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Androgen regulation of the largest subunit of RNA polymerase II in the rat ventral prostate

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Abstract

One of the dramatic changes in the prostate during androgen manipulation is the alteration in cellular content of total RNA — the amount of total RNA in each cell. The abundance of cellular total RNA correlates with the RNA polymerase (RNAP) activity in the prostate. One possible mechanism of androgen regulation of RNAP activity involves the regulation of RNAP expression. Western blot analysis showed that the largest subunit of the RNAP II, an essential component of the transcriptional machinery for mRNA, is indeed regulated by androgens. Castration down-regulates the protein level of RNAP II, whereas androgen replacement up-regulates the protein. However, androgen manipulation does not have consistent effects on the phosphorylation of the C-terminal domain (CTD) of the RNAP II. Androgen regulation of the RNAP II protein expression was also observed in the seminal vesicles but not in the thymus and liver, indicating that androgen regulation of RNAP II protein expression appears to be limited to the male sex accessory organs. These observations suggest that RNAP II plays an essential role in androgen action in male sex accessory organs.

Keywords: Androgen; Prostate; RNA polymerase II

1. Introduction

The prostate is a male sex accessory organ under the influence of androgens, testosterone and dihydrotestosterone [1,2]. Androgen ablation by castration induces massive apoptosis and regression of the prostate [3–5], while androgen replacement leads to rapid cell proliferation in a regressed prostate [1–3] Clearly, androgens play an important role in maintenance of structural and functional integrity of the prostate. Androgens are also involved in the progression of benign prostatic hyperplasia (BPH) and prostate cancer — the two most commonly diagnosed diseases in aging males in US [6]. Thus, studying the androgen action pathway in the prostate will help better understand the mechanisms of the disease progression and may have an impact on the prevention and/or therapy of these two diseases.

Rapid cell proliferation requires active synthesis of both RNA and proteins. It is well established that cellular RNA synthesis is catalyzed by RNA polymerases. There are three types of eukaryotic RNA polymerases — RNA polymerase I (RNAP I), RNA polymerase II (RNAP II), and RNA polymerase III (RNAP III) [7]. RNAP I is responsible for rRNA synthesis; RNAP II is for mRNA synthesis; and RNAP III is for 5S RNA and tRNA synthesis.

The rapid cell proliferation in a regressed prostate following androgen replacement is preceded by a significant increase in cellular content of total RNA [2]. The average amount of RNA per cell increases several fold within 2 days after androgen replacement. Many experiments have been carried out to study the mechanism by which total RNA synthesis is stimulated by androgens. In 1965, Liao et al. [8] have reported that testosterone can rapidly stimulate the RNAP activity of the rat ventral prostate. Davis and Griffiths [9,10] showed that androgens and androgen receptor complexes can stimulate the transcription of the chromatin

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by RNAPs. Mainwaring and Derry [11] suggested that androgen stimulation of polyamine synthesis may enhance the activity of RNAPs. RNA synthesis could also be influenced by activation of the chromatin template, the availability of essential divalent cations and substrates of RNA synthesis such as nucleoside 5′-triphosphates and/or activation of RNAPs [12]. Recent experiments by Kabler et al [13] demonstrated that the enhancement of rRNA synthesis by androgen in the rat ventral prostate is accompanied with increases in RNAP I and UBF — two essential components required in rRNA transcription. Transcription of mRNA is also enhanced by androgen in the rat ventral prostate. However, it is not clear whether the components of mRNA transcription machinery are also increased.

The largest subunit of RNAP II is a key component of the highly complex eukaryotic mRNA transcription machinery [7,14,15]. This eukaryotic protein is conserved evolutionarily and is even homologous to the prokaryotic RNAP. One interesting characteristic of the largest subunit of the eukaryotic RNAP II is its unusual C-terminal domain (CTD) consisting of 26–52 tandem repeats of the sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser [16–18] The largest subunit of RNAP II can adapt two forms designated IIo, which is heavily phosphorylated, and IIa, which is unphosphorylated. IIa is involved in the assembly of the transcription initiation, whereas, IIo catalyzes transcriptional elongation. The activity of RNAP II can be regulated by the amount and/or the phosphorylation of its largest subunit. Rapid changes in cellular total RNA content in the prostate during androgen manipulation have led us to hypothesize that RNAP II is involved in the androgen regulation of mRNA synthesis. This paper addresses the effect of androgens on the largest subunit of RNAP II in the rat ventral prostate.

