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Tissue-Specific and Hormonal Regulation of the Gene for Rat Prostatic Steroid-Binding Protein in Transgenic Mice

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We investigated the tissue-specific and hormonal regulation of the gene for rat prostatic steroid-binding protein by introducing the C3(1) gene with 4-kilobase (kb) upstream and 2-kb downstream flanking sequences into transgenic mice. There was selective expression in the ventral prostate that was stimulated by testosterone, which indicated that the gene together with 6-kb flanking DNA contains the information required for prostate-specific and testosterone-regulated expression.

Prostatic steroid-binding protein is the predominant protein secreted into rat prostatic fluid (7). The protein is an oligomer containing C1, C2, and C3 polypeptides (8) whose expression is stimulated markedly by testosterone (15). In common with other classes of steroid hormone (21), the mechanism whereby androgens regulate the expression of the steroid-binding protein is via effects on rates of gene transcription; in addition, androgens also modulate nuclear RNA turnover (13, 22). There are two nonallelic genes for the C3 polypeptide: C3(1), which is expressed in the ventral prostate, and C3(2) (10, 17). We have demonstrated specific binding of androgen receptors to a region of DNA within 300 base pairs of the C3(1) promoter and within the first intron (18), but functional androgen response elements have yet to be identified. The genes were accurately transcribed after transfection into a number of cell lines that contain androgen receptors, but their expression was not regulated by testosterone (14, 16).

The most likely explanation for the lack of hormonal regulation is the absence of prostate-specific factors in the cell lines that were used. Therefore, in the absence of normal prostate epithelial cell lines for transfection purposes, we have introduced the C3(1) gene into transgenic mice. The approach of gene transfer into the mouse germ line has been used in conjunction with, or as an alternative to, transfection in order to identify and characterize DNA regulatory elements involved in tissue-specific or hormonally regulated gene expression in a number of other genes (3, 11, 19, 20).

We constructed transgenic mouse lines expressing the rat C3(1) gene by microinjecting a 9.5-kilobase (kb) *Bam*HI fragment of the gene (Fig. 1; 17) into the fertilized eggs of C57 × SJL (F1) mice (9). This fragment contains the coding sequences for the C3 subunit, 4.3 kb of 5′-flanking sequence, and 2.2 kb of downstream flanking sequence. Of the 53 mice that developed from these eggs, 5 were transgenic, as determined by Southern analysis of tail DNA by using a 1.1-kb *Pst*I fragment of the C3 gene (Fig. 1A) as a DNA

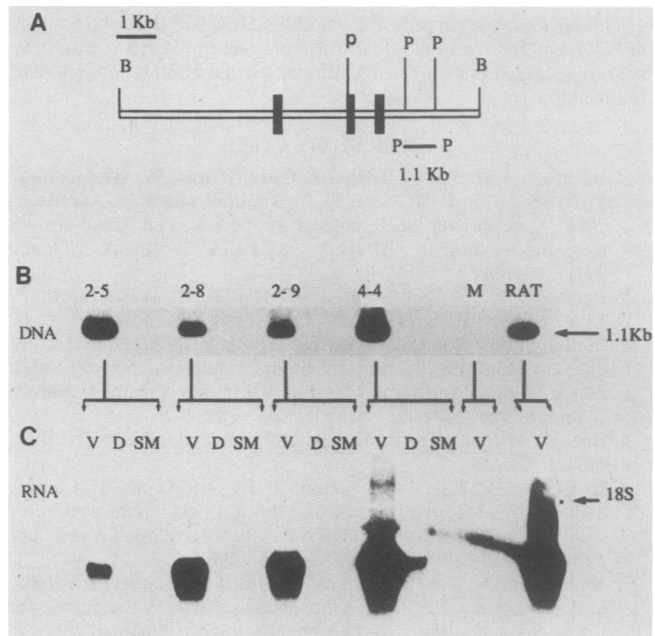

FIG. 1. Identification of the rat C3 transgene and its expression in the ventral prostates of transgenic mice. (A) Restriction map of a 9.5-kb *Bam*HI fragment containing the C3(1) gene that was microinjected into fertilized mouse eggs. B, *Bam*HI; P, *Pst*I. (B) Southern analysis of tail DNA (10 μg) from transgenic offspring of the four founders carrying the rat C3 transgene. The DNA was digested with *Pst*I, and the DNA probe was derived from the 1.1-kb *Pst*I fragment of the rate C3 gene. M, Nontransgenic mouse DNA (10 μg); RAT, 0.23 ng of the C3 *Bam*HI fragment, corresponding to 10 copies of the C3(1) gene, digested with *Pst*I. (C) Northern analysis of total RNA (5 μg) from the ventral prostate (V), dorsal prostate and coagulating gland (D), and seminal vesicle (SM) of 60- to 63-day-old offspring from each of the four lineages and from the ventral prostate of a nontransgenic mouse (M) and a rat. The DNA probe was a 270-bp *Bst*EII-*Bgl*II fragment of the rat C3 cDNA, representing approximately half of the mRNA (17). (The smudge across the gel blot that trails from the rat RNA control resulted from seepage of sample at the time of gel loading.)

