Androgen Repression of the Production of a 29-Kilodalton Protein and Its mRNA in the Rat Ventral Prostate

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The regression of the ventral prostate, after a rat is deprived of androgens by castration, is accompanied by a marked decrease in the prostate’s ability to synthesize RNA and major proteins. Surprisingly, in vitro translation of prostate RNA, isolated from rats 2 days after castration, detects four proteins with Mr of approximately 29,000, 37,000, 46,000, and 49,000 whose message levels increased 4- to 12-fold compared to results from normal rats. According to cDNA dot hybridization analysis, the increase after castration in the level of the 29-kDa protein-mRNA (per unit amount of DNA) was reversed within 6 h by androgen treatment of castrated rats. In contrast, the level of a mRNA in male rat liver, which hybridized to a cloned probe for the prostate 29-kDa protein-mRNA was reduced by castration and increased by androgen treatment.

During an in vitro incubation, the ventral prostates of normal rats were much less efficient than the prostates of rats castrated 2 days earlier in synthesizing a 29-kDa protein. Despite the fact that androgenic manipulation of rats induced very rapid and significant changes in the production of the 29-kDa protein and in the level of its mRNA, the cellular level of this protein in the prostate, as determined by radiolmmunoassay, was maintained at near normal values throughout the 2-week experimental period. Thus, the prostate appears to have a mechanism, based on androgen repression of certain genes, to maintain the cellular levels of the 29-kDa protein and possibly other structurally or functionally important proteins during both the periods of androgen-dependent growth and the castration-induced regression. The loss of such a regulatory mechanism may result in androgen-independent abnormal prostate growth.

Androgens are necessary for maintaining the growth and secretory functions of the ventral prostate in rats. Deprivation of androgens by castration causes a dramatic disintegration, within a week, of cytoplasmic structures, such as the endoplasmic reticulum, ribosomes, and mitochondria in this male accessory sex organ (1, 2). These degradative effects of castration are effectively reversed by the administration of testosterone, which appears to act in the prostate by being converted to 5α-dihydrotestosterone (3, 4).

Androgens have been shown to increase the overall levels of mRNA associated with ribosomes (5), nuclei (6), and whole prostate (7). Many investigators have also shown androgen-dependent increases in mRNA for α-protein (also called prostate-binding protein) (8-10) and sperm-o-binding protein (11, 12) in the rat ventral prostate, for α→globulin in the rat liver (13), and for secretory proteins in rat and guinea pig seminal vesicles (14, 15).

In this report we show that androgens, while stimulating the production of mRNA for many proteins, can also suppress the synthesis of mRNA for a specific protein in the rat ventral prostate. This finding indicates that the androgen-dependent modulation of the growth and functions of a target organ involves both the enhancement and suppression of the expression of different groups of genes.

**EXPERIMENTAL PROCEDURES**

**RESULTS**

**Initial Identification of Androgen-repressed mRNAs**—Androgen-repressed mRNAs were initially identified by the in vitro translation of RNA prepared from rat ventral prostate (Fig. 1). Upon castration the translation products with Mr smaller than 20,000, representing mainly the androgen-dependent secretory proteins, such as α-protein components (8), decreased markedly within 2 days. The production of several other translation products, however, is clearly increased. Their apparent induction was seen within the first day following castration. Four of these translation products had Mr of 29,000, 37,000, 46,000, and 49,000 based on SDS-PAGE. A densitometric scan made of each lane shown in Fig. 1 indicated that 2 days after castration, the percent in-crease in these translation products relative to total products was as much as 12-fold for the 37-kDa translation product and 11-48 kDa), 4- (45 kDa), and 8- (29 kDa) fold for the other translation products.