2. Materials and methods

2.1. Materials

Ethylene diamine tetraacetic acid (EDTA), NaCl, Sodium dodecyl sulfate (SDS), and Tris were from Fisher Biotech (Pittsburgh, PA). Phosphate buffered saline (PBS) solution was from Gibco BRL (Gaithersburg, MD). Phenylmethylsulfonyl fluoride (PMSF), 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), leupeptin, pepstatin, benzanidine, aprotinin, NaF, and Na_3VO_4 were from Sigma (St. Louis, MO). Protran nitrocellulose membrane was purchased from Schleicher and Schuell (Keene, NH).

Two antibodies specific for the largest subunit of RNAP II were used in the present study. One of them is 8WG16 from Promega (no longer available from Promega), which is a monoclonal antibody specifically recognizing the CTD repeats of the RNAP II [19]. The other is ARNA-3, a generous gift from Dr Christoph H Winter. ARNA-3 does not recognize the CTD tail of the RNAP II (personal communication) [20].

2.2. Animals

Young adult male Harlan Sprague–Dawley rats (250–300 g) were castrated in a room dedicated to animal surgery, according to a protocol approved by the Northwestern University Animal Care and Use Committee. Castration removed testes, fat pads, and epididymis. The castrated animals were transferred to Northwestern University Animal Facility for maintenance. Androgen replacement of the 7-day castrated rats was carried out by daily subcutaneous injections of 0.2 ml testosterone propionate dissolved in propylene glycol at 10 mg/ml for up to 7 days. At each experimental condition, at least three rats were sacrificed by decapitation after methoxyflurane anesthesia. The ventral prostate lobes were removed, weighed, frozen in liquid nitrogen, and then pooled together prior to RNA or protein isolation. Other tissues including seminal vesicles, thymus, and liver were also isolated from the same animals.

2.3. Total RNA isolation, poly A^+ RNA selection, and quantification

Total RNA was isolated using the guanidine thiocyanate/CsCl gradient method [21]. Poly A^+ RNA was selected from 0.5 to 1 mg total RNA in Type-7 oligo dT cellulose column (Pharmacia), according to Sambrook et al [22]. RNA quantity was determined by a Beckman UV-spectrophotometer.

2.4. Protein preparation and western blot analysis

Prostate tissue was homogenized using Janke and Kunkel model T25 homogenizer at the top speed in a lysis buffer consisting of 0.4 M KCl, 10 mM EDTA, 25 mM Tris–HCl, pH 8.0, and 1% Triton X-100 and supplemented by the following protease inhibitors — 1 mM PMSF, 10 μM leupeptin, 0.2 mM AEBSF, 1 mM benzanidine, 1 μg/ml aprotinin and 1 μM pepstatin and phosphatase inhibitors; 50 mM NaF and 1 mM Na_3VO_4. Insoluble materials were pelleted by centrifugation at 10 000 × g for 10 min at 4°C. A very small percentage of the protein in the prostate extracts was insoluble. Protein concentration was determined using a BioRad DC protein assay kit. Protein samples were separated on a 6% SDS-PAGE and then transferred onto Protran nitrocellulose membranes. Mouse monoclonal anti-RNAP II primary antibody (Cat # ; E380A with Lot # 240101, Promega) at 1:7500 dilution or

ARNA-3 antibody at 1:1000 dilution in T-TBS plus 1% non-fat milk was used as primary antibody. A secondary antibody linked to horseradish peroxidase (HRP) (Amersham) at 1:5000 dilution was used in a standard western blot procedure for the detection of RNAP II protein in the prostate [18,23]. The enhanced chemiluminescence (ECL) detection method (Amersham) was used to visualize the bound secondary antibody. The 8GW16 antibody was earlier used for the detection of RNAP II protein in western blot [18].

2.5. Densitometric quantitation

The autoradiograms of western blot were quantitated using a Personal Densitometer SI version 4.0 (Molecular Dynamics). The image analysis software used was ImageQuaNTV4.2a.

3. Results

3.1. Androgen replacement increases cellular total RNA content in the ventral prostate of 7-day castrated rats

Total RNA synthesis is important for cell proliferation. Androgen replacement induces rapid cell proliferation preceded by significant increases in cellular total RNA content. We have confirmed the effect of androgen replacement on total RNA content in the 7-day castrated rat ventral prostate. Fig. 1A shows that total RNA per ventral prostate in 7-day castrated rats is 53 ± 4 μg (S.E.M.), which starts to increase about 14 h after androgen replacement and at 48 h after the replacement reaches 262 ± 21 μg (S.E.M.), which is a 5-fold induction. This observation agrees with the results observed by Coffey et al with a different method in the same animal model [2].

