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Genes regulated by androgen in the rat ventral prostate

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ABSTRACT **Genes that are regulated by androgen in the prostate were studied in the rat. Four of the less than 10 genes that are down-regulated by androgen in the ventral prostate of a 7-day castrated rat were identified; their mRNAs decayed with identical kinetics. Twenty-five of the estimated 56 genes that are up-regulated by androgen in the castrated prostate have been isolated. The up-regulated genes fall into two kinetic types. Early genes are significantly up-regulated by 6.5 hr whereas the delayed genes respond mainly after 24 hr from the time of androgen replacement. These androgen-response genes are also regulated in the prostate by castration, indicating that these genes could play important roles in androgen-induced regrowth and/or castration-induced regression of the prostate during hormonal manipulation. A survey of the tissue specificity showed that the androgen-response gene expression program in the prostate is mainly prostate-specific. Total RNA Northern blot analysis detects the expression of about 16 up-regulated genes and 3 down-regulated genes in the prostate only. Four up-regulated genes and one down-regulated gene are regulated by androgen in both the prostate and seminal vesicles but not in other organs. The expression of the remaining androgen-response genes is not limited to the prostate but is only responsive to androgen in the prostate. This survey of the androgen-response gene expression program provides insights into the molecular and cellular mechanisms of androgen action in the prostate.**

Androgen plays an important role in the pathogenesis of two common male diseases—prostate cancer and benign prostatic hyperplasia (BPH) (1). To understand the role of androgen in the pathogenesis of prostate cancer and benign prostatic hyperplasia, it is necessary to understand the mechanism of androgen action in the prostate.

The structural and functional integrity of the prostate requires a constant supply of androgen (2, 3). In the rat model, androgen ablation by castration results in a rapid prostatic involution via apoptosis of glandular epithelial cells (4). Apoptosis is detectable within 1 day, peaks between 3 to 4 days, and then drops to a low level at 7 days after castration. The regressed prostate can be stimulated by androgen replacement to undergo a rapid regrowth until it reaches the normal size. The most rapid cell proliferation occurs between 3 to 5 days after androgen administration.

The androgen receptor (AR) is necessary for androgen action because a frame-shift mutation that disrupts AR function results in testicular-feminized mice (5). AR is a ligand-dependent transcription factor (6) that regulates, either directly or indirectly, the expression of androgen-response genes (7). Thus, androgen-response genes should mediate AR downstream events leading to cellular and morphological changes in the prostate during hormonal manipulation. This model of androgen action was modified (8–10) to include paracrine interactions. Dihydrotestosterone first complexes with AR in stromal cells and subsequently induces the production of stroma-derived growth factor (SDGF). The SDGF then stimulates proliferation of prostatic epithelial cells. Cell culture studies (11) implicate that keratinocyte growth factor (KGF) is a candidate stomal to epithelial cell andromedin. The significance of KGF in androgen-dependent pathway is further supported by the stimulatory effect of KGF and inhibitory effect of a neutralizing anti-KGF antibody in organ culture studies (12). However, KGF knockout mice do not exhibit any detectable abnormality in prostate and seminal vesicle development (13). Although the molecules involved in the stromal–epithelial interactions are not well defined (14), the activity of SDGF should be regulated by androgen in the prostate.

To date, the studies on androgen-response genes are limited. Genes that are up-regulated by androgen in the prostate include prostate-specific antigen (PSA) (15, 16), prostatein C3 (17), sex-limited protein (slp) (18), spermine-binding protein (19, 20), prostate-binding protein C2A (21), cystatin-related protein 2 (22, 23), calreticulin (24), and probasin (25, 26). Androgen ablation-induced genes include clusterin/sulfate glycoprotein-2 (SGP-2)/testosterone repressed prostate mRNA-2 (TRPM-2) (27, 28), transforming growth factor β (29), rat ventral prostate gene 1 (RVP.1) (30), glutathione *S*-transferase (31), c-myc (32), c-fos (33), and matrix carboxyglutamic acid protein (30).

The rat ventral prostate was chosen as a model in this study. The rat prostate consists of ventral, dorsal, and lateral lobes. The ventral prostate lobe is the largest and can be conveniently isolated, providing a sufficient quantity of material for various studies. Androgen action has been extensively investigated in the rat ventral prostate (2). The apoptosis and proliferation in the prostate are androgen-dependent in different mammals including human, mouse, and rat. Thus, the functions of androgen-response genes in apoptosis and/or proliferation in the prostate should be conserved between animal models and human.

Our research strategy for analyzing the androgen-induced gene expression program is similar to ones used to study the gene expression program induced by other hormones, for example, the thyroid hormone-induced amphibian tail resorption program (34–36). First, genes that are up- and down-regulated by androgen in a 7-day castrated rat ventral prostate are isolated by a "gene expression screen" (35), a PCR-based cDNA subtraction method that identifies differentially expressed genes by comparing two (or more) populations of closely related mRNA. One advantage of this method is that it provides an estimation of the total number of differentially expressed genes (35). Second, the identities and potential functions of the isolated genes are determined or predicted by sequencing and searching for homology with genes in the published database. Third, the functions of various androgen-response genes in androgen-dependent apoptosis and/or proliferation are determined by taking advantage of transgenic

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Abbreviation: AR, androgen receptor.

