Steroid-sensitive gene-1 is an androgen-regulated gene expressed in prostatic smooth muscle cells *in vivo*

D Marcantonio¹, L E Chalifour¹, M A Alaoui-Jamali^{1,2} and H T Huynh^{1,2}

¹Department of Medicine, Lady Davis Institute for Medical Research, Sir Mortimer B Davis Jewish General Hospital, McGill University, 3755, Chemin Côte Ste Catherine, Montréal, Québec, Canada H3T 1E2

²Department of Oncology, Lady Davis Institute for Medical Research, Sir Mortimer B Davis Jewish General Hospital, McGill University, 3755, Chemin Côte Ste Catherine, Montréal, Québec, Canada H3T 1E2

(Requests for offprints should be addressed to H T Huynh who is now at Molecular Endocrinology Laboratory, National Cancer Centre, 11 Hospital Drive, Singapore 169610; Email: cmrhth@nccs.com.sg or L E Chalifour; Email: czlc@musica.mcgill.ca)

ABSTRACT

Steroid-sensitive gene-1 (SSG1) is a novel gene we cloned, found regulated by 17 β -estradiol in the rat uterus and mammary gland, and over-expressed in 7,12-dimethylbenz(a)anthracene-induced rat mammary tumors. We show here that SSG1 mRNA and protein expression are regulated by androgens in the rat ventral prostate. Increases in SSG1 mRNA levels were detected by Northern blotting after 24 h and reached a 27-fold peak 96 h following castration, relative to SSG1 mRNA expression in sham-operated rats. Dihydrotestosterone or testosterone supplementation of castrated rats prevented this rise in SSG1 mRNA. In contrast with SSG1 mRNA expression, SSG1 protein was

decreased 16-fold 2 weeks following castration but was at control levels in the prostates of castrated rats receiving dihydrotestosterone or testosterone. Although SSG1 is regulated by androgens *in vivo*, treatment of LnCap cells with dihydrotestosterone, cyproterone acetate or flutamide did not result in the regulation of SSG1 protein levels *in vitro*. Immunofluorescence studies show that SSG1 is mainly expressed in prostatic smooth muscle cells. These results indicate that SSG1 is an androgen-regulated gene that is expressed in the smooth muscle component of the rat ventral prostate *in vivo*.

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INTRODUCTION

Steroid-sensitive gene-1 (SSG1) is a novel gene recently cloned from a rat uterus cDNA library. The SSG1 cDNA of 3.719 kb encodes a putative nuclear protein of 41.8 kDa. SSG1 shares no significant homology with existing sequences yet is highly homologous to mouse, rat and human expressed sequence tags (Marcantonio *et al.* 2001). We previously showed that SSG1 is regulated by 17 β -estradiol (E₂) in the rat uterus and mammary gland in a tissue-specific manner. In the normal rat mammary gland, long term treatment with E₂ induced high levels of SSG1 protein expression in mammary tissue and SSG1 protein levels were consistently overexpressed in the hormone-dependent

7,12-dimethylbenz(a)anthracene-induced (DMBA) rat mammary tumors (Marcantonio *et al.* 2001).

Although SSG1 was initially isolated by its responsiveness to E₂ in the rat uterus and mammary gland, regulation of SSG1 may not be limited to E₂. The prostate is an androgen-dependent organ and circulating androgens are required for prostate development and maintenance (Cunha *et al.* 1987). Furthermore, androgens are implicated in both benign prostate hyperplasia and prostate cancer (Wilding 1995). Eighty to eighty-five percent of the normal rat ventral prostate is composed of androgen-sensitive epithelial cells (Colombel & Buttyan 1995). The minority of non-epithelial cells consist of endothelial cells which form the prostatic vasculature, smooth muscle cells which surround

the glands and some afferent blood vessels, nerves, and fibroblasts (Shabisgh *et al.* 1999). Androgen receptor (AR)-negative fibroblasts and AR-positive smooth muscle cells are the predominant cell types forming the prostatic stroma (Flickinger 1972). The main stromal interaction of adult prostatic epithelium is with the surrounding smooth muscle (Hayward *et al.* 1997).

