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Thanatogen Expression During Involution of the Rat Ventral Prostate After Castration

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ABSTRACT: After castration the rat ventral prostate undergoes regression. This process occurs due to the induction of apoptosis, or active cell death, in the epithelial cells of the gland. Several genes, including TRPM-2, (testosterone repressed prostate message), RVP.1, fos, and myc, have been shown to be induced in the prostate during this process. We have investigated the expression of several other genes that may be associated with apoptosis, including tissue transglutaminase (TGase), poly(ADP)ribose polymerase (PARP), and heat shock protein 27 (Hsp27). Northern hybridization has been used to determine the steady-state mRNA levels of these genes in the ventral prostate after castration, and the time course of induction has been compared to the changes in the steady-state levels of prostate steroid binding protein (PSBP), α-tubulin, and TRPM-2 mRNAs. The results show that the mRNAs for PARP, transglutaminase, and Hsp27, in addition to TRPM-2, are induced by androgen ablation in the rat ventral prostate and reach maximum levels between days 3 and 4 after castration. Using *in situ* hybridization we have established that these genes are expressed in the epithelial cells of the prostate that are known to undergo active cell death; this result suggests that their gene products may be required in the dying cells to ensure that the biochemical and morphological processes of apoptosis are completed appropriately.

Key words: Apoptosis, TRPM-2, clusterin, transglutaminase, poly(ADP)ribose polymerase, heat shock proteins, regression.

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Active cell death (ACD) in hormone-dependent tissues is readily induced following hormonal ablation and by treatment with various agents including anti-androgens, anti-estrogens, and transforming growth factor β_1 (TGFβ_1) (for review see Tenniswood et al, 1992). ACD is known to be an active process that requires protein and RNA synthesis (Bruchovsky et al, 1975; Lee, 1981) and the active participation of a number of genes that must be induced *de novo*. We have coined the term *thanatogen* (from the Greek thanatos—death) to describe these genes (Tenniswood et al, 1994). Thanatogens fall into two broad categories: those that initiate the process (primary thanatogens), and those that ensure that the dying cell is fragmented and phagocytosed appropriately (secondary thanatogens). Apoptotic cells undergo significant changes in cellular morphology that require the activity of a number of thanatogens to ensure that ACD is completed appropriately. In addition, during regression of hormone-dependent tissues such as the prostate, the steady-state levels of a number of mRNAs that are not involved in the apoptotic process may also be induced in the cells that eventually undergo apoptosis. These include genes that may be induced in most of the epithelial cells that die as part of a futile stress response mechanism after hormone ablation and genes that are induced in a subset of the epithelial cells as part of a survival mechanism to ensure that the atrophied tissue can respond to restimulation by the trophic hormone.

In the prostate the nuclear androgen receptor decreases to undetectable levels within the first 12 hours after castration, followed by a marked reduction in prostate size and a dramatic decrease in androgen-dependent protein synthesis (Heyns et al, 1977; Parker and Scrace, 1979; Clark et al, 1983). Total RNA and DNA contents decrease 80–85% within 7 days of castration as a result of epithelial cell death (DeKlerk and Coffey, 1978; English et al, 1985, 1987). Paradoxically, the activity of a number of enzymes increases during this time. For example, the activity of cathepsin D is increased and reaches a peak between 5 and 7 days after castration (Tanabe et al, 1982; Sensibar et al, 1990). The activity of acid ribonuclease (RNase) increases gradually, peaking between 5 and 7 days after castration. Treatment with actinomycin D retards this increase, and it also decreases the rate of prostatic regression (Lee, 1981). The activity of both urokinase and tissue type plasminogen activator increases after castra-