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TABLE 1. Transgenic offspring of the C3 founders

Founder	1st generation (No. transgenic/no. of offspring)		Transmission ratio (%)	No. of gene copies transmitted	2nd generation[a] (no. of transgenic/no. of offspring)	
	Female	Male			Female	Male
2-5	3/10	5/10	40	20–30	11/26	13/23
2-8	0/6	2/9	13	10	13/19	14/28
2-9	6/8	7/15	56	10–15	11/16	5/15
4-4	2/19	7/26	20	50	8/15	2/8

[a] Derived from transgenic male offspring of the first generation mated to normal C57 × SJL (F1) females.

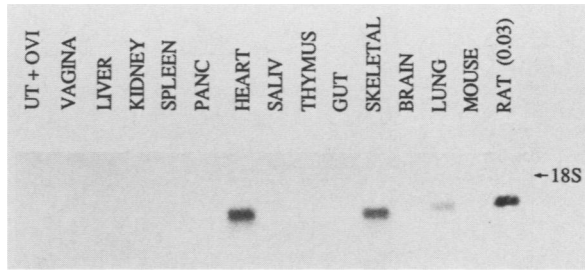

FIG. 3. Expression of the rat C3(1) gene in various tissues of a C3 transgenic female mouse. Total RNA (25 μg) was isolated from tissues of a female from lineage 4-4 and analyzed by Northern blotting. UT + OVI, Uterus plus oviduct.

probe. One transgenic mouse (a female) died; the four other founders (males), designated 2-5, 2-8, 2-9, and 4-4, were used to establish transgenic mouse lines by mating to C57 × SJL (F1) females. Offspring were screened for the transgene by DNA dot blot analysis of tail DNA. The founder males of lineages 2-8 and 4-4 were mosaic in the germ line, as shown by the transmission ratio for the transgene in offspring of these founders (Table 1). All founders transmitted multiple copies of the transgene (Fig. 1B, Table 1) to female and male offspring, ranging from about 10 copies in line 2-8 to 50 copies in line 4-4, as judged by comparison with cloned rat DNA; all lineages contain the complete construct, as determined by Southern analysis with 5′- and 3′-flanking DNA probes of the C3 gene (not shown).

The tissue specificity of C3 transgene expression was analyzed in 60- to 63-day-old male offspring from each of the four transgenic founders by Northern (RNA) blotting. Total RNA was isolated from tissues of a single mouse by the guanidinium isothiocyanate procedure (2), with subsequent fractionation over a CsCl cushion, using a TL-100 ultracentrifuge and the TLA 100.2 rotor (Beckman Instruments, Inc., Fullerton, Calif.). Initially, RNA from the ventral prostate, dorsal prostate, and seminal vesicle from each of the lineages was separated by electrophoresis on a 1% agarose gel that contained 2.2 M formaldehyde (12). The Northern blots were then analyzed for C3 gene expression by using a *Bst*EII-*Bgl*II fragment of the rat C3 cDNA (17). As judged by scanning autoradiographs, each line expressed C3 mRNA in the ventral prostate (Fig. 1) at concentrations ranging from about 1% for line 2-5 to 10% for lines 2-8 and 2-9 to 50% for line 4-4 in comparison with the levels found for rat ventral prostate. Since there was no correlation between the amount of C3 mRNA expression and the number of integrated transgene copies (compare Fig. 1B and C), it is unlikely that all copies of the C3 gene were being expressed in each line. It is doubtful, therefore, whether the transgene contained DNA sequences analogous to those associated with the human β-globin locus which confer position-independent copy number-dependent gene expression (5). C3 gene expression was not detected in the dorsal prostate or seminal vesicle. A more extensive tissue survey (Fig. 2) indicated that expression in the testes was about 5% in line 2-9 and less than 1% in lines 2-8 and 4-4 of the level in the prostate and that expression was lower (<1%) in the heart, pancreas, and salivary gland. In addition, we failed to detect abundant C3 RNA in tissues from female mice, including one that had been treated with testosterone (data not shown). A female of the 4-4 lineage showed low levels (<0.1%) of C3 RNA in the heart, skeletal muscle, and lung (Fig. 3). A female of the 2-8 lineage expressed low levels (<0.1%) of C3 RNA in the pancreas and heart (not shown); thus, females appeared to