Surgical castration results in a rapid decline in serum testosterone levels followed by a gradual depletion of dihydrotestosterone (DHT) in prostatic tissue. Within 6 h following castration, serum testosterone levels are decreased by approximately 98%, DHT is reduced by 35% and after 12 h, DHT in the prostate is reduced to 5% of intact levels (Kyprianou & Isaacs 1988). In the rat, androgen deprivation triggers apoptosis in the androgen-dependent epithelial cells within a day after castration and results in glandular involution with greater than 85% of cellular losses occurring within two weeks (Colombel & Buttyan 1995). Apoptosis is a rare event in prostatic smooth muscle cells following castration (Shabisgh *et al.* 1999). However, castration results in the disintegration and disappearance of smooth muscle bundles and is associated with the loss of prostatic smooth muscle differentiation markers (Hayward *et al.* 1996, 1997).

The prostate and mammary gland share many similarities. Both mammary and prostatic tissue require steroid hormones, estrogens and androgens, for differentiation, growth and function respectively, and both glands undergo involution following hormonal withdrawal (Lopez-Otin & Diamandis 1998). Furthermore, the initial stages of breast/prostate cancer are hormone dependent and are sensitive to anti-estrogen/anti-androgen treatments respectively (Lopez-Otin & Diamandis 1998). In the mammary gland, SSG1 is regulated by E₂. In this study, we set out to determine whether the SSG1 gene is regulated by androgens in the prostate.

MATERIALS AND METHODS

Experimental animals

All animal studies were performed in accordance with the regulations of the Canadian Council of Animal Care and the Animal Care Committee of the Lady Davis Institute for Medical Research. Male Sprague-Dawley rats weighing 300–350 g (Charles River, Québec, Canada) (6 rats/group) were either castrated or 'sham' castrated (scrotal incision only) under ketamine (15 mg/kg) : xylazine (10 mg/kg) anaesthesia. Rats were killed after 6, 24, 48, 96 and 120 h. The ventral prostate was immediately

removed, snap frozen in liquid nitrogen and stored at -80°C until needed.

In a second study, two weeks after castration, rats (3 animals/group) were implanted in the dorsal area with silastic tubing packed with testosterone to release 1.2, 2.4 or 3.6 $\mu\text{g/kg}$ per day or with DHT to release 1.2, 2.4 or 3.6 $\mu\text{g/kg}$ per day. Control rats (3 animals/group), were sham operated or were castrated and received dorsal implants of empty tubing. Rats were killed 21 days after hormone implantation, ventral prostates were collected and tissue was stored at -80°C until needed.

Finasteride, 5 mg tablets (Merck Frosst, Montreal, Quebec, Canada), was dissolved in water. Male Sprague-Dawley rats received 10 mg finasteride/kg body weight by gavage daily for 21 days (3 animals/group) whereas control rats received vehicle by gavage (3 animals/group). At the end of the treatment, ventral prostates were collected, snap frozen in liquid nitrogen and stored at -80°C until needed.

RNA isolation and Northern blot analysis

RNAzol B (Tel-Test Inc., Friendswood, TX, USA) was utilized to extract total RNA from tissue as described by the manufacturer. Total RNA (30–45 μg) was denatured, electrophoresed through a 1% formaldehyde-agarose gel and transferred by downward alkaline capillary blotting onto Zeta Probe GT nylon membranes (BioRad, Montréal, Québec, Canada) using 50 mM NaOH. SSG1 mRNA was visualized by Northern blot hybridization using the entire SSG1 cDNA, radio-labeled with [³²P]α-dCTP by random priming using the T⁷ Quick Prime Kit (Amersham Pharmacia, Baie D'Urfé, Canada), as directed by the manufacturer. Prehybridization, hybridization and washing were performed as previously described in Huynh (1998). Blots were subjected to autoradiography with intensifying screen at -80°C . Blots were stripped and then hybridized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to normalize for minor loading differences.

