Identification of genes expressed in the rat prostate that are modulated differently by castration and Finasteride treatment

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Abstract

In mammals, testosterone and 5 α -dihydrotestosterone (DHT) are the principal male hormones (androgens). Testosterone is the most abundant circulating androgen, and is converted in specific tissues to DHT by the 5 α -reductase enzymes. Although each of these androgens binds to the same receptor protein (androgen receptor, AR), each exerts biologically distinct effects. Theories to explain the specific effects of testosterone and DHT have centered on kinetic differences of binding of androgens to the receptor or differences in the metabolic fates of the two hormones. In the current experiments, differential display PCR (ddPCR) was used to identify genes regulated differently by testosterone and DHT. Adult male rats were treated as follows: castrated, treated with Finasteride (an inhibitor of 5 α -reductase) or left intact for ten days. RNA was prepared from the dissected prostates of these animals and used for ddPCR. Genes exhibiting four distinct patterns of regulation were observed among the mRNAs.

Class 1 genes showed equivalent expression in intact and Finasteride-treated animals, but were absent in castrated animals (mRNAs D1, D2, D6, D10). Class 2 genes showed higher expression in intact animals, intermediate levels following Finasteride treatment, but were absent in castrated animals (mRNA D8). Two classes of gene were particularly intriguing: class 3 showed gene expression only in the intact animal (mRNA D7, D9) and class 4 showed increased gene expression following Finasteride treatment (mRNA D3). While the patterns observed for some of these genes (e.g. D8) suggest that the different biological effects of testosterone and DHT may be due to the lower affinity of the AR for testosterone and limiting tissue concentrations of androgen, our results also suggest that some genes expressed in the rat prostate may be regulated in fundamentally different ways in response to testosterone and DHT.

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Introduction

Testosterone and 5 α -dihydrotestosterone (DHT) are the principal circulating androgens in mammals and exert different actions in the male during embryogenesis and in postnatal life. Studies of animals (Goldstein & Wilson 1972) and humans (Griffin *et al.* 1995, Quigley *et al.* 1995) with defects of the androgen receptor (AR) suggested that both hormones exert their actions via this single receptor protein. Although this receptor mediates the effects of both hormones, genetic and pharmacological data make it clear that the functions of testosterone and DHT are not equivalent. Testosterone is the principal circulating androgen in man and animals; however, the principal steroid hormone found bound to the AR in the nuclei is DHT, the 5 α -reduced metabolite of testosterone (Bruchovsky & Wilson 1968, Grino *et al.* 1990a). Genetic studies of individuals defective in the synthesis of one of the two enzymes that catalyze this conversion (5 α -reductase 2) provides further evidence that this biochemical transfor-

mation is physiologically important (Russell & Wilson 1994). Patients deficient in this enzyme show a phenotype characterized by female external genitalia and the absence of prostatic development, but with normal virilization of the male Wolffian duct-derived internal organs. Equally important, pharmacological studies have demonstrated that the administration of specific inhibitors of the 5 α -reductase enzyme(s) in animals during development causes defects of phenotypic male development that mirror the defects of virilization observed in patients with 5 α -reductase type II deficiency (Imperato-McGinley *et al.* 1985, George & Peterson 1988).

At least three mechanisms might account for the differential actions of these hormones. First, each hormone might have a different allosteric effect on the receptor and thus promote the modulation of different but overlapping sets of androgen responsive genes. Secondly, the formation of DHT in some tissues might be required to enhance a weak signal exerted by testosterone that is present in limiting concentrations (signal amplification). In this latter

theory, testosterone in high concentrations could perform all androgen actions, and DHT formation would be most important in tissues with low androgen content. This latter theory could explain the fact that exogenous DHT can virilize all portions of the urogenital tract in the female embryo (Schultz & Wilson 1974). Finally, the metabolic conversions of testosterone and DHT are not equivalent. Testosterone can be aromatized to estrogen, while DHT cannot. Such differences might allow for the selective regulation of genes by testosterone or DHT owing to selective conversion to metabolites (such as estrogen).