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mouse technology. Fourth, the mechanisms by which androgen-response genes regulate prostatic cell death and/or proliferation are characterized.

This paper describes the identification and characterization of androgen-induced gene expression changes that lead to the regrowth of a 7-day castrated rat ventral prostate. This androgen-induced gene expression program is mainly prostate-specific and involves a limited number of androgen-response genes. We have identified two kinetic types of up-regulated genes and only one for down-regulated genes.

MATERIALS AND METHODS

Animals. Young adult male Harlan Sprague–Dawley rats (250–300 g) were used. The rats were castrated in a room dedicated for animal surgery according to a protocol approved by the Northwestern University Animal Care and Use Committee (Chicago). Testes, fat pads, and epididymis were removed in the castration. The castrated animals were maintained in the Northwestern University animal facility. Treatment of 7-day castrated rats with exogenous androgen was carried out by daily subcutaneous injections of testosterone propionate at 2 mg/rat for up to 7 days. At various times after castration or androgen replacement, at least three rats were sacrificed by decapitation after anesthesia. The ventral prostate lobes were removed, weighed, and immediately frozen in liquid nitrogen prior to RNA isolation.

RNA Isolation and Analysis. Total RNA was isolated using the guanidinium/CsCl gradient method (37). Purified RNA samples were fractionated in a 1% agarose-formaldehyde gel. Ten micrograms of total RNA sample was loaded in each lane. After electrophoresis, RNA was transferred to a nylon membrane by capillary blotting and then cross-linked to the membrane by UV irradiation. Northern blot hybridization of the membrane was carried out at 42°C overnight in a buffer containing 5× SSPE, 2× Denhart's solution, 0.1% SDS, 100 μg/ml denatured salmon sperm DNA, and 50% formamide in the presence of DNA probes labeled by random priming. The membrane was then washed at room temperature with 1× SSC and 0.1% SDS for 20 min followed by three 20-min washes at 65°C with 0.2× SSC and 0.1% SDS.

Synthesis and Screening of ZAP-cDNA Library. Poly(A)$^+$ RNA was selected from the total rat ventral prostate RNA using an oligo(dT) column (Pharmacia, type 7) for cDNA synthesis. ZAP-cDNA library was constructed and screened according to the instructions provided by Stratagene.

PCR-Based cDNA Subtraction. cDNA subtraction was carried out essentially as described by Wang and Brown (35), except where noted below. The synthesis of cDNA, restriction digestion, linker ligation, and PCR amplification was as described previously (35). The photobiotinylation of driver DNA was performed using a 125-W lamp at a distance of 7.5 cm for 15 min. The subtractive hybridization reaction was scaled down to 75% of the original reaction size (35). The removal of driver after subtractive hybridization was carried out by adding 200 μg streptavidin followed by three extractions with phenol/chloroform and one with chloroform extraction. After three rounds of PCR-based subtraction, the highly enriched cDNAs were cloned into pBluescript (Stratagene) for screening.

Sequencing Analysis. Distinct cDNA fragments identified from the PCR-based subtraction library were sequenced using an ABI automated sequencing machine with fluorescent dye terminators. The sequence homology was searched in the GenBank database (38).

RESULTS

Identification of Genes That Are Up- or Down-Regulated by Androgen in a 7-Day Castrated Rat Ventral Prostate. There are two alternative approaches for identifying androgen-response genes from the rat ventral prostate using the gene expression screen method: first, finding genes that are up- or down-regulated in the prostate by castration, or second, finding genes that are up- or down-regulated by androgen replacement in 7-day castrated rats. We chose the second approach. One major advantage of this choice is that the induction of androgen-response genes is not affected by other factor(s) or hormone(s) secreted from the testis. In contrast, castration may cause gene expression changes that are regulated by nonandrogen testicular factor(s) (39, 40). Furthermore, the induction of androgen-response genes in the prostate by hormone replacement should be more rapid than that by castration because the decay of androgen after castration takes time. The half-life of dihydrotestosterone in the rat ventral prostate is longer than 6 hr (41). The rapid induction of androgen-response genes could facilitate subtractive identification of early androgen-response genes.

The purpose of choosing the 14 hr of androgen replacement is to isolate early androgen-response genes. The expression of known early androgen-response genes including prostatein C3 gene is induced within several hours and then maintained after androgen treatment (17). Thus, genes that are up- or down-regulated by androgen within 14 hr in a 7-day castrated rat ventral prostate should include prostatein C3 and other early response genes.

The 48-hr time point after androgen replacement in a 7-day castrated rat ventral prostate was chosen for isolation of delayed androgen-response genes because this is the period of time sufficient to initiate DNA synthesis (2, 3, 42). It is important to point out that the number of nuclei remains the same within 48 hr after androgen replacement in a 7-day castrated rat ventral prostate (2). The ratio between stromal and epithelial cells should not be altered within this period of time. Thus, changes in gene expression induced by androgen within 48 hr in a 7-day castrated prostate are not a result of changes in the composition of cell populations.