**Preparation of a cDNA Library**—The in vitro translation...
assay indirectly measures mRNA levels via their translatability, and so factors that influence translation may affect accurate measurements of the message levels. In order to obtain a more quantitative measurement of the levels of at least one of these mRNAs, we prepared and cloned cDNAs to these mRNAs. Randomly selected clones from a cDNA library, prepared from poly(A)+ RNA enriched by gradient centrifugation, indirectly measures mRNA levels via their translatability into proteins (Fig. 2).

To demonstrate that the antibodies cross-reacted with the protein encoded by the androgen-repressed mRNA, we initially compared the SDS-PAGE patterns of radioactive proteins produced during the in vitro incubation with L-[3H]leucine of mince prostate RNA isolated from normal, castrated, and androgen-treated castrated rats. As shown in Fig. 3, an approximate 10-fold increase in the amount of 29-kDa protein-mRNA per unit of RNA was seen 2 days after castration as compared to normal. This increase was suppressed by androgen treatment. A control experiment using pBR322 as the probe showed no significant hybridization.

Within 3 days after castration or androgen injection prostate RNA levels are altered significantly (6, 16), the increase in the 29-kDa protein-mRNA per unit of RNA may reflect no change in the level of this mRNA per cell but only an increased proportion of the 29-kDa protein due to declining levels of abundant mRNAs which require androgens for their induction. Therefore, the level of the mRNA for the 29-kDa protein was normalized per unit of DNA, since DNA levels do not change significantly within 3 days after castration (16), and normalization based on DNA content should also account for changes in cell number in the prostate during the experimental period. Even after normalization there is more than a 3-fold increase in this mRNA per unit amount of DNA 2 days after castration (Fig. 4). The castration-induced increase was seen as early as 1 day after castration, while 3 days the levels gradually decreased. The castration-induced increase was rapidly reversed by injection of 5α-dihydrotestosterone. Essentially all the castration-induced increase was abolished within 12 h of androgen injection. In three experiments we have observed that within 1 day after androgen treatment the mRNA level of the 29-kDa protein decreased to about 20% below the level seen in the intact animal but returned to normal levels thereafter. The level of this mRNA decreased to about 75% of the normal level 7 days after castration. Injection of 5α-dihydrotestosterone at this time resulted in a rapid decline in the level of this mRNA within 24 h of treatment. After 5 days of continuous 5α-dihydrotestosterone treatment, the level of this mRNA then increased to 90% of normal values. If androgen was injected at the time of castration, the mRNA level was maintained at the normal value throughout the 2-week experimental period.

After castration, the amount of RNA per unit of DNA in the ventral prostate decreased, by about 50% in 2 days, and continued to decrease to about 15% by the 12th day. As expected (2, 6, 16), the RNA content of the prostate increased after castrated rats were treated with androgen (Fig. 4).

Identification of 29-kDa Protein-mRNA in Other Tissues—The 29-kDa protein-mRNA was detected by dot hybridization in liver, brain, and uterus, but not in kidney. In normal male rat liver, there was as much 29-kDa protein-mRNA per unit of DNA as in normal prostate. Within 2 days following castration, the level of this mRNA in male liver was reproducibly reduced to about 40% of the normal value. This decrease was reversed by androgen injection. Female liver contained only about 20–40% of the level found in male liver.

Identification and Purification of 29-kDa Protein Product of Androgen-repressed mRNA—To isolate the protein encoded by the androgen-repressed mRNA, we initially compared the SDS-PAGE patterns of radioactive proteins produced during the in vitro incubation with L-[3H]leucine of mince prostate RNA isolated from normal and castrated rats. Cytosol fractions from the incubated prostates were prepared and radioactively labeled proteins produced were analyzed by SDS-PAGE (Fig. 5). In this experiment, we found that the synthesis of a radioactive 29-kDa protein was more prominent in the prostate of rats castrated 2 days earlier than that found in the prostate of normal rats. Many other proteins were synthesized in equal or lower amounts in castrated rat prostates. The radioactive 29-kDa protein passed through both DEAE-Sepahcel and phosphocellulose columns when applied in buffers with pH ranging from 6.2 to 9.0. At pH 7.6, the protein also did not bind tightly to DNA or concanavalin-A Sepharose. After passing through these columns, the protein was at least 90% pure on the basis of SDS-PAGE (Fig. 6). According to Sephadex G-100 chromatography, the protein appeared to exist as an oligomer, probably a dimer or trimer. The pI of the protein was 7.2. The protein was not iodinated well using NaI[125] and chloramine T, indicating that it may not have exposed tyrosine residues. The protein does not dissolve readily into an aqueous buffer once lyophilized.