Although androgen replacement increased the amount of total RNA about 5-fold within 2 days, the changes in the wet weight of the ventral prostate during the same treatment is much less significant (Fig. 1B). The wet weight of the 7-day castrated prostate is 68 ± 7 mg (S.E.M.). After 2 days of androgen replacement, the prostate wet weight increased about 34% and reached 91 ± 6 mg (S.E.M.).

From the above observations, the amount of total RNA relative to prostate wet weight is 0.79 μg/mg of the tissue in 7-day castrated rats and the RNA concentration is increased to 2.88 μg/mg of the tissue 48 h after androgen replacement in the castrated animals. This is a 3.6-fold increase in total RNA concentration in the prostate. Clearly, the increase in the total RNA quantity in each ventral prostate is mainly attributed to the elevation in the total RNA concentration rather than the increase in tissue size. Since there is little or no change in the number of nuclei in the castrated prostate within 48 h after androgen replacement [1], the average cellular total RNA content is dramatically increased by androgen replacement.

Using type-7 oligo-dT cellulose column, about 4% of poly A^+ RNA was selected from the total RNA of the 7-day castrated ventral prostate or androgen treated prostate of 7-day castrated rats. The 4% poly A^+ RNA yield from prostatic total RNA was reproducible in two independent experiments. Thus, androgen-induced increase in total RNA results from a coordinated synthesis of rRNA and mRNA rather than solely from the synthesis of rRNA.

3.2. Androgen regulates the expression of the largest subunit of RNAP II in the rat ventral prostate

RNAPs are responsible for the synthesis of RNA. Thus, androgen regulation of RNA synthesis may be mediated through RNAPs. We used 8WG16 and ARNA-3 — two antibodies against the largest subunit of RNAP II, to study the effect of androgen on RNAP II in the prostate during hormonal manipulation. Fig. 2A shows the western blot analysis using both 8WG16 and ARNA-3. Both antibodies showed similar western

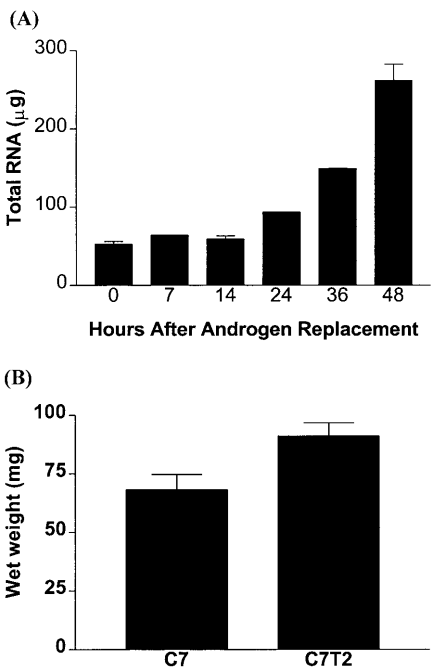

Fig. 1. The effect of androgen replacement on total RNA content (A) and wet weight (B) of the ventral prostate of rats castrated for 7-days prior to androgen treatment. Total RNAs were extracted from at least four prostates. The RNA extraction from prostate treated with testosterone propionate for 0, 14, and 48 h were repeated three times and standard error means (S.E.M.) were indicated. The wet weights of the ventral prostate treated with testosterone propionate for 0 h (C7) or 48 h (C7T2) were determined by dissecting ten animals in each group. The S.E.M. of the wet weight was also indicated.