Protein extraction and Western blot analysis

Total protein was extracted from rat prostate tissue as described in Marcantonio *et al.* (2001). Rabbit polyclonal SSG1 antibody was created using synthetic peptides corresponding to predicted amino acids 3 to 29 (TVRGPSVSEQLYPLPR KEQQREKPQA) of the rat SSG1 gene and characterized as described in Marcantonio *et al.* (2001). Total protein, 75 μg , was subject to SDS-PAGE through a 10% polyacrylamide gel and

electrotransferred onto nitrocellulose membranes (BioRad) according to standard protocol. The membranes were blocked by incubation in TTBS (150 mM NaCl, 10 mM Tris-HCl, pH 7.4 and 0.1% Tween-20) buffer containing 7% dried milk for 1 h and then probed with affinity-purified anti-SSG1 rabbit polyclonal antibody in 1% milk in TTBS, at a concentration of 1 µg/ml at room temperature for 1 h. After four 15-min washes with TTBS, membranes were incubated with a peroxidase-conjugated secondary goat anti-rabbit antibody (Amersham Pharmacia). Immune complexes were detected using the enhanced chemiluminescence detection reagents (Amersham Pharmacia), as recommended by the manufacturer. Blots were stripped and then immunoblotted with anti- α -tubulin antibody as described above to normalize for protein loading.

Immunofluorescence

Normal prostate tissue was fixed in 10% buffered formalin for 24 h, dehydrated and embedded in paraffin. Tissue sections, 5 µm in thickness, were prepared and mounted on poly-L-lysine coated slides. Tissue sections were deparaffinized and rehydrated by successive incubations in toluene, 100% ethanol, 95% ethanol and finally in water. Prostate sections were equilibrated in phosphate-buffered saline (PBS). Deparaffinized tissue sections were blocked for 1.5 h at room temperature with blocking solution containing 0.2% gelatin, 2% bovine serum albumin and 2% normal goat serum in PBS. Samples were incubated with primary antibody, anti-SSG1, at 20 µg/µl, 10 µg/µl or 5 µg/µl blocking solution overnight at 4 °C in a humid chamber. For the negative control, tissue sections were incubated in blocking solution overnight at 4 °C in a humid chamber in the absence of primary antibody. Unbound primary antibody was removed by washing three times for 5 min each time with PBS containing 0.1% bovine serum albumin. Samples were incubated with secondary antibody, goat anti-rabbit IgG conjugated to Texas Red at a concentration of 1 µg/ml. Tissue sections were washed in PBS containing 0.1% bovine serum albumin and then with PBS alone. Samples were mounted and photographs were taken at $\times 400$ magnification using an Olympus BH2 microscope equipped with epifluorescence.

Cell culture

LnCap cells were maintained in RPMI media supplemented with 10% fetal calf serum. Cells were plated in RPMI media containing 5% fetal calf

serum on 100 mm plates. At a confluence of 60–70%, cells were washed twice in 5% charcoal stripped serum-phenol red free RPMI and incubated in this media. Twenty-four hours later, LnCap cells were treated with DHT, cyproterone acetate or flutamide for 72 h.

RNA and protein quantitation

The optical density of the hybridization signals was quantified by densitometric scanning (HPScanJet, Scion Image Quant Release Beta 3 Software; NIH, Bethesda, MD, USA) of the autoradiograms. Levels of mRNA expression were corrected for minor loading differences by normalizing to GAPDH mRNA expression. Levels of protein expression were corrected for by normalizing to α -tubulin protein levels. Statistical analyses were performed using Graph Pad Prism and a *P* value of <0.05 was considered significant.

RESULTS

Castration induces SSG1 mRNA expression in the rat ventral prostate

The effect of castration-induced androgen ablation on SSG1 mRNA expression was determined in the rat ventral prostate (Fig. 1A). Northern blotting of SSG1 mRNA expression indicates that SSG1 mRNA expression is low in sham-castrated rats (SC) at 6 h and did not significantly differ at 48, 96 and 120 h post sham castration (data not shown). Following castration, there was a significant 2-fold increase ($P<0.05$) in SSG1 mRNA expression at 24 h. SSG1 mRNA levels peaked at 96 h post castration and were 27-fold greater than levels of SSG1 mRNA expression in sham-operated rats (Fig. 1B).