The first step in the gene expression screen is the isolation of poly(A)$^+$ RNA from a 7-day castrated rat ventral prostate and the castrated prostate treated with testosterone propionate for 14 or 48 hr. According to procedures described in the *Materials and Methods* and in previous publications (35, 36), we have constructed four subtracted cDNA libraries. The first subtraction library (UP14) is enriched for cDNA sequences that are up-regulated at 14 hr after the androgen replacement. The second library (DOWN14) is enriched for cDNA sequences that are down-regulated at 14 hr after androgen replacement. The third library (UP48) is enriched for cDNA sequences that are up-regulated at 48 hr after the androgen replacement. The fourth library (DOWN48) is enriched for cDNA sequences that are down-regulated at 48 hr after the androgen replacement. The enriched UP- and DOWN-cDNAs do not hybridize to each other, indicating that the subtractive enrichment is effective.

The four enriched, PCR-based cDNA libraries were converted into plasmid libraries by inserting cDNA fragments into the pBluescript (Stratagene). Screening these cDNA libraries has led to the identification of 33 distinct small cDNA fragments from genes that are up-regulated by androgen and 5 distinct fragments from genes that are down-regulated by androgen. Androgen regulation of genes corresponding to each fragment was verified by Northern blot analysis.

Time Course of Androgen-Induced Gene Expression in the Prostate. To get an overview of the gene expression cascade induced by androgen replacement in the prostate prior to cell proliferation, we analyzed the induction kinetics of each identified up- and down-regulated gene in the prostate by Northern blot. Time courses of four representative up-regulated genes and one down-regulated gene are shown in Fig. 1.

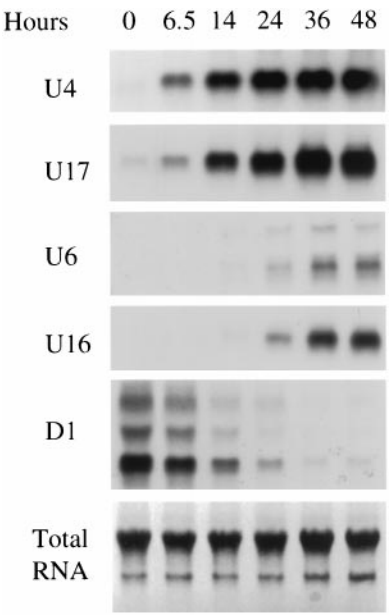

FIG. 1. Northern blot analysis of total RNA from the ventral prostate of 7-day castrated rats treated with testosterone propionate for indicated number of hours. Representative androgen-response genes are indicated. The amount and quality of total RNA loaded in the gels were examined by staining the transferred nylon membrane with methylene blue (53).

The down-regulation of all of the identified down-regulated genes is induced with the same time course, and one example, D1, is shown in Fig. 1. The down-regulation occurs within 14 hr after androgen replacement and the level of mRNA decreases over 90% within 24 hr. This observation suggests that androgen replacement induces only one kinetic type of down-regulation in a 7-day castrated rat ventral prostate.

The time course of up-regulated genes can be classified into at least 2 different classes. The early-response genes are defined as those that are up-regulated within 14 hr after androgen replacement, for example, genes U4 and U17 (Fig. 1). In contrast, the delayed-response genes, for example, genes U6 and U16 (Fig. 1), are up-regulated about 24 hr after the induction of the early-response genes. Nineteen out of 25 identified androgen-response genes are early genes whereas the remaining 6 are delayed genes (Table 1).

Tissue Specificity of Androgen-Response Genes. We have studied the expression of various androgen-response genes in the ventral prostate, seminal vesicles, liver, brain, kidney, heart, and muscle in testis-intact rats, 7-day castrated rats, and 7-day castrated rats with androgen replacement (Fig. 2 and Table 2). The expression of various androgen-response genes was also examined in the testis (result not shown). In this survey, only the prostate and seminal vesicles are male accessory sex organs that undergo massive regression upon castration and rapid cell proliferation upon androgen replacement. The results shown in Fig. 2 and Table 2 indicate that the regulation of androgen-response genes by androgen is mainly prostate-specific. Total RNA Northern blot analysis detects the expression of about 16 up-regulated genes and 3 down-regulated genes in the prostate only. Four up-regulated genes and one down-regulated gene are responsive to androgen in both the prostate and seminal vesicles but not in other organs. The rest of the androgen-response genes are expressed in many tissues but are only inducible by androgen in the prostate.