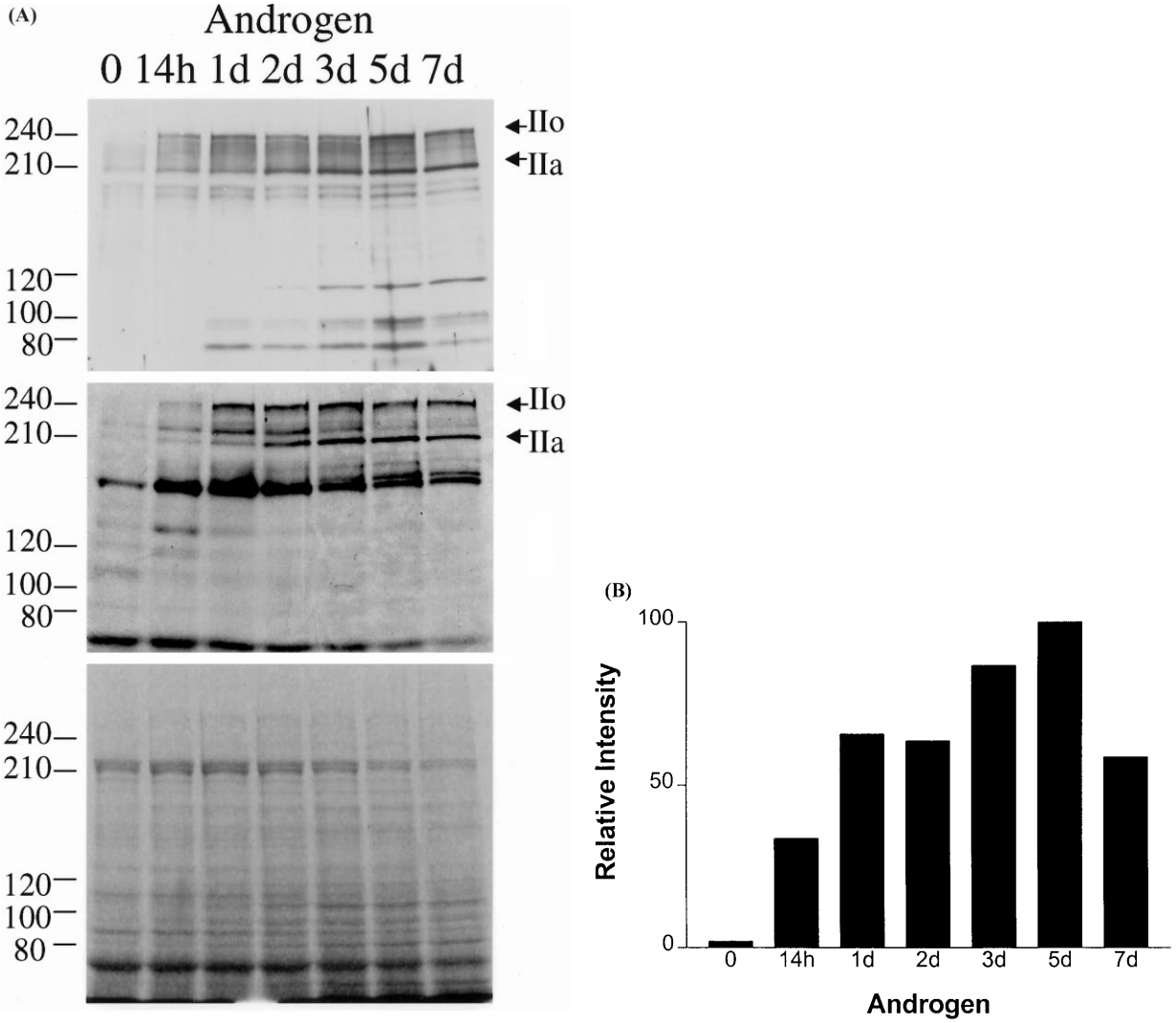

Fig. 2. (A) Effect of androgen replacement on RNAP II largest subunit in the rat ventral prostate. Protein extracts were prepared from the ventral prostate of 7-day castrated rats treated with testosterone propionate for indicated number of hours (h) or days (d). The protein extract was loaded in each lane of the 6% SDS-PAGE gel. The RNAP II largest subunit was detected by western blot using 8WG16 monoclonal antibody (top panel) or ARNA-3 antibody (middle panel). The phosphorylated form (IIo) and unphosphorylated form (IIa) of the RNAP II largest subunits migrate at 240 and 210 kDa, respectively. The RNAP II migrating between 210 and 240 kDa most likely reflects partial phosphorylation of the CTD tail of the RNAP II. The western blot also detected bands below 210 kDa. The western blot experiments were repeated three times with similar results. The loading of the protein extract was revealed by staining with Coomassie Brilliant Blue R-250 (bottom panel). The molecular weight (kDa) was indicated at the left of each panel. (B) Densitometric quantitation of the western blot analysis with the 8WG16 monoclonal antibody.

blot results. The western blot with 8WG16 was quantitated using a densitometer and the result is shown in Fig. 2B. This experiment shows that androgen replacement can significantly increase the amount of the largest subunit of RNAP II. The increase is initiated within 14 h and reaches the maximum at 3–5 days after androgen replacement.

We have also studied the effect of castration on the largest subunit of RNAP II using 8WG16 antibody (Fig. 3A). Fig. 3B shows the densitometric quantitation of the western blot. The level of RNAP II is reduced within 1 day after castration. The most significant down-regulation of RNAP II largest subunit occurs between 1 and 2 days after castration.