SSG1 mRNA and protein levels are regulated in the ventral prostates of castrated rats receiving androgen supplementation

The involuted ventral prostate of the long-term castrated rat can be regenerated following testosterone or DHT supplementation (Colombel & Buttyan 1995). In our study rats castrated 2 weeks earlier received either 0, 1.2, 2.4 or 3.6 µg/kg per day DHT or testosterone for 21 days. In agreement with the results shown in Fig. 1, SSG1 mRNA expression was low but detectable in the prostate of sham-operated rats (Fig. 2, lane SC). Two weeks following castration, SSG1 mRNA levels in the ventral prostate of castrated rats were significantly increased by 8-fold ($P<0.01$) when compared with

FIGURE 1. Effect of castration on SSG1 gene expression in the ventral prostate. (A) The 3.8 kb SSG1 mRNA was detected by Northern blot analysis in the ventral prostate of sham-castrated rats (SC, $n=6$) and in the prostate of rats at 6, 24, 48, 96 and 120 h following castration ($n=6$). SSG1 expression in sham-castrated rats is shown only at 6 h as SSG1 mRNA expression did not significantly differ at 24, 48, 96 and 120 h post sham castration. GAPDH mRNA expression is also indicated and representative blots are shown. (B) Expression of SSG1 mRNA was quantitated by densitometric scanning of autoradiograms, normalized to GAPDH mRNA expression and is plotted. Values are expressed in arbitrary units with the means $\pm$ s.e. shown. Statistical significance ($P<0.05$) is indicated by an asterisk.

levels of SSG1 mRNA expression in control prostates (Fig. 2, lanes C). Northern blot analysis indicates that levels of SSG1 mRNA expression in the two-week castrated rats receiving DHT or testosterone were at low levels and were similar to SSG1 mRNA expression in sham-operated prostate tissue. Although DHT and testosterone vary in potency, both androgens were effective in decreasing SSG1 mRNA expression to levels expressed in the sham-operated rat.

In contrast to SSG1 mRNA, SSG1 protein expression in the rat ventral prostate of sham-castrated rats was significantly greater ($P<0.01$) than levels of SSG1 protein expression in the prostates of castrated rats (Fig. 3). Castration resulted in a 16-fold decrease in the levels of

FIGURE 2. Effect of castration and androgen replacement on SSG1 mRNA in the ventral prostate. (A) Northern blotting was performed on 30 µg total RNA extracted from the ventral prostate of sham-castrated (SC, $n=3$), 2-week-castrated (C, $n=3$) or 2-week-castrated rats treated with 1.2, 2.4 or 3.6 µg/kg per day DHT or 1.2, 2.4 or 3.6 µg/kg per day testosterone (T) ($n=3$), for 21 days. Blots were hybridized to radioactive SSG1- and GAPDH-specific cDNA probes. Representative blots are shown. (B) SSG1 and GAPDH mRNA expression was quantitated by densitometric scanning of autoradiograms and SSG1 mRNA expression was normalized to GAPDH mRNA expression. Values are expressed in arbitrary units with the means $\pm$ s.e. shown. Statistical significance, when compared with SSG1 expression in sham-castrated samples ($P<0.01$), is indicated by an asterisk.

SSG1 protein expression in the ventral prostate. In the ventral prostate of castrated animals receiving 1.2, 2.4 or 3.6 µg/kg per day DHT, SSG1 protein expression was similar to that of sham-operated rats. Although SSG1 protein expression in castrated rats receiving 1.2 or 2.4 µg/kg per day testosterone was significantly higher ($P>0.01$) than SSG1 protein expression in castrated rats, testosterone was not as effective as DHT in restoring SSG1 protein levels to that expressed in control tissue. These results indicate that SSG1 protein expression is regulated by androgens and that SSG1 mRNA and protein expression, in response to androgen withdrawal and replacement in the ventral prostate, are discordantly regulated.

FIGURE 3. Effect of castration and androgen replacement on SSG1 protein expression in the ventral prostate. (A) 75 µg total protein extracted from the ventral prostate of sham-castrated (SC, $n=3$), 2-week-castrated (C, $n=3$) or 2-week-castrated rats treated with 1.2, 2.4 or 3.6 µg/kg per day DHT or 1.2 or 2.4 µg/kg per day testosterone (T, $n=3$), for 21 days, were separated on a 10% SDS-PAGE gel. Blots were probed with anti-SSG1 or anti- α -tubulin antibodies as indicated. A representative blot is shown. (B) SSG1 and α -tubulin expression were quantitated by densitometric scanning of the autoradiograms. SSG1 expression was normalized to α -tubulin expression and is plotted. Values are expressed in arbitrary units with the means $\pm$ S.E. shown. Statistical significance ($P<0.01$), when compared with protein expression in the ventral prostate of castrated rats, is indicated by an asterisk.