Identities of Androgen-Response Genes. Each independent cDNA fragment was sequenced. This analysis has generated 200–300 bases of sequence information for each of the fragments, which allowed us to determine the identity of 11 up-regulated genes and 1 down-regulated gene (Table 3). Six

Table 1. Summary of androgen-response genes in the rat ventral prostate

Gene	mRNA size, kb	No. of fragments	Fold induction	mRNA abundance	Kinetic type
U1	4 + 1.2	3	>20	H	E
U2	1.5	2	>20	H	E
U3	1	1	>20	M	E
U4	1	2	>20	H	E
U5	2	2	>20	M	E
U6	4 + 1.3	1	>20	M	D
U7	1.8	1	15	M	E
U8	1.6	1	>20	M	E
U9	1.1	1	15	M	E
U10	6	2	10	L	E
U11	8 + 2.5	1	10	L	D
U12	8	2	>20	H	E
U13	4 + 2.5	2	>20	L	E
U14	3	1	>20	L	E
U15	2 + 1	1	>20	L	E
U16	1.3	1	>20	H	D
U17	1.1	1	>20	H	E
U18	3	1	10	L	D
U19	2	1	10	M	E
U20	5	1	10	L	E
U21	3	1	15	M	E
U22	8	1	>20	M	E
U23	1.4	1	15	M	E
U24	4	1	15	L	D
U25	2	1	10	M	D
D1	2	2	15	H	
D2	1.5	1	10	L	
D3	1.1	1	10	L	
D4	2.5	1	10	L	

Genes that are up-regulated by androgen are named U1 to U25, and genes that are down-regulated by androgen are named D1 to D4. The induction fold before and after 48 hr of androgen replacement in the prostate of a 7-day castrated rat on Northern blot is determined by using Kodak Digital Science 1D IMAGE ANALYSIS Software. The abundance of mRNA was estimated by comparing Northern blot and genomic Southern blot signals when hybridized in same reaction. We have assumed that each cell contains about 500,000 mRNA molecules of 2.5-kb average size and that the haploid genome size of the rat is 3×10^9 bp. Kinetic type: E, early; D, delayed.

of these genes are known to be up-regulated by androgen in the rat prostate; these encode prostate-binding protein C2A (21), spermine-binding protein (19), probasin (26), cystatin-related protein 1 (22), cystatin-related protein 2 (22), and prostatein C3 (17). The other five genes were not previously known to be up-regulated by androgen in the prostate; these encode calreticulin (43), farnesyl pyrophosphate synthetase (44), LDL-R (45), adrenomedullin (46), and histo-blood group A transferase (47). Sixteen independent cDNA fragments were isolated from 14 unknown genes that are up-regulated by androgen in the prostate. Two different cDNA fragments were isolated from SGP-2/TRPM-2 (27, 28), which is known to be repressed by androgen in the prostate. Three cDNA fragments were isolated from three unknown genes that are down-regulated by androgen in the prostate.

The Complexity of the Androgen-Induced Gene Expression Program. The gene expression screening method has an advantage of estimating the total number of genes that are similarly induced (35). This estimation is based on the isolation of independent cDNA fragments that are derived from the same gene. Different cDNA fragments are assigned to the same gene if they hybridize to the same high-molecular-weight cDNA clone isolated from a ZAP cDNA library (35). For up-regulated genes, as shown in Table 1, one distinct cDNA fragment was isolated from each of 18 different genes, two fragments from each of 6 genes, and three fragments from 1

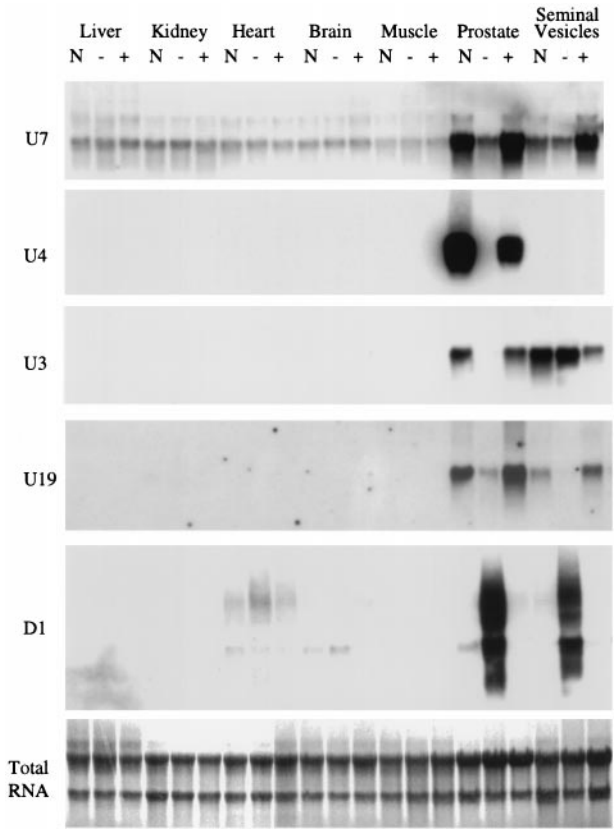

FIG. 2. Northern blot analysis of tissue specificity of indicated androgen-response genes in the rat during hormonal manipulation. N, tissue from the testis-intact rats; −, tissue from 7-day castrated rats; +, tissue from the rats castrated for 7 days followed by androgen replacement for an additional 2 days. The amount and quality of total RNA loaded in the gels were examined by staining the transferred membrane with methylene blue.

gene, U1. By applying Poisson distribution analysis (35), we predict that androgen up-regulates about 56 genes in the ventral prostate of a 7-day castrated rat. Most of the genes that are known to be responsive to androgen in the ventral prostate of a 7-day castrated rat are found in our collection, further suggesting that the complexity of the androgen-induced gene expression program is limited.