3.3. The phosphorylation status of RNAP II largest subunit during hormonal manipulation

RNAP II largest subunit can exist in two forms. The unphosphorylated form, designated IIa, is involved in transcription initiation. The heavily phosphorylated form, designated IIo, functions in transcription elongation. The phosphorylation of RNAP II can be regulated during cell proliferation [18]. Figs. 2 and 3 and Fig. 4 showed that both IIa and IIo forms are present in the rat ventral prostate, seminal vesicles, thymus, and liver. Interestingly, western blot detected the largest subunit of RNAP II with molecular weights in between the IIa and IIo, indicating that RNAP II can be

partially phosphorylated at their CTD repeats. The ratio of IIa and IIo forms in different prostate samples varies slightly and the variation in the ratio during androgen manipulation is not reproducible. Thus, the phosphorylation of RNAP II largest subunit was not consistently influenced by androgen manipulation. Both IIa and IIo forms of the RNAP II can be detected using either 8WG16 or ARNA-3 antibody (Fig. 2A).

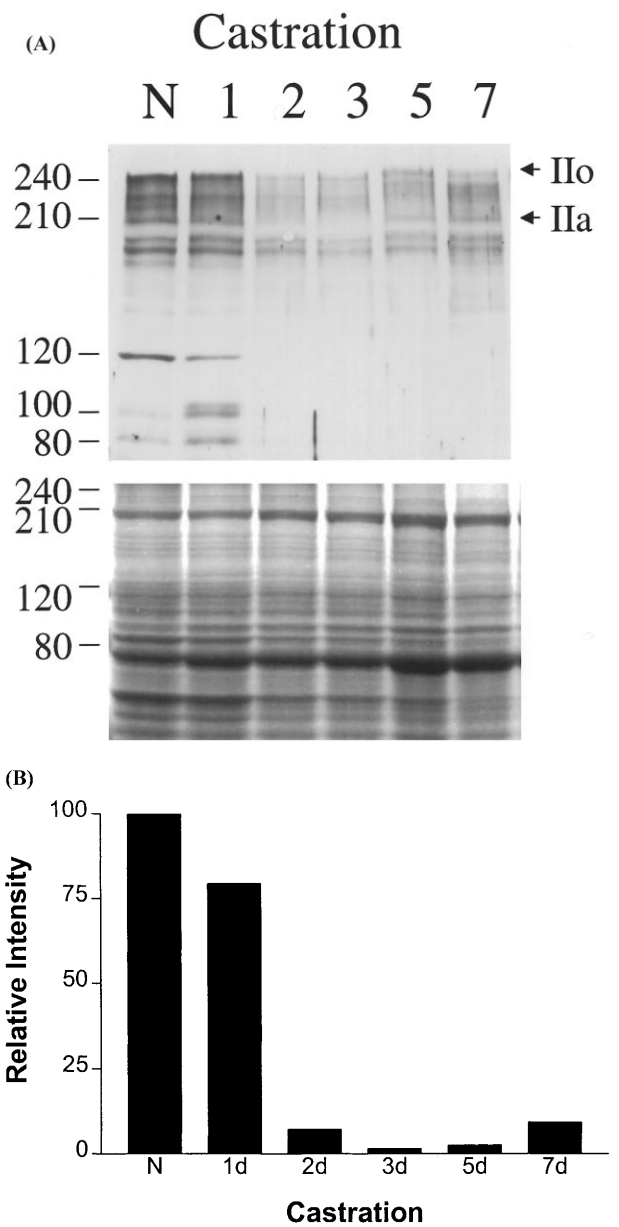

Fig. 3. (A) Effect of castration on RNAP II largest subunit in the rat ventral prostate. Protein extracts were prepared from the ventral prostate of the testis-intact rats and the rats castrated for indicated number of days (d). As described in Fig. 2, the RNAP II largest subunit was detected by western blot using 8WG16 monoclonal antibody specific for the CTD of the RNAP II largest subunit (top panel). The western blot was repeated three times with similar results. Lower panel shows the loading of the protein extract revealed by staining with Coomassie Brilliant Blue R-250. (B) Densitometric quantitation of the western blot analysis.