Finasteride does not regulate SSG1 mRNA or protein levels in the rat ventral prostate *in vivo*

In androgen-dependent target cells, 5 α -reductase converts testosterone to the more potent androgen DHT (Gormley 1992). Finasteride, a steroid analogue, competitively inhibits 5 α -reductase activity resulting in an increase in intraprostatic testosterone levels by about 10-fold and the suppression of serum and intra-prostatic DHT concentrations to castration-equivalent levels (Gormley 1992). SSG1 mRNA and protein expression were measured in the ventral prostates of intact

rats treated with 10 mg/kg finasteride daily for 21 days. As indicated in Fig. 4A and B, SSG1 mRNA expression does not significantly differ in the prostates of control-treated rats versus SSG1 mRNA expression in the prostates of finasteride-treated rats. Furthermore, SSG1 protein expression in control-treated prostates did not significantly differ when compared with SSG1 expression in finasteride-treated samples (Fig. 4C and D) although treatment with this antiandrogen resulted in a significant ($P<0.05$) decrease in ventral prostate weight (data not shown).

Immunofluorescent localization of SSG1 in the rat ventral prostate

The site of SSG1 protein expression in the normal rat ventral prostate was investigated by immunofluorescent analysis using a SSG1-specific antibody (Fig. 5). A strong immunofluorescent signal was predominantly located to the prostatic smooth muscle cells (panels A and B, arrowhead) and to the vascular smooth muscle cells (panel B, arrow) of the rat ventral prostate. Immunofluorescent labeling was also observed at lower levels in the epithelial compartment of the ventral prostate (panels A and B, star). In panel C, immunofluorescent labeling was performed in the absence of primary anti-SSG1 antibody to demonstrate the non-specific component of the immunofluorescent signal.

Treatment with androgens and antiandrogens *in vitro* does not regulate SSG1 protein expression

LnCap prostate cancer cells of epithelial origin do not express detectable levels of SSG1 mRNA by Northern blot analysis and SSG1 mRNA remained undetectable following DHT treatment (data not shown). Nonetheless, Western blot analysis indicates that LnCap cells express SSG1 protein. Therefore, LnCap cells were treated with DHT (0, 1, 5 or 50 nM), cyproterone acetate, a steroidal anti-androgen, (0, 10, 50 or 500 nM) or flutamide, a non-steroidal antiandrogen, (0, 10 or 500 nM) for 72 h. As shown in Fig. 6A and B, SSG1 protein levels remain unchanged following androgen or antiandrogen treatment *in vitro*.

DISCUSSION

We previously demonstrated that SSG1 is regulated by E₂ in the rat mammary gland *in vivo*. SSG1 protein expression is induced in the rat mammary

FIGURE 4. SSG1 mRNA and protein levels following finasteride treatment in the rat ventral prostate. Rats ($n=3$) were treated with 0 or 10 mg finasteride/kg body weight daily for 21 days as indicated. A representative Northern blot analysis of SSG1 expression is shown in panel A. In panel B, levels of SSG1 mRNA expression were quantitated by densitometric scanning of autoradiograms and normalized to 28S mRNA levels. Values are expressed in arbitrary units with the means $\pm$ s.e. shown. A representative Western blot analysis of SSG1 protein expression in the ventral prostates of control and finasteride-treated rats is shown in C. SSG1 protein levels were quantitated by densitometry and normalized to α -tubulin protein expression (D). Results are expressed in arbitrary units with the means $\pm$ s.e. shown.

gland following a two-week exposure to super-physiological levels of E_2 and is decreased following the removal of the E_2 stimulus. Furthermore, SSG1 is overexpressed in the hormone-dependent DMBA-induced rat mammary tumors, suggesting that SSG1 may play a role in estrogen functions related to mammary carcinogenesis or may be a marker for breast cancer (Marcantonio *et al.* 2001). The objective of this study was to determine whether SSG1 would be regulated by steroid hormones in the prostate. Therefore the influence of androgens on the regulation of SSG1 in the ventral

prostate was studied by investigating the mRNA expression pattern of SSG1 following castration.