In previous studies, we demonstrated that a model androgen responsive gene, MMTV-CAT, is regulated in cells deficient in androgen metabolizing enzymes in a way that is most consistent with 5 α -reductase performing an amplifying function. That is, although the maximum reporter gene activation by testosterone and DHT was the same, differences were evident in the kinetics of gene activation effected by testosterone and DHT (Deslypere *et al.* 1992). Although these studies established the existence of one type of regulation of genes by testosterone and DHT, they offered no information regarding the relevance of these findings to a larger group of androgen-responsive genes in androgen target tissues. The current studies seek to examine these alternative models of gene regulation by testosterone and DHT in a more general fashion. To this end, we have used differential display PCR to identify genes that are expressed in the rat prostate in intact (normal levels of testosterone and DHT), castrated (low levels of testosterone and DHT), and Finasteride-treated (low levels of DHT) animals in patterns that suggest differential control by testosterone or DHT.

Materials and Methods

Treatment protocols and RNA extraction

Twenty mature male rats (each weighing approximately 200 g) were treated as follows: three were kept intact, ten were castrated and seven were treated with Finasteride (50 mg/kg/day s.c. in 20% ethanol in triolein oil). Following the treatment period, the rats were killed and the prostates were dissected, weighed, and used to prepare RNA. RNA was isolated using the Ultraspec RNA isolation kit (Biotecx Laboratories, Inc., Houston, TX, USA). The wet weights and RNA yields from each of the treatment regimens are shown in Table 1. As reported previously (George 1997), the concentration of DHT was reduced by 90% in the prostates of animals treated with Finasteride.

Differential display PCR

The display PCR protocol used in these studies is derived from that described by Liang and Pardee (1992). cDNA

was synthesized from total rat prostate RNA using 1–2 μ g total RNA in 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM MgCl₂, 0.01% gelatin, 1 nmol each of the primers (T₁₁CA, T₁₁GG, T₁₁GC, and T₁₁AT), and 800 μ M of four deoxyribonucleoside triphosphates (dNTPs). The reactions were incubated at 94 °C for 2 min, cooled, and the reverse transcription initiated by the addition of 2 units AMV reverse transcriptase (AMV-RT; Promega, Madison, WI, USA) and incubation at 40 °C for 1 h. Following this period, the reactions were incubated at 70 °C for 10 min and 94 °C for 5 min. Two units Ribonuclease H (Gibco BRL, Gaithersburg, MD, USA) were added to the reactions and the incubation continued at 37 °C for an additional 1 h. The reactions were then incubated at 94 °C for 5 min and either kept at –20 °C or used in the PCR reaction.

The cDNAs resulting from these reactions were then amplified by low stringency PCR using the previously described buffer, 1 nmol each of the primers described above, 1 nmol each of the 10 bp random primers (CTGATCCATG, CCAACGATGC, and AGAACAGTCA), and 800 μ M of the dNTPs. [³²P]dCTP (15 μ Ci, 3000 Ci/mmol; Amersham, Arlington Heights, IL, USA) was included to label the product of the PCR reaction. Native Taq polymerase (2.5–5 units; Perkin Elmer, Branchburg, NJ, USA) was added to the reactions followed by a 2 min incubation at 94 °C. Each PCR cycle consisted of a 30 s denaturation at 94 °C, followed by 1 min annealing at 42 °C and 30 s extension at 72 °C. After 40 cycles, the final products were extended for 5 min at 72 °C with subsequent incubations of 10 min at 98 °C and 15 min at 27 °C. The amplified cDNAs were precipitated with ethanol from 0.3 M sodium acetate, resuspended, and counted. Approximately 16 000 counts per minute per sample were applied to a 6% Long Ranger (AT Biochem, Malvern, PA, USA) gel cast using 1 $\times$ TBE. The RTS label-ready 100 bp DNA ladder from Gibco BRL was included in each gel as size standard. After electrophoresis, the gels were dried and exposed to X-ray film.

cDNA reamplification cloning and sequencing

After comparing the patterns visualized by autoradiography, individual bands were selected for study. This selection was based on apparent decreases in expression in response to castration or treatment with Finasteride. The gel fragments corresponding to these bands were cut out and eluted in water after heating at 68 °C for 1 h. The individual cDNA fragments were reamplified using aliquots of the eluted gel slices as template using the same primers and amplification parameters as those used to produce them through either display PCR or RT-PCR (40 cycles). The amplified products were purified by electrophoresis on agarose gels and cloned using the TA cloning vectors (Invitrogen, San Diego, CA, USA).