The down-regulated gene expression program appears to be less complex. For down-regulated genes, one distinct cDNA fragment was isolated from each of three different genes and two fragments from one gene (Table 1). The same statistical analysis would predict that androgen down-regulates less than 10 genes in the ventral prostate of 7-day castrated rats.

Our collection includes only genes that are similarly regulated by androgen in the prostate, that is, with a minimum of 10-fold induction and at least 10–20 copy per cell in average. Our estimation does not consider genes that are expressed at extremely low abundance or low magnitude (<5-fold) of induction, that lack a poly(A) tail, and genes that are not regulated at the mRNA level.

DISCUSSION

Studying the androgen-response gene expression program in the prostate will greatly facilitate further analysis of androgen-dependent cellular processes, including apoptosis, proliferation, and differentiation, in the prostate. Comprehensive identification of androgen-response genes will allow us to study individual androgen-response genes in the context of knowing many other androgen-response genes. The experiments presented in this paper were designed to obtain an overview of the kinetics, complexity, and tissue-specificity of androgen-response genes in the prostate using the rat as a model system.

Table 2. Tissue-specificity survey of androgen-response genes

	P			SV								
Gene	N	C	+T	N	C	+T	B	Ht	K	Lv	Ms	Ts
U1	H	–	H	–	–	–	–	–	–	–	–	–
U2	H	–	H	–	–	–	–	–	–	–	–	–
U3	M	–	M	M	M	L	–	–	–	–	–	–
U4	H	–	H	–	–	–	–	–	–	–	–	–
U5	M	–	M	–	–	–	–	–	–	–	–	–
U6	M	–	M	–	–	–	–	–	–	–	–	L
U7	M	L	M	L	L	M	L	L	L	L	L	L
U8	M	L	M	L	L	L	L	L	L	L	L	L
U9	M	L	M	–	–	L	L	L	L	L	L	M
U10	L	–	L	–	–	–	–	–	–	–	–	–
U11	L	–	L	–	–	–	–	–	–	–	–	–
U12	H	L	H	–	–	–	–	–	–	–	–	L
U13	L	–	L	–	–	–	–	–	–	–	–	–
U14	L	–	L	–	–	–	–	–	–	–	–	–
U15	L	–	L	–	–	–	–	–	–	–	–	–
U16	H	–	H	–	–	–	–	–	–	–	–	–
U17	H	L	H	–	–	–	–	–	–	–	–	–
U18	L	–	L	–	–	–	–	–	–	–	–	–
U19	M	L	M	L	–	M	–	–	–	–	–	L
U20	L	–	L	–	–	–	–	–	–	–	–	–
U21	M	L	M	L	L	M	L	L	L	L	L	L
U22	M	L	M	–	–	–	–	–	–	–	–	–
U23	M	L	M	L	L	L	L	L	L	L	L	L
U24	L	–	L	–	–	–	–	–	–	–	–	–
U25	M	–	M	–	–	–	–	–	–	–	–	–
D1	L	H	L	L	H	L	L	L	L	L	L	L
D2	–	L	–	–	–	–	–	–	–	–	–	–
D3	–	L	–	–	–	–	–	–	–	–	–	–
D4	–	L	–	–	–	–	–	–	–	–	–	–

The abundance of mRNAs was estimated by comparing Northern blot signals with genomic Southern blots as described in Table 1. In the prostate (P) and seminal vesicles (SV) samples, N represents the tissue from the testis–intact rats; C represents the tissue from 7–day castrated rats; and +T represents the tissue from the rats castrated for 7 days followed by testosterone for an additional 2 days. Androgen had no detectable effect on the expression of androgen–response genes in brain (B), heart (Ht), kidney (K), liver (Lv), and muscle (Ms). The expression of androgen–response genes in the testis (Ts) was also determined by Northern blot analysis. High–abundance (H) mRNA is greater than 500 copies per cell; medium–abundance (M) mRNA ranges from 50 to 500 copies per cell; and low–abundance (L) mRNA is about 20 copies per cell. –, mRNA is not detectable with total RNA Northern blot analysis.

Table 3. Identities of androgen-response genes in the rat ventral prostate

Gene	Product	Reference
U1	Prostate-binding protein C2A	21
U2	Spermine-binding protein	19
U3	Probasin	26
U4	Prostatein C3	17
U5	Cystatin-related protein 1	22
U6	Cystatin-related protein 2	22
U7	Calreticulin	43
U8	Adrenomedullin	46
U9	Farnesyl pyrophosphate synthase	44
U10	LDL-receptor	45
U11	Histo-blood group A transferase	47
D1	SGP-2/TRPM-2	27, 28

Each gene matches 100% with corresponding genes except U11, which matches 80% at the nucleic acid level with the histo-blood group A transferase gene.