Using DEAE-Sepahcel chromatography and SDS-PAGE (Fig. 6), we followed the production of the radioactively labeled 29-kDa protein during the incubation of minced prostate tissue with L-[3H]leucine. The incorporation of radioactivity into the 29-kDa protein was linear up to 6 h of incubation. Addition of cycloheximide (10 μg/ml incubation mixture) reduced the incorporation of the radioactive amino acid into the protein by 95%, indicating that the incorporation was dependent on protein synthesis.

Within 2 days after castration, the production of this protein per unit amount of total radioactive proteins made or total tissue protein present in the rat prostate increased by about 150–250%. This increase leveled off afterward. The castration-induced increase per unit of DNA (or per cell) was about 80% (Fig. 7). Since we did not analyze the amino acid pool in the prostate, the actual change in the production rate may be different from these values.

Radioimmunoassays of 29-kDa Protein—Antibodies to the purified 29-kDa protein were raised in rabbits and tested for their immunological specificity by immunoblot analysis using 125I-protein A. Antibodies and 125I-protein A predominantly bound to a protein with M, of about 29,000 when the nitrocellulose filter was incubated with the antisera of rabbits immunized with the 29-kDa protein (Fig. 8A). No protein A binding occurred when the filter was incubated with preimmune rabbit sera. Amido black staining of the nitrocellulose after transfer indicated a representative transfer of proteins of all molecular sizes (Fig. 8, B and C).

To demonstrate that the antibodies cross-reacted with the protein encoded by the androgen-repressed mRNA, the mRNA was first isolated from a prostate RNA preparation by hybridization to the plasmid containing its cDNA and then translated in vitro. When the translated products were immunoprecipitated and analyzed by SDS-PAGE, the major immunoprecipitated protein was found to have a M, of 29,000.
Androgen-repressed Gene Expression

The level of the 29-kDa protein in rat ventral prostate was measured by radioimmunoassay and immunoblotting. The 29-kDa protein represented about 1% of the cytosol protein in the prostate. About 75% of this protein was in the cytosol fraction with the rest being in the extracellular fluid. No significant amount of the protein was found in 0.4 M KCl extracts of nuclei or other cellular particulate fractions. The level of the 29-kDa protein per unit amount of cytosol protein gradually increased to about 200% of the normal value 12 days following castration. By comparison, the levels of many other major prostatic secretory proteins decrease to less than 10% of normal within 5 days after castration (12). These effects of castration were reversed by injection of castrated rats with 5a-dihydrotestosterone (Fig. 4B).

The level of the 29-kDa protein per unit amount of DNA was maintained at about the same level throughout the 2-week experimental period shown in Fig. 4B. During this period androgen manipulation of rats caused rapid and dramatic changes in the mRNA level (Fig. 4A) and the prostate's ability to synthesize the radioactive 29-kDa protein (Fig. 7). The protein represented about 2% of the soluble protein in liver. No significant change in the 29-kDa protein level was found in the liver upon castration. In the kidney, the mRNA for this protein was not detected by dot hybridization analysis, but a low level of the 29-kDa protein was detected by radioimmunoassay. The 29-kDa protein was not detected in rat sera.