Clones were isolated with the Qiagen plasmid isolation kit (Chatsworth, CA, USA) and those containing inserts were sequenced using the DNA Sequenase kit from US Biochemical Corp. (Cleveland, OH, USA).

High stringency RT-PCR reactions

The RT-PCR reactions consisted of obtaining a specific cDNA from RNA using specific primers and amplification of such cDNA by PCR. In these reactions, cDNA was prepared by priming 1–2 µg total prostate RNA with 1 nmol of a specific T-tailed primer using the previously described AMV-RT reaction conditions. The resulting cDNA was used in a high stringency RT-PCR amplification reaction using a specific set of oligonucleotide primers (25 nucleotides in length) derived from the nucleic acid sequence of the individual cDNAs. Although the high stringency reactions employed the same buffer conditions as described above, the amplification conditions differed considerably. The reactions were incubated at 95 °C for 5 min and at 70 °C for 5 min. One unit Perfect Match (Stratagene, La Jolla, CA, USA) and 2.5–5 units native Taq polymerase were then added to the reactions followed by a 2 min incubation at 94 °C. The PCR cycle of amplification consisted of 30 s denaturation at 94 °C, followed by 30 s annealing at 70 °C and 1 min extension at 72 °C. After 25 cycles the final products were extended, as previously described. For C3 and AR RT-PCR reactions the following primers were used: CTGGCTGCAGTAT TCTAGATGAAG (C3 sense), GGCTGTTGACAGCT TTCAGTGTTA (C3 antisense) and CATGAAAGCA CTGCTACTCTTCAG (AR sense), GCTCACCATA TGGGACTTGATTAG (AR antisense) respectively. The cDNAs were precipitated and applied to a 6% sequencing PAGE cast using 1 × TBE buffer. After electrophoresis the gels were dried and exposed to X-ray film.

Results

Treatment protocols

In these studies, RNA was prepared from prostate tissue that was exposed to a range of hormonal environments: stimulation by DHT and testosterone (intact male rats), predominantly testosterone (Finasteride-treated male rats), or by neither DHT nor testosterone (castrated male rats). As outlined previously (George 1997), under the conditions used in this Finasteride treatment regimen, prostatic DHT concentrations fell by 90%, while intra-prostatic testosterone concentrations rose 3- to 5-fold. Of note, each of the treatment regimens used in these experiments was continued for a total of ten days. This protocol was designed to minimize the contribution from acute changes in gene expression caused by the changes in cellular composition that occur in the prostate immediately following androgen withdrawal or administration.

Table 1 Average prostate weights (means ± S.D.) and RNA yields of prostates from intact, castrated and Finasteride-treated intact rats

Treatment	Number of prostates	Average prostate weight (mg)	Total RNA yield (µg)
Intact	3	230 ± 54	2090
Castrated	10	20 ± 2	176
Intact, treated with Finasteride	7	89 ± 10	1136

The characteristics of the harvested tissues and RNA yields in one such experiment are shown in Table 1.

Differential display PCR and RT-PCR

Differential display PCR was used to analyze these RNA samples using 12 different combinations of oligonucleotide primers, as outlined above. A portion of a representative gel from one such experiment is shown in Fig. 1. Although very large numbers of changes are evident between the different samples in experiments using each primer set, our analysis centered on identifying mRNAs that appeared to reflect potential differences of regulation by testosterone and DHT. In this selection process, we focused our efforts on those genes that exhibited decreased expression in response to the two forms of androgen deprivation. Portions of the dried gel corresponding to the bands of interest were cut from the gel, eluted, reamplified, cloned and sequenced. A total of 25 possible candidates were eluted and 14 of these could be amplified. Of these 14 fragments, 8 partial cDNAs were cloned and characterized in detail. A summary of the characteristics of these clones and their patterns of regulation as inferred from the differential display PCR gel are shown in Table 2. The derived nucleotide sequences of the partial cDNA clones are presented in Table 3.