The Androgen-Response Gene Expression Program Leading to the Regrowth of the Prostate. We have isolated 25 of about 56 genes that are up-regulated by androgen and 4 of less than 10 genes that are down-regulated by androgen within 48 hr after hormone replacement in the ventral prostate of 7-day castrated rats. The limited complexity of the androgen-response gene expression program is further supported by the observation that most, if not all, genes that are known to be dramatically regulated by androgen in the rat prostate were identified in this androgen-response gene collection (Table 3). It is important to point out that this estimation is limited to genes that are induced within 48 hr after androgen replacement. It is possible that more genes are induced after 48 hr when cells undergo proliferation; for example, many proliferation-associated genes may be induced after 48 hr of androgen replacement.

Northern blot analysis of the time course of androgen induction of all the isolated androgen-response genes shows that androgen replacement induces two kinetic types of up-regulation and one type of down-regulation in the prostate of 7-day castrated rats (Fig. 1 and Table 1). Because cell proliferation occurs within 48 hr of androgen replacement (2, 3, 42), the two kinetic types of up-regulation and one type of down-regulation should be sufficient to initiate proliferation in the prostate. The early up-regulated genes are expected to be direct androgen-response genes. In fact, known early genes including probasin and prostatein C3 are regulated directly by androgen (17). Although the delayed response genes are expected to be indirectly regulated by androgen, the promoter region of one delayed response gene, cystatin-related protein 2, contains androgen-receptor-binding elements that are functional in transfection experiments (48). This result indicates that delayed genes could also respond directly to androgen. The mechanism of down-regulation is not clear, and it is not known whether the down-regulation is a direct or an indirect response to androgen.

No transiently regulated genes were found in our screen for androgen-response genes. Every up-regulated gene remains activated, whereas every down-regulated gene remains repressed as long as androgen is present. This agrees with the observation that a constant supply of androgen is required for the functional and structural integrity of the prostate.

The overall complexity and regulation of the androgen-regulated gene expression program in the ventral prostate of a 7-day castrated rat appear to be very similar to that of the thyroid hormone-regulated gene expression program in the tail of *Xenopus* tadpole (35, 36), although different genes are regulated by these two hormones. The up-regulation is more complicated than down-regulation in both gene expression programs. There are two kinetic types of up-regulation and one type of down-regulation in both systems. These similarities may reflect the fact that receptors for both hormones belong to the same superfamily. Thus, the mechanisms of regulating gene expression by these two hormones are likely to be conserved.

One interesting observation about androgen-response genes is that genes up-regulated by androgen are regulated dramatically during both androgen ablation and androgen replacement. In contrast, genes up-regulated by castration in the prostate are not necessarily down-regulated by androgen replacement in a 7-day castrated prostate (49). One possible explanation is that cells expressing castration-induced genes are dying and they are eliminated in the castrated prostate. As a result, the number of cells that express castration-induced genes declines after castration. Thus, the level of castration-induced mRNA decreases after the peak of apoptosis events. Many castration-induced genes were not identified in this screen, because these genes are not dramatically regulated by androgen replacement. Some of these genes, such as transforming growth factor β and calmodulin, are down-regulated moderately by androgen replacement whereas others, such as c-myc, H-ras, and tissue transglutaminase, are up-regulated moderately by androgen replacement in the prostate of a 7-day castrated rat (49).

Functions of Androgen-Response Genes in the Prostate. Androgen regulates three major cellular processes in the prostate: proliferation, differentiation, and apoptosis. Thus, androgen-response genes should participate in the regulation of these cellular events during hormonal manipulation. The induction of androgen up-regulated genes correlates with cell proliferation and differentiation in the prostate of a 7-day castrated rat. Further characterization showed that these genes are expressed in the normal prostate and are down-regulated by castration (Fig. 2 and Table 2), indicating that these genes may also play important roles in suppressing castration-induced apoptosis. These genes are likely to control various androgen-dependent cellular and morphological processes. Identification of these genes makes it possible to predict and to test their functional importance in androgen action.

Ca^{2+} influx is known to be involved in castration-induced apoptosis in the prostate (50, 51). Identification of calreticulin as an androgen-response gene in the prostate implies that this protein has potential to modulate intracellular Ca^{2+} levels in the prostate because calreticulin is a major intracellular Ca^{2+}-binding protein in nonmuscle cells. Further analysis suggests that down-regulation of calreticulin by castration correlates with apoptosis of prostatic epithelial cells (N. Zhu and Z.W., unpublished data).

Schaffner *et al.* showed in 1978 that androgen regulates cholesterol synthesis in the rat ventral prostate (52). We have identified two androgen-response genes that encode farnesyl pyrophosphate synthase (FPPS) and LDL-R (Table 3). FPPS is required for cholesterol synthesis, and LDL-R is involved in cholesterol transport. Thus, it is very likely that androgen controls cholesterol level in the prostate by regulating the expression of FPPS and LDL-R.