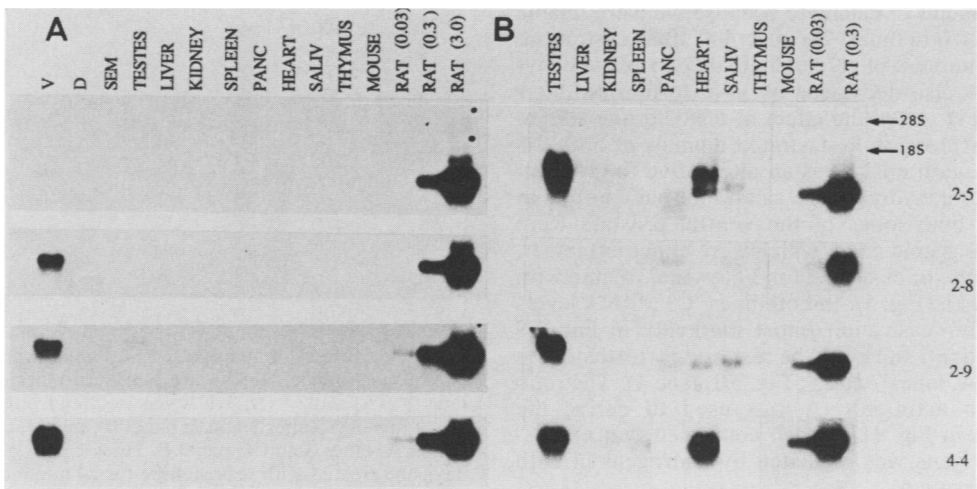

FIG. 2. Expression of the rat C3(1) gene in various tissues of C3 transgenic mice. (A) Northern analysis of total RNA (3 μg) from the ventral prostate and other tissues of offspring from the four founder lineages. (B) As for panel A, but with 25 μg of total RNA loaded onto the gel. SEM, Seminal vesicle; PANC, pancreas; SALIV, salivary gland; MOUSE, nontransgenic mouse ventral prostate total RNA (25 μg); RAT, rat ventral prostate total RNA (0.03, 0.3, and 3 μg).

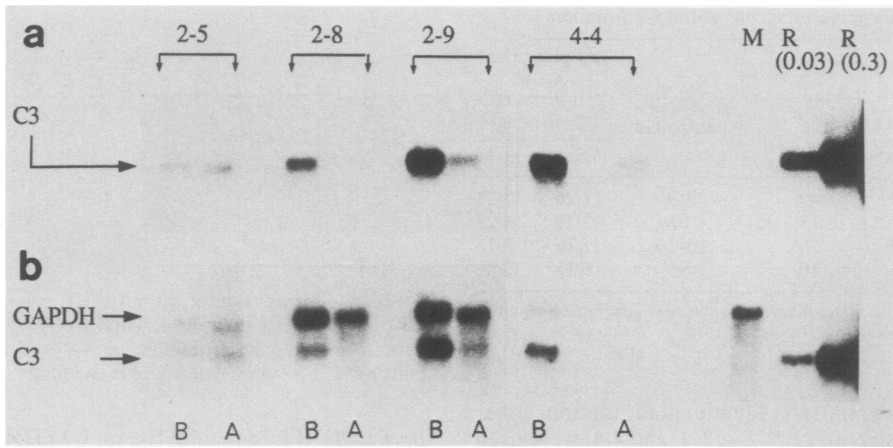

FIG. 4. Effect of castration on expression of the rat C3(1) gene in the ventral prostate of C3 transgenic mice. Equivalent amounts of RNA from each lineage, as judged by ethidium bromide staining, were analyzed by Northern blotting for C3 mRNA expression (a) and glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA (b). Total RNA from 67- to 72-day-old mice of each lineage was isolated before castration (lanes B; from left lobe of ventral prostate) and 3 days after castration (lanes A; from right lobe of ventral prostate). Shown on the right are ventral prostate RNAs from a nontransgenic mouse (M) and a rat (R).

be similar to males of equivalent lineages with respect to the pattern of ectopic C3 RNA expression. Therefore, although mice do not express a homologous gene for rat prostatic steroid-binding protein, in three of four transgenic mouse lines the C3(1) gene was expressed selectively in the ventral prostate. We conclude that the gene, together with 6 kb of flanking DNA, contains the necessary information required for prostate specific expression.