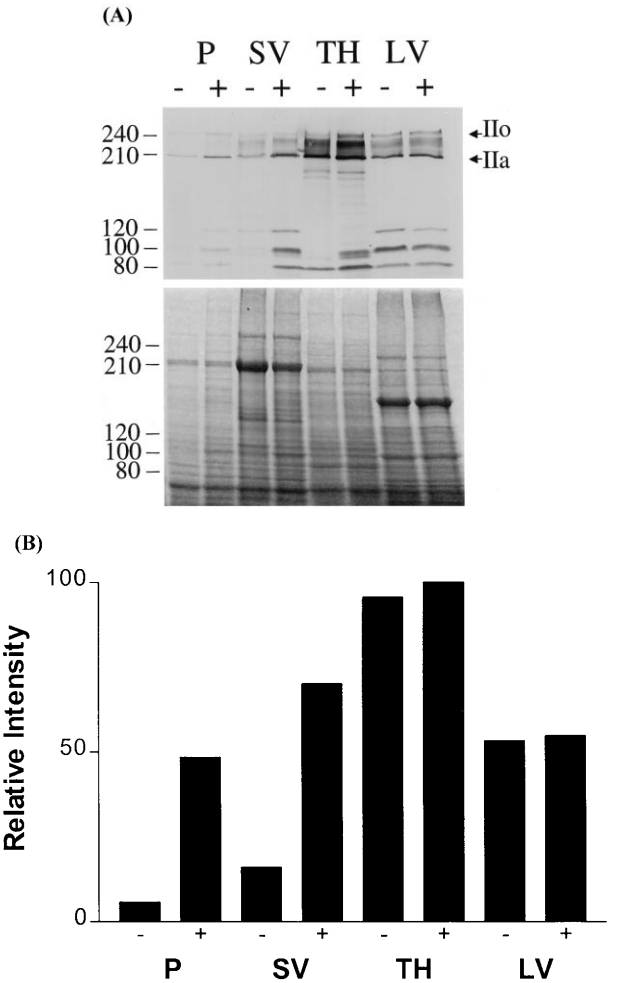

Fig. 4. (A) Effect of androgen on RNAP II largest subunit in the ventral prostate, seminal vesicles, thymus, and liver. Protein extracts were prepared from the ventral prostate (P), seminal vesicles (SV), thymus (TH), and liver (LV) of the 7-day castrated rats (−) and the 7-day castrated rats treated with testosterone for 2 days (+). As described in Fig. 2, the RNAP II largest subunit was detected by western blot using 8WG16 monoclonal antibody specific for RNAP II largest subunit (top panel). The western blot was repeated twice with similar results. Lower panel shows the loading of the protein extract revealed by staining with Coomassie Brilliant Blue R-250. (B) Densitometric quantitation of the western blot analysis.

3.4. Androgen regulates the expression of the largest subunit of RNAP II in male sex accessory organs but not in other organs

The effect of androgens on male sex accessory organs is different from that on other tissues. One major difference is that androgen manipulation induces massive apoptosis or proliferation in male sex accessory organs but not in tissues such as liver and thymus. Thus, we anticipate that androgen regulates RNAP II in male sex accessory organs only. Fig. 4A shows the western blot analysis of RNAP II in the ventral prostate, seminal vesicles, thymus, and liver of castrated animals with or without androgen replacement. Fig. 4B

shows the densitometric quantitation of the western blot. The result in Fig. 4 shows that the largest subunit of RNAP II is induced by androgen in the prostate and seminal vesicles. Androgen replacement had little or no effect on the largest subunit of RNAP II in the liver and thymus.

4. Discussion

The present research has shown that androgens regulate the protein level of the largest subunit RNA polymerase II in the prostate. The increase of cellular RNA content, the amount of total RNA per cell, represents an essential step leading to cell proliferation in a regressed prostate. Thus, it is important to study the mechanism by which androgens regulate prostatic RNA synthesis. Our studies show that the up-regulation of the largest subunit of RNAP II correlates with the increase of cellular RNA in the prostate. Therefore, this up-regulation is likely to be a key step in androgen-induced regrowth in the prostate.

Although androgens reproducibly up-regulate the protein levels of the largest subunit of RNAP II, the phosphorylation of the RNAP II was not influenced consistently in the same manipulation. To inhibit potential dephosphorylation of RNAP II, we added phosphatase inhibitors in the preparation of the protein extracts. However, we can not rule out the possibility that the variations in the distributions of IIa and IIo forms were due to incomplete inhibition of phosphatases.

One interesting observation in the western blot analysis using the 8WG16 monoclonal antibody (Fig. 2A, Fig. 3A, Fig. 4A) was the presence of readily detectable immunoreactive protein products with molecular weights of 190, 180, 120, 100, and 80 kDa. Some of these proteins are present in the normal prostate and the regrown prostate, but not in the regressed prostate. It is likely that these bands are unrelated to RNAP II and may represent some androgen-regulated proteins that have an epitope recognized by the 8WG16 antibody used in the western blot analysis. The fact that these low molecular weight bands were not detected with the ARNA-3 antibody (Fig. 2A) supports this explanation. However, we can not rule out the possibility that these bands may represent RNAP II degradation products and the degradation process of the RNAP II in the regressed prostate is altered relative to that in the normal and regrown prostates. It is possible that some RNAP II degradation products may react with the 8WG16 antibody but not the ARNA-3 antibody since these antibodies recognize different epitopes of RNAP II.