Androgen-Regulated Gene Expression Program in the Prostate Is Mainly Prostate-Specific. Given that AR is expressed in many tissues, one question is why androgen manipulation induces dramatic regression or regrowth only in the male accessory sex organs. Identification and characterization of this collection of prostatic androgen-response genes provide important insights into this question. A simple explanation is that androgen-response genes in male accessory sex organs are not responsive to androgen in other organs. This idea is supported by our tissue-specificity survey, which indicates that prostate androgen-response genes are not regulated in brain, heart, kidney, liver, and muscle (Fig. 2 and Table 2). This observation argues that the androgen-response gene expression program in male accessory sex organs is not established in other organs during development.

Another question in androgen action is whether or not the mechanism by which androgen manipulation controls regression and regrowth is shared among male accessory sex organs. Our tissue-specificity survey showed that only a few prostate androgen-response genes are also regulated by androgen in the seminal vesicles. If the mechanism of androgen-dependent cell death and/or proliferation is shared among various male accessory sex organs, only those few genes responsive to androgen in both the prostate and seminal vesicles could be involved in apoptosis and/or proliferation in male accessory sex organs. Otherwise, the mechanism by which androgen manipulation controls apoptosis and proliferation in different male accessory sex organs would be very different. Characterization of genes that are regulated by androgen in both the prostate and seminal vesicles is likely to provide an answer to this question.

The observation that the androgen-regulated gene expression program in the prostate is mainly prostate-specific has significant implications in elucidating the role of androgen in

the pathogenesis of prostate cancer and benign prostatic hyperplasia. A possible explanation for the fact that androgen is an important risk factor for diseases in the prostate but not in other organs, including other male accessory sex organs, is that most of the androgen-response genes in the prostate are prostate-specific. The activity of selective androgen-response genes in the prostate may be responsible for the high risk of developing prostate cancer and benign prostatic hyperplasia.

