Mol. Endocrinol.* **6,** 589–597.
9. Nagata, K., Ozawa, S., Miyata, M., Shimada, M., Gong, D. W., Yamazoe, Y. & Kato, R. (1993) *J. Biol. Chem.* **268,** 24720–24725.
10. Chatterjee, B., Majumdar, D., Ozbilen, O., Murty, C. V. R. & Roy, A. K. (1987) *J. Biol. Chem.* **262,** 822–825.
11. Surh, Y. J., Liem, A., Miller, E. C. & Miller, J. A. (1991) *Biochem. Pharmacol.* **41,** 213–221.
12. Falany, C. N. & Wilborn, T. W. (1994) *Adv. Pharmacol.* **27,** 301–329.
13. Watabe, T., Ogura, T., Satsukawa, M., Okuda, H. & Hiratsuka, A. (1994) *Chem. Biol. Interact.* **92,** 87–105.
14. Miller, J. A. (1994) *Chem. Biol. Interact.* **92,** 329–341.
15. Okuda, H., Nojima, H., Watabe, N. & Watabe, T. (1989) *Biochem. Pharmacol.* **38,** 3003–3009.
16. Dipple, A. (1976) in *Chemical Carcinogens*, ACS Monogr. 173, ed. Searle, C. E. (Am. Chem. Soc., Washington, DC), pp. 245–314.
17. Roy, A. K. (1977) *Eur. J. Biochem.* **73,** 537–543.
18. Song, C. S., Rao, T. R., Demyan, W. F., Mancini, M. A., Chatterjee, B. & Roy, A. K. (1991) *Endocrinology* **128,** 349–356.
19. Supakar, P. C., Song, C. S., Jung, M. H., Slomczynska, M. A., Kim, J. M., Vellanoweth, R. L., Chatterjee, B. & Roy, A. K. (1993) *J. Biol. Chem.* **268,** 26400–26408.
20. Supakar, P. C., Jung, M. H., Song, C. S., Chatterjee, B. & Roy, A. K. (1995) *J. Biol. Chem.* **270,** 837–842.
21. Roy, A. K., Vellanoweth, R. L., Chen, S., Supakar, P. C., Jung, M. H., Song, C. S. & Chatterjee, B. (1995) *Exp. Gerontol.*, in press.
22. Wang, Y., DeMayo, J. L., Hahn, T. M., Finegold, M. J., Konecki, D. S., Lichter-Konecki, U. & Woo, S. L. (1992) *J. Biol. Chem.* **267,** 15105–15110.
23. Hogan, B., Costantini, F. & Lacy, E. (1986) *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Lab. Press., Plainview, NY).
24. Chang, C. S., Kokontis, J. & Liao, S. T. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7211–7215.
25. Tan, J. A., Joseph, D. R., Quarmby, V. E., Lubahn, D. B., Sar, M., French, F. S. & Wilson, E. M. (1988) *Mol. Endocrinol.* **2,** 1276–1285.
26. Walter, C. A., Nasr-Schirf, D. & Luna, V. J. (1989) *BioTechniques* **7,** 1065–1070.
27. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
28. Tso, J. Y., Sun, X. H., Kao, T. H., Recce, K. S. & Wu, R. (1985) *Nucleic Acids Res.* **13,** 2485–2502.
29. Chatterjee, B., Song, C. S., Kim, J. M. & Roy, A. K. (1994) *Chem. Biol. Interact.* **92,** 273–279.
30. Mancini, M. A., Song, C. S., Rao, T. R., Chatterjee, B. & Roy, A. K. (1992) *Endocrinology* **131,** 1541–1546.
31. Prins, G. S., Birch, L. & Greene, G. L. (1991) *Endocrinology* **129,** 3187–3199.
32. Palmiter, R. D., Sandgren, E. P., Avarbock, M. R., Allen, D. D. & Brinster, R. L. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 478–482.
33. Lubahn, D. B., Moyer, J. S., Golding, T. S., Couse, J. F., Korach, K. S. & Smithies, O. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11162–11166.
34. Bingham, P. M., Scott, M. O., Wang, S., McPhaul, M. J., Wilson, E. M., Garbern, J. Y., Merry, D. E. & Fischbeck, K. H. (1995) *Nat. Genet.* **9,** 191–196.
35. Kemppainen, J. A., Lane, M. V., Sar, M. & Wilson, E. M. (1992) *J. Biol. Chem.* **267,** 968–974.
36. Chan, J., Song, C. S., Chatterjee, B. & Roy, A. K. (1995) *77th Endocrine Society Meeting* (Am. Chem. Soc., Washington, DC), (abstr.).
37. Roy, A. K. & Chatterjee, B. (1995) *Crit. Rev. Eukaryotic Gene Exp.* **5,** 157–176.
38. Power, R. F., Mani, S. K., Codina, J., Conneeley, O. M. & O'Malley, B. W. (1991) *Science* **254,** 1636–1639.
39. Schwartz, A. G., Whitcomb, J. M., Nyce, J. W., Lewbart, M. L. & Pashko, L. L. (1988) *Adv. Cancer Res.* **51,** 391–424.
40. Jensen, E. V., Suzuki, T., Kawashima, T., Stumpf, W. E., Junglelut, P. W. & Desombre, E. R. (1968) *Proc. Natl. Acad. Sci. USA* **59,** 632–638.
41. Sheridan, P. J., Buchanan, J. M., Anselmo, V. C. & Martin, P. M. (1979) *Nature (London)* **282,** 579–582.
42. Gorski, J., Malayer, J. R., Gregg, D. W. & Lundeen, S. G. (1994) *Endocr. J.* **2,** 99–100.
43. Guiochon-Mantel, A., Lescop, P., Christin-Maitre, S., Loosfelt, H., Perrot-Applanat, M. & Milgrom, E. (1991) *EMBO J.* **10,** 3851–3859.