DISCUSSION

Anderson and his associates (17) have analyzed rat ventral prostate proteins by two-dimensional gel electrophoresis and identified several proteins whose relative levels increase upon castration and decrease after androgen treatment. Proteins were identified by staining gels with Coomassie blue; thus, it is not known whether these differences reflect changes in the synthesis of these proteins and their mRNAs or more complex nongenomic processes. Rennie et al. (18) studied the effect of castration and 5a-dihydrotestosterone treatment on plasminogen activator activity in the rat ventral prostate. The activity of plasminogen activators increased 10-fold 7 days after castration; subsequent 5a-dihydrotestosterone treatment reduced the activity of this class of proteolytic enzymes. Enzymes of this type may be involved in the involution of prostatic tissue upon castration (18, 19). Although de novo synthesis of plasminogen activators was not demonstrated, it was postulated that androgens may repress the gene(s) for this enzyme activity. Castration and loss of occupied androgen receptors would lead to derepression of this gene. Very recently, Montpeit et al. (20) have also described androgen-repressed mRNAs in the rat ventral prostate. They identified by in vitro translation several mRNAs whose levels increased after castration. Differential Northern hybridization analysis, using labeled cDNA prepared from prostate mRNA of normal and castrated rats, was used to demonstrate increases in the levels of two prostatic mRNAs (approximately 1 and 2 kilobases long) after castration. The peak level of these mRNAs were reached 4 (2-kilobase mRNA) and 8 (1-kilobase mRNA) days after castration. Therefore, these mRNAs may be distinct from the mRNA for the 29-kDa protein described here, which has a peak mRNA level at 2–3 days after castration and a mRNA of approximately 1.2 kilobases.

Hiremath et al. (21) reported previously that the concentrations of certain mRNAs in the poly(A)+ RNA fraction of the ventral prostates of castrated rats were higher than that in the equivalent fraction of normal rats. These investigators suggested that these mRNAs were stable androgen-insensitive mRNAs (transcription products of cellular housekeeping genes) and attributed the difference to the preferential loss after castration of the relatively short-lived mRNAs for the androgen-sensitive proteins. Our present study, however, showed that, at least for the 29-kDa protein-mRNA, there was an actual increase in the amount of the mRNA and in the apparent rate of synthesis of the 29-kDa protein per unit amount of the prostate or DNA. This finding indicates that deprivation of androgens by castration can cause either an increase in the synthesis or a decrease in the degradation of mRNA in the ventral prostate.

The decrease in the 29-kDa protein-mRNA, after an injection of castrated rats with androgens, occurred rather rapidly (Fig. 4A); a loss of about 50% of the mRNA was seen within 6–8 h after androgen administration. The half-life of this mRNA, therefore, appeared to be comparable to or shorter than the half-life of mRNAs for androgen-stimulated secretory proteins, such as a-protein components which have a half-life of about 6–12 h (9). The possibility that androgens may repress the synthesis of mRNA for certain proteins in the prostate is interesting since androgens have been shown to enhance the general synthesis of RNA and proteins in the prostate (2, 7). The alternative possibility that androgens accelerate the degradation of the 29-kDa protein-mRNA cannot be excluded, although it has been suggested that androgens and other steroid hormones can confer stability on the gene transcripts coding for steroid hormone-induced proteins, such as a-protein (22) and ovalbumin (23).

The increase in the cellular level of the mRNA and in the synthesis of the 29-kDa protein in the prostate after castration were not accompanied by a significant change in the amount of the 29-kDa protein in the prostate (Fig. 4B). This may suggest that the stimulatory effect of castration on the synthesis of the protein and its mRNA may be needed to compensate for the gradual but general impairment of the protein synthesizing machinery (1, 2) and increases in the degradative activities of hydrolytic enzymes (19) in the prostate after castration. The 3- to 4-fold increase in the mRNA level for the 29-kDa protein 2 days after castration (Fig. 4A) appeared to provide only an 80% increase in the rate of production of this protein by the prostate (Fig. 7) when normalized per unit amount of DNA. This increase was probably necessary to maintain the protein level close to normal values while the prostate continues to regress in the absence of androgens.