The increases in total RNA in the rat ventral prostate could result from increases in cellular total RNA and/or increases in the number of cells. Androgen replacement increased the wet weight of the ventral prostate about 34%, from 68 to 91 mg within 48 h. This increase is likely due to the increases in cell dimension because the number of cells remains virtually unchanged within 2 days after androgen replacement [1]. These observations collectively indicate that the total RNA content per cell on average in the ventral prostate was increased about 5-fold by androgen replacement.

The 5-fold increase in total RNA makes significantly more rRNA available for ribosome assembly and leads to more efficient protein synthesis. Concurrently, the increased mRNA synthesis provides more templates for protein synthesis. Johnsonbaugh et al. [24] showed that polyribosome formation was significantly stimulated by testosterone replacement in the castrated prostate. The newly synthesized RNAs were present in all components of polyribosomes after the replacement. These observations further substantiate the significance of coordinated increases in rRNA and mRNA upon androgen induction in the prostate.

The increase in the amount of total cellular RNA is different from the induction of specific androgen-response genes. The induction of androgen-response gene mRNAs is above the increase in total RNA content in prostatic cells. It is important to point out that some of the androgen-response genes are down-regulated during the hormonal manipulation [25]. The up- or down-regulation of androgen-response genes is mediated through the androgen receptor, a ligand-dependent transcription factor, either directly or indirectly. The induction of androgen-response genes is thought to play important roles in controlling cell proliferation, death, and differentiation during hormonal manipulation. The increase in total cellular RNA is essential for efficient protein synthesis that prepares the cells to undergo rapid proliferation and differentiation. Both the induction of specific androgen-response genes and the elevation of total cellular RNA are likely to be important to the proliferation and differentiation of prostatic cells.

Since RNAP II is only responsible for the transcription of mRNAs, the increased synthesis of other types of RNAs such as rRNA by androgens would require the increased activities of other types of RNAPs. As demonstrated by elegant studies of Kabler et al [13], RNAP I is indeed regulated by androgens in the prostate. Therefore, androgens initiate coordinated increases in total RNA synthesis by increasing the protein levels of RNAP I and II in the prostate. It will be interesting to determine whether the mechanisms of androgen regulation for both enzymes are similar in the prostate.

There are two major types of cells in the prostate — epithelial and stromal cells. Epithelial and stromal cells respond to androgen differently. In addition, epithelial cells in various regions of the prostatic ductal system

respond to androgen heterogeneously [26]. It is likely that the cellular RNA content varies in different cells, with some of them above 5-fold induction, whereas others less than 5-fold. We would like to propose that the cellular proliferation rate correlates with the induction of total RNA synthesis and that fast-proliferating cells have more active total RNA synthesis than slow-proliferating cells in the prostate.

The tissue-specificity study shows that androgens regulate the largest subunit of RNAP II in the prostate and seminal vesicles but not in thymus and liver, suggesting that androgen regulation of RNAP II occurs in male sex accessory organs only. This result suggests that androgen-regulation of the largest subunit of RNAP II is a critical step in androgen action in the prostate and other male sex accessory organs. The mechanism by which androgens regulate tissue-specific expression of RNAP II could be relevant to androgen regulation of cell proliferation and/or apoptosis in the male sex accessory organs. Any novel approaches that can specifically inhibit androgen-induction of RNAP II should inhibit cell proliferation and cause significant regression of male sex accessory organs because RNAP II inhibitors, such as α-amanitins, are cytotoxic [27]. Understanding the mechanism of tissue-specific activation of RNAP II by androgens may lead to the finding of novel targets for the inhibition of prostate growth.

Transcriptional regulation is highly complex. The rate-limiting step for transcription of Pol II genes is in general, at the step of transcription initiation which involves the assembly of the transcription initiation complex that includes RNAP II. RNAP II is a critical component of the transcription initiation complex and its availability could be important for the transcription of Pol II genes. The Pol II transcription is regulated by transcription factors, including general transcription factors. It is possible that some of these transcription factors are regulated by androgens. As revealed earlier, UBF, a transcription factor involved in the initiation of rRNA synthesis, is regulated by androgens [13]. Additional studies will be needed to address androgen regulation of other transcription factors in the prostate.