The level of expression of these clones as visualized on the display PCR gels suggested that we have isolated clones that are expressed at different levels under the different treatment conditions. As the display PCR experiments employed degenerate primers and low stringency conditions it was possible that the changes observed were a reflection of changes in the overall cellular composition of expressed RNAs and might not accurately reflect the abundance of the specific mRNAs under study. For this reason, the expression levels of each of the clones was examined further using a high stringency RT-PCR assay that employed specific primers derived from the nucleotide sequence of each clone. In these experiments, the specific primers were derived from the segments 5' to the poly A tail and distinct from those used in the original display PCR. Representative gels from such experiments are shown in Fig. 2 and the results of all such experiments

Figure 1 Differential display PCR of mRNAs expressed in rat prostates dissected from intact male rats (I), castrated male rats (C), and intact male rats treated with Finasteride (F). Similar quantities of labeled cDNA (approximately 1.6×10^5 disintegrations per minute) were loaded in each lane and electrophoresed on a 6% denaturing polyacrylamide gel. Following electrophoresis, the gels were dried and autoradiographed. The autoradiograph shows the analysis of the same RNA preparations using four different primer pairs (pair 1: T₁₁CA/AGAACAGTCA; pair 2: T₁₁GG/CTGATCCATG; pair 3: T₁₁GG/CCAACGATGC; pair 4: T₁₁GG/AGAACAGTCA). The white arrows indicate the position of the amplified bands corresponding to clones D9 and D1 in the differential display autoradiograph.

are tabulated in Table 2. The results indicate that the patterns of expression inferred from the results of the low stringency differential display PCR reactions were

accurate and only minor discrepancies were evident compared with the results of the high stringency RT-PCR reactions.

Table 2 cDNA clone characteristics and patterns of regulation

Clone	Pattern observed in display PCR experiments			cDNA fragment		Pattern observed in high stringency RT-PCR	Pattern type
	I	C	F	Size (bp)	Unique		
D1	+	—	+	359	Yes	Same ^a	1
D2	2+	+/-	2+	289	Yes	Same ^a	1
D3	+	+	3+	303	Yes	Same ^a	4
D6	2+	—	+	211	Yes	1+—2+ ^a	1
D7	+	—	—	224	Yes	Same ^a	3
D8	2+	—	+	225	Yes	Same ^a	2
D9	+	—	—	479	Yes	Same ^a	3
D10	+	—	+	293	Yes	Same ^a	1

The patterns of expression of 8 different cDNA clones as visualized by display PCR are indicated on the left using a semiquantitative scale (— (mRNA not detected), + (least) to 3+ (most)) to grade the intensity of bands observed. The characteristics of the isolated cDNA fragments are presented in the center. The pattern of expression inferred from 'hot' RT-PCR experiments using specific primers (based on the insert nucleotide sequence) and stringent PCR conditions is shown to the right. Superscript a indicates that the bands visualized in the hot PCR reaction have been shown to have the expected structure by sequence analysis of bands isolated from the RT-PCR reactions. I, intact rats; C, castrated rats; F, Finasteride-treated intact rats.

Discussion

Testosterone and DHT are the major circulating androgens in human and in other vertebrates. Although each hormone displays potent effects in biological assays of androgen action (e.g. prostate growth), under specific conditions each hormone displays properties that suggest roles in the modulation of specific functions. It is clear that DHT is crucial for the normal development of the male external genitalia and of the prostate (Imperato-McGinley *et al.* 1985, George & Peterson 1988). In contrast, testosterone appears to play essential roles in promoting spermatogenesis and Wolffian duct development, as well as gonadotropin regulation.

Although several potential explanations could account for the different biological effects of testosterone and DHT, three models seem most plausible. In the first model, both testosterone and DHT are capable of regulating androgen responsive genes to the same maximal extent and the biological differences evident between the activities of the two hormones would be due to kinetic differences in the binding of testosterone and DHT by the AR and limiting concentrations of testosterone and DHT at the cellular level (Veyssiere *et al.* 1982, Grino *et al.* 1990b). In this scenario, even in intact animals the tissue concentrations of androgens are below those needed to saturate the AR. In this context, the formation of DHT—which is more avidly bound by the receptor—would explain the dependence of some phenomenon on the formation of DHT. A second model postulates that although many genes can be regulated both by testosterone and DHT, the conformation of the two receptor-hormone complexes are not identical and that these differences confer specific regulation by only testosterone-AR or DHT-AR complexes on selected

genes (Fang *et al.* 1969). Such selective regulation of genes by the two androgens has not yet been described. Finally, differences in the metabolism of DHT and testosterone might account for their different biological effects. In this model, metabolic transformations of testosterone and/or DHT would lead to the formation of metabolites that possess unique biological properties. Although the aromatization of testosterone to form estradiol is the most obvious example, other transformations could be involved.