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1. Kozlowski, J. & Grayhack, J. (1991) in *Adult and Pediatric Urology*, eds. Gillenwater, J., Grayhack, J., Howards, S & Duckett, J. (Mosby Year Book, Chicago), 2nd Ed., pp. 1277–1393.
2. Bruchovsky, N., Lesser, B., Van Doorn, E. & Craven, S. (1975) *Vitam. Horm.* **33,** 61–102.
3. Coffey, D., Shimazaki, J. & Williams-Ashman, H. (1968) *Arch. Biochem. Biophys.* **124,** 184–198.
4. English, H. F., Santen, R. J. & Isaacs, J. T. (1987) *Prostate* **11,** 229–242.
5. He, W., Kumar, M. & Tindall, D. (1991) *Nucleic Acids Res.* **19,** 2373–2378.
6. Zhou, Z., Wong, C., Sar, M. & Wilson, E. (1994) *Recent Prog. Horm. Res.* **49,** 249–274.
7. Mainwaring, W. (1977) in *Monographs in Endocrinology* (Springer, New York), pp. 1–178.
8. Cunha, G. (1984) *Prog. Clin. Biol. Res.* **145,** 81–102.
9. Cunha, G., Alarid, E., Turner, T., Donjacour, A., Boutin, E. & Foster, B. (1992) *J. Androl.* **13,** 465–475.
10. Tenniswood, M. (1986) *Prostate* **9,** 375–385.
11. Yan, G., Fukabori, Y., Nikolaropoulos, S., Wang, F. & McKeehan, W. (1992) *Mol. Endocrinol.* **6,** 2123–2128.
12. Sugimura, Y., Foster, B. A., Hom, Y. K., Lipschutz, J. H., Rubin, J. S., Finch, P. W., Aaronson, S. A., Hayashi, N., Kawamura, J. & Cunha, G. R. (1996) *Int. J. Dev. Biol.* **40,** 941–951.
13. Guo, L., Degenstein, L. & Fuchs, E. (1996) *Genes Dev.* **10,** 165–175.
14. Sensibar, J. (1995) *Microscopy Res. Tech.* **30,** 342–350.
15. Riegman, P., Vlietstra, R., van der Korput, J., Brinkmann, A. & Trapman, J. (1991) *Mol. Endocrinol.* **5,** 1921–1930.
16. Young, C., Montgomery, B., Andrews, P., Qui, S., Bilhartz, D. & Tindall, D. (1991) *Cancer Res.* **51,** 3748–3752.
17. Tan, J., Marschke, K., Ho, K., Perry, S., Wilson, E. & French, F. (1992) *J. Biol. Chem.* **267,** 4456–4466.
18. Adler, A., Danielsen, M. & Robins, D. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 11660–11663.
19. Hiipakka, R., Chen, C., Schilling, K., Oberhauser, A., Saltzman, A. & Liao, S. (1984) *Biochem. J.* **218,** 563–571.
20. Chapman, M., Qu, N., Pascoe, S., Chen, W., Apostol, C., Gordon, D. & Miesfeld, R. (1995) *Mol. Cell. Endocrinol.* **108,** R1–7.
21. Claessens, F., Dirckx, L., Delaey, B., Decourt, J., Hemschoote, K., Peeters, B. & Rombauts, W. (1989) *J. Mol. Endocrinol.* **3,** 93–103.
22. Devos, A., DeClercq, N., Vercaeren, I., Heyns, W., Rombauts, W. & Peeters, B. (1993) *Gene* **125,** 159–167.
23. Winderickx, J., Vercaeren, I., Verhoeven, G. & Heyns, W. (1994) *J. Steroid Biochem. Mol. Biol.* **48,** 165–170.
24. Bruchovsky, N., Snoek, R., Rennie, P., Akakura, K., Goldenberg, L. & Gleave, M. (1996) *Prostate* **6,** 13–21.
25. Kasper, S., Rennie, P., Bruchovsky, N., Sheppard, P., Cheng, H., Lin, L., Shiu, R., Snoek, R. & Matusik, R. (1994) *J. Biol. Chem.* **269,** 31763–31769.
26. Rennie, P., Bruchovsky, N., Leco, K., Sheppard, P., McQueen, S., Cheng, H., Snoek, R., Hamel, A., Bock, M., MacDonald, B., Nickel, B., Chang, C., Liao, S., Cattini, P. & Matusik, R. (1993) *Mol. Endocrinol.* **7,** 23–36.
27. Sensibar, J., Griswold, M., Sylvester, S., Buttyan, R., Bardin, C., Cheng, C., Dudek, S. & Lee, C. (1991) *Endocrinology* **128,** 2091–2102.
28. Montpetit, M., Lawless, K. & Tenniswood, M. (1986) *Prostate* **8,** 25–36.
29. Kyprianou, N. & Isaacs, J. (1989) *Mol. Endocrinol.* **3,** 1515–1522.
30. Briehl, M. & Miesfeld, R. (1991) *Mol. Endocrinol.* **5,** 1381–1388.
31. Chang, C., Saltzman, A., Sorensen, N., Hiipakka, R. & Liao, S. (1987) *J. Biol. Chem.* **262,** 11901–11903.
32. Quarmby, V., Beckman, W., Jr., Wilson, E. & French, F. (1987) *Mol. Endocrinol.* **1,** 865–874.
33. Buttyan, R., Zakeri, Z., Lockshin, R. & Wolgemuth, D. (1988) *Mol. Endocrinol.* **2,** 650–657.
34. Brown, D., Wang, Z., Furlow, J., Kanamori, A., Schwartzman, R., Remo, B. & Pinder, A. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 1924–1929.
35. Wang, Z. & Brown, D. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 11505–11509.
36. Wang, Z. & Brown, D. (1993) *J. Biol. Chem.* **268,** 16270–16278.
37. Chirgwin, J., Przbyla, A., MacDonald, R. & Rutter, W. (1979) *Biochemistry* **18,** 5294–5299.
38. Altschul, S., Gish, W., Miller, W., Myers, E. & Lipman, D. (1990) *J. Mol. Biol.* **215,** 403–410.
39. Dalton, D., Lee, C., Huprikar, S., Chmiel, J. & Grayhack, J. (1990) *Prostate* **16,** 225–233.
40. Darras, F., Lee, C., Huprikar, S., Rademaker, A. & Grayhack, J. (1992) *J. Urol.* **148,** 432–440.
41. Kyprianou, N. & Isaacs, J. (1988) *Endocrinology* **122,** 552–562.
42. Chung, L. & Coffey, D. (1971) *Biochim. Biophys. Acta* **247,** 584–596.
43. Krause, K. H. & Michalak, M. (1997) *Cell* **88,** 439–443.
44. Wilkin, D., Kutsunai, S. & Edwards, P. (1990) *J. Biol. Chem.* **265,** 4607–4614.
45. Lee, L., Mohler, W., Schafer, B., Freudenberger, J., Byrne-Connolly, N., Eager, K., Mosley, S., Leighton, J., Thrift, R., Davis, R. & Tanaka, R. (1989) *Nucleic Acids Res.* **17,** 1259–1260.
46. Kanazawa, H., Kurihara, N., Hirata, K., Kudoh, S., Kawaguchi, T. & Takeda, T. (1994) *Biochem. Biophys. Res. Commun.* **205,** 251–254.
47. Yamamoto, F., Marken, J., Tsuji, T., White, T., Clausen, H. & Hakomori, S. (1990) *J. Biol. Chem.* **265,** 1146–1151.
48. Devos, A., Claessens, F., Alen, P., Winderickx, J., Heyns, W., Rombauts, W. & Peeters, B. (1997) *Mol. Endocrinol.* **11,** 1033–1043.
49. Furuya, Y. & Isaacs, J. (1993) *Endocrinology* **133,** 2660–2666.
50. Connor, J., Sawczuk, I., Benson, M., Tomashefsky, P., O'Toole, K., Olsson, C. & Buttyan, R. (1988) *Prostate* **13,** 119–130.
51. Martikainen, P. & Isaacs, J. (1990) *Prostate* **17,** 175–187.
52. Singhal, A., Bonner, D. & Schaffner, C. (1978) *Proc. Soc. Exp. Biol. Med.* **159,** 1–5.
53. Herrin, D. & Schmidt, G. (1988) *Biotechniques* **6,** 196–197, 199–200.