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References

[1] N. Bruchovsky, B. Lesser, E.V. Doorn, S. Craven, Hormonal effects on cell proliferation in rat prostate, Vitam. Horm. 33 (1975) 61–102.
[2] D. Coffey, J. Shimazaki, H. Williams-Ashman, Polymerization of deoxyribonucleotides in relation to androgen-induced prostatic growth, Arch. Biochem. Biophys. 124 (1968) 184–198.
[3] H.F. English, J.R. Drago, R.J. Santen, Cellular response to androgen depletion and repletion in the rat ventral prostate: autoradiography and morphometric analysis, Prostate 7 (1985) 41–51.
[4] H. English, N. Kyprianou, J. Isaacs, Relationship between DNA fragmentation and apoptosis in the programmed cell death in the rat prostate following castration, Prostate 15 (1989) 233–250.
[5] N. Kyprianou, J. Isaacs, Activation of programmed cell death in the rat ventral prostate after castration, Endocrinology 122 (1988) 552–562.
[6] J. Kozlowski, J. Grayhack, Carcinoma of The Prostate, Mosby Year Book, Chicago, 1991.
[7] B. Lewin, Genes VI, Oxford, New York, 1997.
[8] S. Liao, K. Leininger, D. Sagher, R. Barton, Rapid effect of testosterone on ribonucleic acid polymerase activity of rat ventral prostate, Endocrinology 77 (1965) 763–765.
[9] P. Davies, K. Griffiths, Influence of steroid-receptor complexes on transcription by human hypertrophied prostatic RNA polymerases, Mol. Cell. Endocrinol. 5 (1976) 269–288.
[10] P. Davies, K. Griffiths, Similarities between 5 alpha-dihydrotestosterone-receptor complexes from human and rat prostatic tissue: effects on RNA polymerase activity, Mol. Cell. Endocrinol. 3 (1975) 143–164.
[11] W.I. Mainwaring, N.S. Derry, Enhanced transcription of rRNA genes by purified androgen receptor complexes in vitro, J. Steroid Biochem. 19 (1983) 101–108.
[12] W. Mainwaring, The Mechanism of Action of Androgen, Springer, New York, 1977.
[13] R.L. Kabler, A. Srinivasan, L.J. Taylor, J. Mowad, L.I. Rothblum, A.H. Cavanaugh, Androgen regulation of ribosomal RNA synthesis in LNCaP cells and rat prostate, J. Steroid Biochem. Mol. Biol. 59 (1996) 431–439.
[14] M. Sawadogo, A. Sentenac, RNA polymerase B (II) and general transcription factors, Annu. Rev. Biochem. 59 (1990) 711–754.
[15] R.A. Young, RNA polymerase II, Annu. Rev. Biochem. 60 (1991) 689–715.
[16] J.L. Corden, Tails of RNA polymerase II, Trends Biochem. Sci. 15 (1990) 383–387.
[17] J.L. Corden, RNA polymerase II transcription cycles, Curr. Opin. Genet. Dev. 3 (1993) 213–218.
[18] M.F. Dubois, V.T. Nguyen, M.E. Dahmus, G. Pages, J. Pouyssegur, O. Bensaude, Enhanced phosphorylation of the C-terminal domain of RNA polymerase II upon serum stimulation of quiescent cells: possible involvement of MAP kinases, EMBO J. 13 (1994) 4787–4797.
[19] N.E. Thompson, D.B. Aronson, R.R. Burgess, Purification of eukaryotic RNA polymerase II by immunoaffinity chromatography. Elution of active enzyme with protein stabilizing agents from a polyol-responsive monoclonal antibody, J. Biol. Chem. 265 (1990) 7069–7077.
[20] A. Kramer, R. Haars, R. Kabisch, H. Will, F.A. Bautz, E.K. Bautz, Monoclonal antibody directed against RNA polymerase II of *Drosophila melanogaster*, Mol. Gen. Genet. 180 (1980) 193–199.
[21] J. Chirgwin, A. Przbyla, R. MacDonald, W. Rutter, Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease, Biochemistry 18 (1979) 5294–5299.

[22] J. Sambrook, E. Fritsh, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989.
[23] D.M. Bollag, S.J. Edelsten, Protein Methods, Wiley-Liss, 1991.
[24] R.E. Johnsonbaugh, F.G. Dalldorf, F.S. French, S.N. Nayfeh, Androgen action in the rat ventral prostate: effect of castration and testosterone treatment on polyribosomes, J. Steroid Biochem. 7 (1976) 73–79.
[25] Z. Wang, R. Tufts, R. Haleem, X. Cai, Genes regulated by androgen in the rat ventral prostate, Proc. Natl. Acad. Sci. USA 94 (1997) 12999–13004.
[26] C. Lee, J. Sensibar, S. Dudek, R. Hiipakka, S. Liao, Prostatic ductal system in rats: regional variation in morphological and functional activities, Biol. Reprod. 43 (1990) 1079–1086.
[27] S. Muraoka, N. Fukamachi, K. Mizumoto, T. Shinozawa, Detection and identification of amanitins in the wood-rotting fungi *Galerina fasciculata* and *Galerina helvoliceps*, Appl. Environ. Microbiol. 65 (1999) 4207–4210.