To date, the only work to examine the mechanisms by which testosterone and DHT act to regulate androgen-responsive genes has been performed using a model androgen-responsive gene, MMTV-CAT (Deslypere *et al.* 1992). Although the results of this study clearly demonstrate that this model gene is regulated by androgen in a manner most consistent with the first model described above, it has not been clear how representative these results are compared with the larger number of androgen-regulated genes expressed in androgen target tissues. Furthermore, while a number of studies have used differential display to examine the differential expression of genes in prostate tissue or prostatic cells (see, for example, Blok *et al.* 1995 or Chapman *et al.* 1995), only a single report (Lin & Chang 1997) has reported the isolation of genes differentially regulated by testosterone and DHT.

The current study was designed to begin to address these questions in a more general fashion using a tissue (the rat prostate) in which the importance of DHT formation is well established (Imperato-McGinley *et al.* 1985, George & Peterson 1988). Three features have been incorporated into this study to simplify the analysis of results. First, to minimize changes in gene expression caused by fluctuations in hormone levels (e.g. following androgen administration to castrated animals), we have compared mRNAs expressed in the rat prostate following no treatment

Table 3 Sequences of cDNA fragment clones isolated from differential display PCR

	Sequence
Clone	
D1	<p>GCTTTTGAGAAATATATCGGGTAAGGTTGAAAAATTAGACCTGAATA AATTCATTAATACAGAACCATACAGTGAAGACGATAAAATTTTATTGCGGCA ATTAAGAAAATTATTGCCTAATGAAATAACAAAGTATTTAAACCGTAATTCTC CGTTCAGTGGTTTTTGGGAAAACATTATTCAACAGCATGATGATGAGCTACC TGAAGAAACCAGAAAGCTGATTAACGAATACCTTCACCCAAAAATAAAAAA ATATTAGCAGAATTAAGTGAAAGCAATTTTGCATTTTATCTGCCGGATAAAA AATCTTTTACAACCTGCTAACTCAGAAAAGCTGAATTTATTGCAGAGCCTGC TGTGCCTGAGTTTTACGTTAATTATGCTAATGCTAAGTATGAGATTGAATGT AAGGTAAAGGCATCC(A)₁₁</p>
D2	<p>CCATATAAGGAAAGCCGGGTAAGAAGCCTAACATATATACCCGGTAT TGCTTGCCGTATGCAGTTGTATTATTTCTCAACTGTAATGTTTTTGATTGA CTCACTTCTTCTAAATCAATACCAAAAGAAATATCATAGCAAACAGGAATTT GTATGACTTTTTTTCTTGCGTCATTCGAGATGGGGTTCTTGCTCAACGAG AAATCTCTCGAATTTATTAAGACAAGATTTTCTGCACGAACCTCGAAATGA CGATTGATTCTTTTACCGCATTTTCAATTTGCTCTTTATAAATTGATAAGGT TGGAATGCTTTTGATAAAATAACATAAGCATCGTCG(A)₁₁</p>
D3	<p>CCGGCAGACATTGACATCTCTCCATAGTACAGCCCTTGGCTGCAGA GACTCCTCAGATCTGAGAGGCTGAACAGAGACTGGTAATATGCGGCCAGC GAAGGGTCATCTCTCTGAATGAGAAGGGGCTTCTGTTCCAGAAATTCCTTG AAAAATGTCTCTACTTTGATGGTGATATTAACCTTCAAAGAACTACTGG GGCTGTCAAAGTTAATACAGAAAGCGTATTAGGTAGCTCCTCTTACCTG CTTGCAAGGAACAGACTCTTCTCATTCTCATCCCCGTGGGGCTTCACTTTCT TTGGCATCGTTGGCG(A)₁₁</p>
D6	<p>GAAATGAAGAAGCTGGCTCAACTTCTCCAAGATTCTAGTAAGACTTTT GATTAAAATTATTCCATTCTTTTACAGGCTCTAGATCTTTAATTCTTCTCA GTAAAAAGTCTGTAAATAATTTTAAAGTCTTTCCTAATGGTGTTCCAGAGA AAACCCGCCTGGTCCGAAACCGTCTGTAAACATCTAGCGTGAAATGTGAAAA TTTCCAGTATTCAAATAAATCTCTATCAATCCAAAATTCAAAACCATCTACCA AACCAATCATAGCATCGTTGGCC(A)₁₁</p>
D7	<p>CATTGATGCCAGGAGCCTTCCCTGGTCATACTTCATGCAACCACTCT ACGATTATACATAGATTTCACTGTAGTTTATGTGTGAATCCACTTCTCTAAG GGTCTAATCACTGAACAAGCTACAACCAAAACATAGCCATTGTCCCAAGTTT TATAAACTGTATATGCCGTTTTATTGTTAATTGAATTGACTAATAGACAAGTC ATTTATTCTACATGTACATG(A)₁₁</p>