We investigated the effect of androgens on C3 gene expression in 67- to 72-day-old mice from each of the four lineages. The left lobe of the ventral prostate was removed from each mouse, the mouse was castrated, and 3 days later the right lobe was removed. Total RNA was isolated from each lobe, and the yield was estimated by running a sample on a nondenaturing agarose gel in the presence of ethidium bromide. The total RNA yield at the time of castration was about four- to sixfold greater than after castration for 3 days. When equivalent amounts of total RNA were loaded onto gels, we found that C3 RNA was markedly reduced after castration in lines 2-8, 2-9, and 4-4 (Fig. 4), but the fold reduction was difficult to calculate because we were unable to detect the RNA in lines 2-8 and 4-4 after castration. Surprisingly, the amount of glyceraldehyde phosphate dehydrogenase mRNA also decreased by two- to fourfold after castration. We next tested the effect of testosterone administration on C3 expression in castrated animals of lines 2-8 and 2-9 by using actin mRNA as an alternative to glyceraldehyde phosphate dehydrogenase as an internal control. In this experiment, both lobes of the ventral prostate were removed from transgenic mice, which were either left intact, castrated for 3 days, or castrated for 3 days and treated with testosterone for 24 h (Fig. 5). In both lines, C3 mRNA levels were reduced after castration (most markedly in line 2-8 [Fig. 5A, lanes 3 to 6]) and could be restored by testosterone treatment (Fig. 5A, lanes 7 and 8; Fig. 5B, lane 4). The ratio of C3 mRNA to actin mRNA was used to derive the histogram shown in Fig. 5C, which confirmed that expression of the C3(1) gene was regulated by androgens in both lines of transgenic mice.

We conclude that the C3 gene, together with 6 kb of flanking DNA, contains the information required for both testosterone-regulated and tissue-specific expression. Although it is conceivable that the genes contain androgen

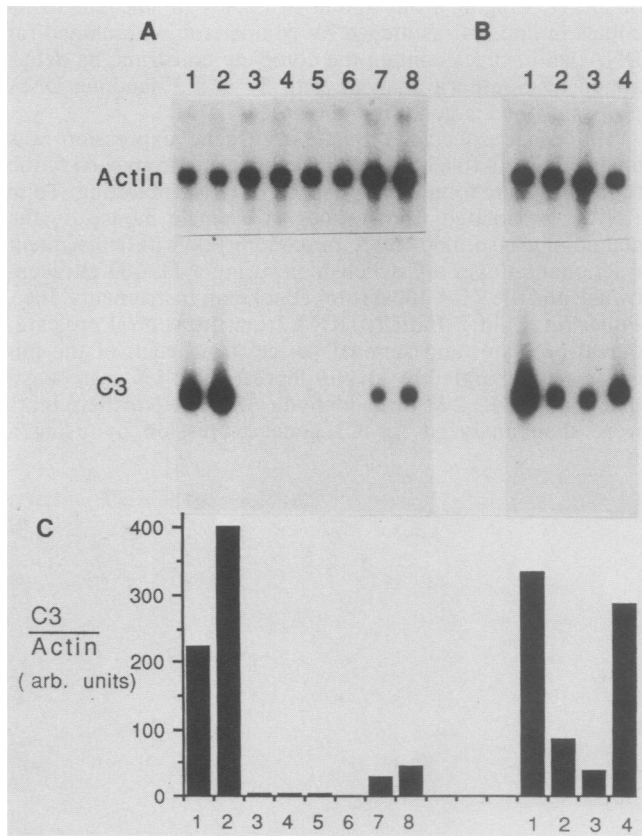

FIG. 5. Effects of androgens on C3 gene expression in prostates of transgenic mice. Shown are Northern blots of total RNA from lines 2-8 (A) and 2-9 (B). RNA was isolated from mice that were left intact (panel A, lanes 1 and 2; panel B, lane 1), castrated for 3 days (panel A, lanes 3 and 4; panel B, lanes 2 and 3), or castrated for 3 days and treated with testosterone for 24 h (panel A, lanes 7 and 8; panel B, lane 4). Autoradiographs were scanned on an LKB UltraScan XL to derive an integrated peak area for actin and C3 mRNA and thereby generate the C3/actin ratio shown in the histogram (C). Numbers under the histogram correspond to the lane numbers in the autoradiographs.

response elements similar to those identified in mouse mammary tumor virus (1, 4, 6), these results with the entire gene do not distinguish between transcriptional and posttranscriptional effects on gene expression. Our future work will therefore be directed toward mapping the boundaries of DNA regulatory elements involved in tissue-specific and hormonal regulation by characterizing C3 fusion genes.

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