Whether the mechanism involved in the selective repression of the level of certain mRNAs is dependent on a direct interaction of an androgen-receptor complex with a specific gene is not clear. It is not inconceivable that some of the androgen-receptor complexes stimulate the expression of a group of genes while other complexes inhibit the transcription of other genes. Other investigators have shown that the transcription of different eukaryotic genes can be stimulated or repressed by the same protein (24–26). Another possibility is that the transcription of the 29-kDa protein gene may become less efficient because of androgen stimulation of the transcription of a proximal gene. Emerman and Temin (27) have shown that active transcription of genes can repress the transcription of proximal genes. It is also possible that androgens stimulate the synthesis of a protein that represses the expression of the 29-kDa protein gene. Such a mechanism is very similar to that proposed to account for the killing of lymphocytes by glucocorticoids (28).

We have not studied whether the 29-kDa protein is present...
in epithelial cells which are specialized in producing large quantities of androgen-dependent secretory proteins or in stromal cells that appear to play important roles in regulating epithelial cell activities (29). Total cell number in the rat ventral prostate remains constant for about 3 days after castration (6, 30). The number of epithelial cells decreases by about 15% during this period (9, 31). Therefore, the rapid and large changes in the level of the 29-kDa protein-mRNA, 3 days following castration or after injection of androgens, is not due to changes in the proportion of the two cell types. Since the amount of the 29-kDa protein per unit of DNA is maintained at a constant level for 12 days after castration (Fig. 4B) while the proportion of epithelial cells falls progressively to about 40% (9) or less (16) within 7 days, this protein may be distributed rather evenly in the epithelial and stromal cells (9).

The cellular functions of the 29-kDa and other proteins encoded by androgen-repressed mRNAs are not known. It is intriguing to suggest that these proteins may be involved in the mechanism through which the prostate regulates its own growth and functions. For example, a key cellular activity of the prostate may be dependent on the level of some of these proteins. Overgrowth (hyperplasia or hypertrophy) of the prostate is prevented by keeping the level of these proteins at a constant level through repression of the synthesis of their mRNAs during the androgen-dependent growing phase of prostate cells. Proteins that may play such a role may be enzymes or their regulatory factors but specific structural components of cellular organelles, such as cytoskeleton, nuclear matrix, cellular membranes, or chromatin may also be good candidates.

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Androgen-repressed Gene Expression

SUPPLEMENTARY MATERIAL TO:
AN ANDROGEN-DEPENDENT PRODUCTION OF A 29-BEDOLLATION-RELATED PROTEIN AND ITS mRNA IN THE RAT VENTRAL PROSTATE

BY

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EXPERIMENTAL PROCEDURES

Materials

P11 phosphatase activity was obtained from Sigma, Hydroxyapatite (Bio-Gel HTP), soybean lysate, and 5α-reductase were obtained from Bio-Rad. Sigma-Aldrich (now Genentech) A medium was purchased from Sigma. Reverse transcription was from Life Science. Sigma-Aldrich has been reconstituted and analyzed in the products of Bovine Prostate Serum (hereafter referred to as BPS serum). The 24 hour assay was then applied to a hydroxyapatite column (10 mL) and the samples were eluted with 10% of the initial volume and the eluent eluted in 2.5 mL fractions. The 24 hour assay was performed using heat-activated and needle dissection and RNA isolation procedures.

Tissue dissection

Mice and female rats of the Sprague-Dawley strain living Animal Laboratories, Oregon, ME were used. Tissue was removed from the ventral prostate of male rats before dissection. RNA from the prostate was prepared using a combination of 5% and 3% of the prostate RiboPure Kit (Ambion) as described above. The prostate was immediately dissected using a combination of RNAse-free DTT and RNAse inhibitor. The prostate was then centrifuged at 14,000 rpm for 90 min at 4°C. 24 hour assay was performed using heat-activated and needle dissection and RNA isolation procedures.