(testosterone and DHT present), castration (neither present), or treatment with Finasteride, an inhibitor of 5 α -reductase (predominantly testosterone present). It is

important to note that the protocol employed for the administration of Finasteride in this study has been previously described and shown to result in defined levels of

Table 3 Continued

	Sequence
Clone D8	<p>GTAATAATAAA<u>ACATTCTTCTCAGATGACTTCTTGC</u>ACACACAAAAAT GATTCTGCAAGCACAAACAGAACTTATTTCCAAGTTAAGCACTGTGTGTACCA AAAGAAAAAGAAAATTATCATTAATAAACCCATTCAATTATTTTTCTAAAGA CTAAGAACTGATTCCTTTCATCAATTTCAATTAATGTTTAACACTTTTAAAAATC AGATT<u>TACAATTCCATAACAGGTCTGCGT</u>G(A)₁₁</p>
D9	<p>CCAACGATGCGGAACCACTGAAGTACATGGTGGGTTTTAGAAAT<u>CTC</u> <u>AGTATTTCTTTACCTAGATA</u>CTTGCCCAATGTGAGAACTGTACCAGATGTCA GCCTCTGTATGTCCTCACTATTCTCCCACTTGCTTTCTCTGTATTATTCATTC ACATGGCTCCAGGCCTGGTTCTCGTCTCATTTCCTTGATGTTAAATTTTTAT TTGTTAACTATTAATATTTATAATTAATATTTATCTGTTATTTCTGCTAACTTT TATTTCTTTAATTTTTCATTTTTGTTTTGACAACCTCTGGTTTTTTATTTGGTGCA CGTTGGCATGCATGCACCAACGATGCGGAACCACTGAAGTACATGGTGGGT TTTAGAAATCTCAGTATTTCTTTACCTAGATACTTGCCCAATGTGAGAACTGT CCAGATGTCAGCCTCTGTATGTCCTCACTATTCTCCCACTTGCTTTCTCTGT TATTCATTACATGGCTCCAGGCCTGGTCTCGTCTCATTTTCCTTGATGTTTA ATTTTATTTGTTAACTATTAATATTTATAATTAATTTATCTGTATTCTGCTAA CTTTTATTCTTTAATTTTCATTTTTGGTTTGACAACCTCTCC(A)₁₁</p>
D10	<p>GGTGAA<u>ACAGAGGCAAAGCACGTGGTTAC</u>AGCTCTAGAGCTGGCAA GCGGCAAAATACCAAGGGGCAACTGGAACCTATGGCAGGGTTTGTAAGG AGATGGCCAAAGGTCTTAAACATGTCAGACTGAGAAAATGGAAGGCCAAAGA GGGATTTAAAAAGAGAGATTGACGGGGTTGGGGATTTAGCTCAGCGATAG AGCGCTTGCCTAGGAAGCGCAAGGTCTGGGTTTCGGTCCCCAGCTCCGAA AAAAAGACCCAAAAAAGAGAGAGACAGAGAGGTTGACACTT GCAACATACAAAACAACATGACATGAGTGTGTGTGTGCACATTTGTGTGTG ATGAGCGACTGTGTGTATGAGAGAGAAAGTGTGTGTGTGAGAGAGTGTAT GTGTGTATGTGAGTGAAGTGTCTG(A)₁₁</p>