In our studies, castration-induced androgen ablation increased SSG1 mRNA levels in a time-dependent manner when compared with SSG1 mRNA levels in sham-operated animals. Treatment of rats with regressed prostates with DHT or testosterone decreased SSG1 mRNA expression and was similar to levels of SSG1 expression in sham-castrated controls. These *in vivo* studies demonstrate that SSG1 mRNA expression is regulated, at least in part, by DHT and

FIGURE 5. Immunofluorescent localization of SSG1 protein in the rat ventral prostate. Formalin-fixed, paraffin-embedded rat ventral prostate tissue sections were used in these studies. In panels A and B, the SSG1 protein was immunofluorescently labeled using an anti-SSG1 specific antibody and goat anti-rabbit IgG conjugated to Texas Red. Arrowheads indicate the SSG1 protein localized to the prostatic smooth muscle cells whereas SSG1 located to vascular smooth muscle is indicated by an arrow. SSG1 located to the epithelial cells of the prostate is indicated by a star. The negative experimental control in which primary antibody was omitted is shown in panel C.

testosterone in the rat ventral prostate and suggests that androgens may act to negatively regulate SSG1 mRNA expression. This is the first demonstration that SSG1 is regulated by androgens in a male reproductive tissue. In contrast to the SSG1 mRNA results, SSG1 protein levels dramatically decreased after castration. Androgen supplementation returned SSG1 to control levels of protein expression in the castrated rat. Thus, in the prostate, SSG1 mRNA and protein expression are discordantly regulated in response to androgens. We previously showed that SSG1 mRNA and protein expression were also discordantly regulated in the rat mammary gland following estrogen treatment (Marcantonio *et al.* 2001). Furthermore, a discordant pattern of AR mRNA and protein regulation in response to androgens was observed in LnCap prostate cancer cells and the AR-positive MDA453 breast cancer cells (Yeap *et al.* 1999). In LnCap and MDA453 cells, DHT treatment decreased total AR mRNA, but increased AR protein expression (Yeap *et al.* 1999). In LnCap cells, DHT down-regulated AR mRNA transcription but stabilized AR mRNA. In contrast, DHT destabilized AR mRNA yet had

no effect on AR mRNA transcription in MDA453 cells (Yeap *et al.* 1999). This suggests an important role for post-transcriptional pathways in the regulation of gene expression by androgens which ultimately affect mRNA and/or protein expression. Although the mechanism of SSG1 regulation by androgens in the ventral prostate has not been determined, we propose that SSG1 undergoes tissue-specific translational and/or post-translational modifications that may ultimately affect SSG1 expression in the prostate.

Androgens play an important role in the male reproductive system; however, estrogens are also produced in the male (Griffin 1992). Seventy-five to ninety percent of male estrogen is formed by the peripheral aromatization of testosterone to E_2 and androstenedione to estrone in the adipose tissue. The remaining 10–25% of E_2 and estrone is produced by the testes (Farnsworth 1999). In the rat ventral prostate, estrogen-receptor alpha is localized to stromal cells, both fibroblasts and smooth muscle cells, which are the primary target of estrogens, whereas estrogen-receptor beta is expressed in the luminal epithelial cells (Prins & Birch

FIGURE 6. SSG1 protein levels are unchanged in LnCap cells following treatment with DHT or antiandrogens. (A) Western blotting was performed on 75 μ g total protein extracted from LnCap cells treated with nanomolar concentrations of DHT, cyproterone acetate or flutamide for 72 h using an anti-SSG1 or an anti- α -tubulin antibody as indicated ($n=3$). A representative Western blot is shown. (B) SSG1 and α -tubulin protein expression were quantitated by densitometric scanning of the autoradiograms. SSG1 expression was normalized to that of α -tubulin and results are plotted. Values are expressed in arbitrary units with the means $\pm$ s.e. shown.