Analysis of DNA Content in Genitourinary Tissues

Tissue was homogenized using a Polytron (Brinkmann-Wiener) at a setting of 6 on for 10 min at 4°C. The mixture was then applied to a hydroxyapatite column (10 mL) and the samples were eluted with 10% of the initial volume and the eluent eluted in 2.5 mL fractions. The 24 hour assay was performed using heat-activated and needle dissection and RNA isolation procedures.

Binding of Dihydrotestosterone

24 hour assay was performed using heat-activated and needle dissection and RNA isolation procedures. The 24 hour assay was performed using heat-activated and needle dissection and RNA isolation procedures.
Androgen-repressed Gene Expression

Figure 3. Testosterone analysis of prostate RNA from normal, castrated, and androgen-treated rodents. (A) Total RNA from normal prostate was extracted with phenol/chloroform and then precipitated with ethanol. The RNA was then resuspended in diethyl pyrocarbonate-treated water and analyzed by gel electrophoresis. The positions of the RNA marker bands are indicated. (B) Androgen-repressed gene expression was analyzed by Northern blotting. The filter was hybridized with a 32P-labeled cDNA probe for the androgen-repressed mRNA to the zinc-rich domain of the 29K arm protein. The filter was washed and exposed to x-ray film. Prostate RNAs were prepared from normal rats, rats castrated 2 days previously, rats castrated and then reimplanted with 2 mg of testosterone enanthate on the day of castration and the following day, or rats castrated and then injected with 1 ml of oil vehicle (controls) for 2 days. The amount of RNA applied to each lane is shown.

Figure 4. Effect of castration on the expression of prostate RNA from normal, castrated, and androgen-treated rodents. (A) Androgen-repressed gene expression was analyzed by Northern blotting. The filter was hybridized with a 32P-labeled cDNA probe for the androgen-repressed mRNA to the zinc-rich domain of the 29K arm protein. The filter was washed and exposed to x-ray film. Prostate RNAs were prepared from normal rats, rats castrated 2 days previously, rats castrated and then reimplanted with 2 mg of testosterone enanthate on the day of castration and the following day, or rats castrated and then injected with 1 ml of oil vehicle (controls) for 2 days. The amount of RNA applied to each lane is shown.

Figure 5. Effect of castration on the expression of prostate RNA from normal, castrated, and androgen-treated rodents. (A) Androgen-repressed gene expression was analyzed by Northern blotting. The filter was hybridized with a 32P-labeled cDNA probe for the androgen-repressed mRNA to the zinc-rich domain of the 29K arm protein. The filter was washed and exposed to x-ray film. Prostate RNAs were prepared from normal rats, rats castrated 2 days previously, rats castrated and then reimplanted with 2 mg of testosterone enanthate on the day of castration and the following day, or rats castrated and then injected with 1 ml of oil vehicle (controls) for 2 days. The amount of RNA applied to each lane is shown.

Figure 6. Effect of androgen replacement on the expression of prostate RNA from normal, castrated, and androgen-treated rodents. (A) Androgen-repressed gene expression was analyzed by Northern blotting. The filter was hybridized with a 32P-labeled cDNA probe for the androgen-repressed mRNA to the zinc-rich domain of the 29K arm protein. The filter was washed and exposed to x-ray film. Prostate RNAs were prepared from normal rats, rats castrated 2 days previously, rats castrated and then reimplanted with 2 mg of testosterone enanthate on the day of castration and the following day, or rats castrated and then injected with 1 ml of oil vehicle (controls) for 2 days. The amount of RNA applied to each lane is shown.