The nucleotide sequence of each of the cloned cDNA fragments is presented. In each instance, the segment depicted is followed by repeated adenosine residues derived from the poly-A tail. The clone designations are those defined in Table 2. The specific primers used for high stringency RT-PCR of each clone are underlined.

testosterone and DHT in serum and in the prostate itself (George 1997). In the prostates of animals treated in this fashion, DHT levels were reduced by 90%, while testosterone levels increased 3- to 5-fold. Secondly, we have attempted to avoid the contributions of the well known changes in the cell composition of prostate that occur following castration and androgen administration in these studies by using a 10-day treatment regimen prior to tissue harvesting. This has been done in order to minimize changes in mRNA directly related to alterations in the tissue composition, caused by either apoptosis or regrowth. Finally, we have focused our attention on identifying genes that show increased expression in the intact or

Finasteride-treated animals, compared with the castrated rats. Although this condition certainly will result in the exclusion of genes regulated negatively by androgen from our study, this criterion is also likely to exclude genes that appear to increase in abundance following castration only as a result of the large scale changes occurring in the level of highly expressed RNAs in the prostate.

The eight genes isolated and analyzed in this study display four different patterns of regulation. Pattern types class 1 (D1, D2, D6, D10) and class 2 (D8) all show detectable expression of transcript in the RNA prepared from both the intact and Finasteride-treated animals (see Table 2). Both patterns are consistent with model 1 (see

Figure 2 RT-PCR of specific mRNAs expressed from rat prostates from intact male rats (I), castrated male rats (C) and intact male rats treated with Finasteride (F). The autoradiograph shows the analysis of the same RNA preparations using four sets of specific primers. The arrows indicate the positions of the amplified bands corresponding to fragments of the D9, D3, D6, and D8 mRNAs (identities of these bands have been proven by elution of the visualized bands from the gel, reamplification, and nucleotide sequence analysis). This autoradiograph demonstrates the four different patterns of mRNA expression that we have encountered. D9, pattern 3; D3, pattern 4; D6, pattern 1; D8, pattern 2. For comparison, assays of AR and C3 mRNA in these same RNA specimens using RT-PCR assays are shown in the two right-hand panels.

above), in which differences in the level of expression of genes in response to testosterone and DHT could be accounted for on the basis of differences in the kinetics of testosterone and DHT binding by the AR and limiting androgen concentrations. Together these two patterns account for over 50% of the genes that we have studied in detail (Table 2) or by inspection of our original display PCR gels (see Fig. 1).

In contrast, genes displaying the pattern termed class 3 (cDNAs D7 and D9) are detected only in RNA prepared from the prostates of intact animals. While these genes are candidates to be genes that are only regulated in response to 5α -dihydrotestosterone, it is also possible that these genes are exhibiting the same type of pattern observed for clone D8, but with a level of expression in the Finasteride-treated animals that is below the limits of sensitivity of the assay used. Further studies will be required to discriminate between these two possibilities.

The pattern observed for gene D3 (class 4) is quite unexpected, with the highest levels of expression being observed in RNA prepared from the prostates of Finasteride-treated animals. Although this pattern could be potentially explained in a number of different ways, selective regulation by testosterone or one of its metabolites (e.g. estradiol or androstenediol) or by the increased intraprostatic levels of testosterone that result from the Finasteride treatment regimen seem to be the most likely. Of interest, two mRNAs that are known to be regulated

by androgen – those encoding C3 and the AR – exhibit this pattern of regulation in RT-PCR assays.

In summary, we report the isolation and characterization of partial cDNAs representing genes that show four different patterns of regulation in response to castration and Finasteride treatment. Although it is impossible to predict whether any of the genes that we have identified will show absolute or selective regulation by testosterone or DHT, it is clear that we have identified genes that show substantially different patterns of androgen regulation in castrated and Finasteride-treated animals. While the patterns observed for many of these genes suggest that the different biological effects of testosterone and DHT may be due to the lower affinity of the AR and limiting tissue concentrations of androgen, our results also suggest that some genes expressed in the rat prostate may be regulated in fundamentally different ways in response to testosterone and DHT. Further studies will be required to identify the sites of expression of these genes within the prostate and the mechanisms that are responsible for these apparent differences in expression in response to testosterone and DHT.

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