1997, Chang *et al.* 1999, Chang & Prins 1999). In our study finasteride treatment of rats did not significantly alter SSG1 mRNA or protein expression in the rat ventral prostate *in vivo*. Finasteride is an anti-androgen which inhibits the activity of 5 α -reductase required for the conversion of testosterone to the more potent androgen DHT (Gormley 1992). Although finasteride reduces the levels of intraprostatic DHT, treatment with this anti-androgen is not equivalent to castration-induced androgen ablation. Finasteride treatment results in a 10-fold increase in intraprostatic testosterone concentration in rats and in humans, which can be metabolized to estrogens (Geller 1990, McConnell *et al.* 1992). Therefore the lack of significant regulation of SSG1 expression in response to

finasteride in the ventral prostate of intact rats suggests that SSG1 is regulated by testosterone but also potentially by estrogens. With increasing age, the ratio of androgens to estrogens is decreased due to the rise in estrogen production and is accompanied by the development of benign prostatic hyperplasia, a stromal disease associated with increased serum E₂ level (Levine *et al.* 1997). Therefore, the role of estrogens in SSG1 modulation in the rat ventral prostate warrants further investigation in view of the potential role of estrogens in prostate disease.

The SSG1 protein was highly expressed in the prostatic smooth muscle cells and the smooth muscle component of the vasculature *in vivo*. Prostatic smooth muscle cells are AR-positive, and

are the primary target of androgen action in the adult prostate stroma (Gerdes *et al.* 1996). The adult prostate requires androgens to maintain smooth muscle cell differentiation and is probably dependent on expression of the AR in prostatic smooth muscle (Hayward *et al.* 1997). It has been proposed that the differentiated prostatic smooth muscle of the adult prostate stroma is a key player in the microenvironment of the prostate epithelium (Cunha *et al.* 1996). Thus, prostatic smooth muscle cells maintain epithelial structure and function and conversely, prostatic epithelium maintains smooth muscle differentiation (Cunha *et al.* 1996). Furthermore, the interaction between stroma and epithelium via stimulatory and inhibitory peptides, such as epidermal growth factor (EGF), transforming growth factor (TGF)- α , TGF- β , insulin-like growth factors (IGFs) and fibroblast growth factors (FGFs) maintains normal prostate growth (Hiramatsu *et al.* 1988, Hofer *et al.* 1991, Kyprianou & Isaacs 1989, Peehl *et al.* 1995, Story 1995). Damage to the prostatic epithelium may result in aberrant epithelial to smooth muscle signaling. Consequently, smooth muscle cells de-differentiate resulting in abnormal smooth muscle to epithelial signaling (Hayward *et al.* 1997). Due to the lack of androgen-responsive prostate stromal cell lines, advances in the identification of key androgen-regulated prostate stromal genes has been limited. This is despite the fact that characterization of androgen-regulated gene expression in prostate smooth muscle is critical to the study of the mechanisms involved in developmental differentiation and growth control of the prostate, as well as in the progression of benign and neoplastic prostatic disease (Gerdes *et al.* 1996). Thus, our finding that SSG1 is an androgen-responsive gene *in vivo* and is expressed in prostatic smooth muscle is of importance. SSG1 was not regulated by DHT in the androgen-responsive LnCap prostate cancer cells which are of epithelial origin and, thus, androgen responsiveness may be limited to prostatic smooth muscle cells. Although the role of androgens is important, alone they are insufficient to maintain normal prostate homeostasis. In adulthood, normal prostatic growth and differentiation are mediated by the local synthesis and action of paracrine signaling molecules (Lee 1997). Prostatic homeostasis requires complex interactions between EGF, TGF- α , TGF- β , IGFs and FGFs with their receptors, which may be regulated by androgens or independently by other factors (Hiramatsu *et al.* 1988, Hofer *et al.* 1991, Kyprianou & Isaacs 1989, Peehl *et al.* 1995, Story 1995). Therefore peptide growth factors may also contribute to SSG1 regulation following castration and androgen

replacement in the rat ventral prostate. At this time, the function of SSG1 is unknown. In the prostate, androgens regulate three major cellular processes: proliferation, differentiation and apoptosis. Therefore, SSG1 may well be involved in the regulation of these cellular events in response to hormonal manipulation *in vivo*.

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