Figure 7. Effect of androgen replacement on the expression of prostate RNA from normal, castrated, and androgen-treated rodents. (A) Androgen-repressed gene expression was analyzed by Northern blotting. The filter was hybridized with a 32P-labeled cDNA probe for the androgen-repressed mRNA to the zinc-rich domain of the 29K arm protein. The filter was washed and exposed to x-ray film. Prostate RNAs were prepared from normal rats, rats castrated 2 days previously, rats castrated and then reimplanted with 2 mg of testosterone enanthate on the day of castration and the following day, or rats castrated and then injected with 1 ml of oil vehicle (controls) for 2 days. The amount of RNA applied to each lane is shown.

Figure 8. Histogram. Androgen-repressed gene expression was analyzed by Northern blotting. The filter was hybridized with a 32P-labeled cDNA probe for the androgen-repressed mRNA to the zinc-rich domain of the 29K arm protein. The filter was washed and exposed to x-ray film. Prostate RNAs were prepared from normal rats, rats castrated 2 days previously, rats castrated and then reimplanted with 2 mg of testosterone enanthate on the day of castration and the following day, or rats castrated and then injected with 1 ml of oil vehicle (controls) for 2 days. The amount of RNA applied to each lane is shown.

Figure 9. Histogram. Androgen-repressed gene expression was analyzed by Northern blotting. The filter was hybridized with a 32P-labeled cDNA probe for the androgen-repressed mRNA to the zinc-rich domain of the 29K arm protein. The filter was washed and exposed to x-ray film. Prostate RNAs were prepared from normal rats, rats castrated 2 days previously, rats castrated and then reimplanted with 2 mg of testosterone enanthate on the day of castration and the following day, or rats castrated and then injected with 1 ml of oil vehicle (controls) for 2 days. The amount of RNA applied to each lane is shown.

Figure 10. Histogram. Androgen-repressed gene expression was analyzed by Northern blotting. The filter was hybridized with a 32P-labeled cDNA probe for the androgen-repressed mRNA to the zinc-rich domain of the 29K arm protein. The filter was washed and exposed to x-ray film. Prostate RNAs were prepared from normal rats, rats castrated 2 days previously, rats castrated and then reimplanted with 2 mg of testosterone enanthate on the day of castration and the following day, or rats castrated and then injected with 1 ml of oil vehicle (controls) for 2 days. The amount of RNA applied to each lane is shown.

Figure 11. Histogram. Androgen-repressed gene expression was analyzed by Northern blotting. The filter was hybridized with a 32P-labeled cDNA probe for the androgen-repressed mRNA to the zinc-rich domain of the 29K arm protein. The filter was washed and exposed to x-ray film. Prostate RNAs were prepared from normal rats, rats castrated 2 days previously, rats castrated and then reimplanted with 2 mg of testosterone enanthate on the day of castration and the following day, or rats castrated and then injected with 1 ml of oil vehicle (controls) for 2 days. The amount of RNA applied to each lane is shown.

Figure 12. Histogram. Androgen-repressed gene expression was analyzed by Northern blotting. The filter was hybridized with a 32P-labeled cDNA probe for the androgen-repressed mRNA to the zinc-rich domain of the 29K arm protein. The filter was washed and exposed to x-ray film. Prostate RNAs were prepared from normal rats, rats castrated 2 days previously, rats castrated and then reimplanted with 2 mg of testosterone enanthate on the day of castration and the following day, or rats castrated and then injected with 1 ml of oil vehicle (controls) for 2 days. The amount of RNA applied to each lane is shown.

Figure 13. Histogram. Androgen-repressed gene expression was analyzed by Northern blotting. The filter was hybridized with a 32P-labeled cDNA probe for the androgen-repressed mRNA to the zinc-rich domain of the 29K arm protein. The filter was washed and exposed to x-ray film. Prostate RNAs were prepared from normal rats, rats castrated 2 days previously, rats castrated and then reimplanted with 2 mg of testosterone enanthate on the day of castration and the following day, or rats castrated and then injected with 1 ml of oil vehicle (controls) for 2 days. The amount of RNA applied to each lane is shown.