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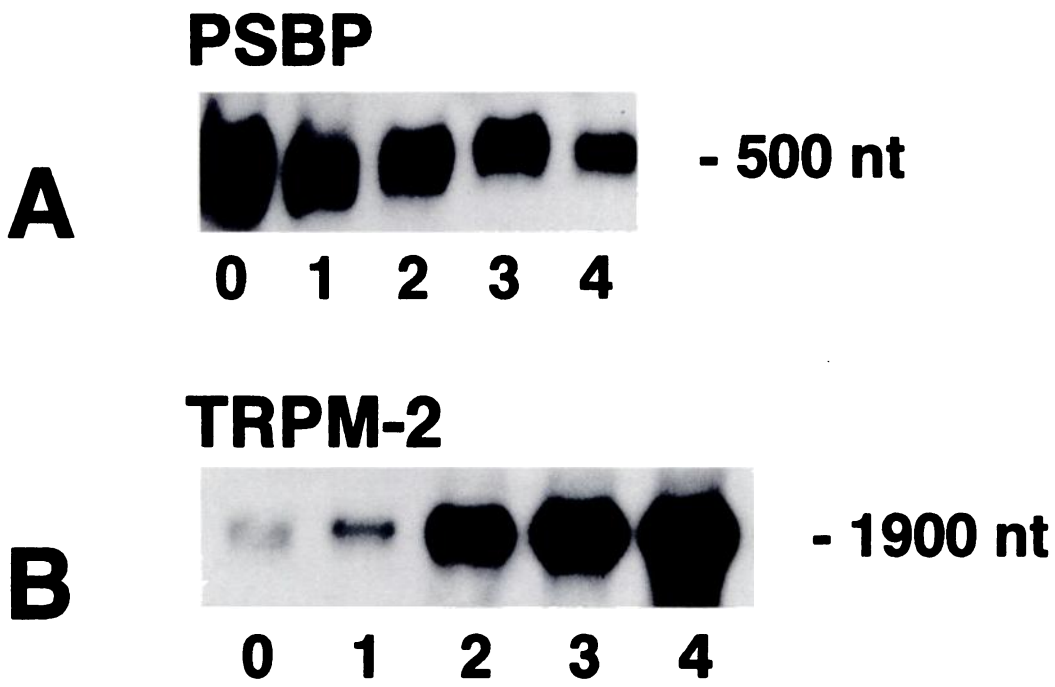


FIG. 1. Expression of PSBP and TRPM-2 in the rat ventral prostate after castration. Mature male adult rats were castrated and sacrificed on the days indicated. Poly(A)$^+$ RNA was isolated from the rat ventral prostate as described in the Materials and Methods section. Poly(A)$^+$ RNA was loaded at 2 μg per lane, electrophoresed on formaldehyde gels, transferred to nylon membranes, and hybridized to radiolabeled cDNA probes (1 × 10^7 cpm) specific for PSBP (panel A), exposed for 4 hours, and TRPM-2 (panel B), exposed for 18 hours.

tion, and the peak activity correlates with the maximum rate of cell death in the gland (Freeman et al, 1990). In addition to the increase in these enzymatic activities, several "castration-induced" mRNA species have already been identified in the prostate (Montpetit et al, 1986; Lee and Sensibar, 1987), including the mRNA coding for the Yb1 subunit of glutathione S-transferase (Chang et al, 1987), TRPM-2 (clusterin/SGP-2) (Léger et al, 1987; Bettuzzi et al, 1989; Rouleau et al, 1990), and RVP.1 (Briehl and Miesfeld, 1991). Other genes, such as fos, myc, and Hsp70, are also induced after castration (Buttyan et al, 1988); however, to date only the expression of TRPM-2 (clusterin/SGP-2) mRNA and cathepsin D have been localized by *in situ* hybridization or immunohistochemistry to the luminal epithelial cells in the distal regions of the prostatic ducts that undergo ACD (Rouleau et al, 1990; Sensibar et al, 1990, 1991).

Using Northern analysis and *in situ* hybridization, we have examined the expression of three additional genes during prostatic regression: tissue transglutaminase (TGase), poly(ADP)ribose polymerase (PARP), and heat shock protein 27 (Hsp27). Each of these genes may play a role in ACD, although whether they represent primary or secondary thantogens remains to be determined. TGase enzymatically cross-links proteins by the formation of ε-(γ-glutamyl)lysine bonds. Immunohistochemical methods have been used to localize the activity of TGase to the apoptotic bodies produced from the fragmentation of dying hepatocytes after lead nitrate-induced hyperplasia and subsequent regression (Fesus et al, 1987). PARP is a nuclear enzyme that is tightly bound to chromatin and catalyzes the poly(ADP)ribosylation of structural chromosomal proteins including histones, non-histones (Burzio et al, 1979; Poirier et al, 1982), and nuclear enzymes (Ohashi et al, 1983). PARP is highly stimulated by DNA strand breakage (Ferro and Olivera, 1982; Zahradka and Ebisuzaki, 1982), an event that occurs in apoptotic cells during the formation of a nucleosomal ladder after activation of an endogenous Ca^{2+}/Mg^{2+} endonuclease (Wyllie et al, 1986; Kyprianou et al, 1988). Hsp27 belongs to a family of proteins induced in response to environmental stress. It is induced in mammalian cells in the presence of arsenite and amino acid analogs as well as during hyperthermia (Welch, 1985; Crete and Landry, 1990). Following serum stimulation of growth, exposure to tumor promoters, or calcium ionophores, there is an increase in the phosphorylation state of Hsp27 without increased synthesis of the protein (Welch, 1985). In Chinese hamster O23 cells the induction of thermotolerance is correlated with an increased constitutive expression of the Hsp27 mRNA (Chretien and Landry, 1988), suggesting that Hsp 27 may serve a survival function in these cells.

We have examined the expression of these genes during prostatic regression induced by castration to determine whether they are involved in ACD in the prostate.

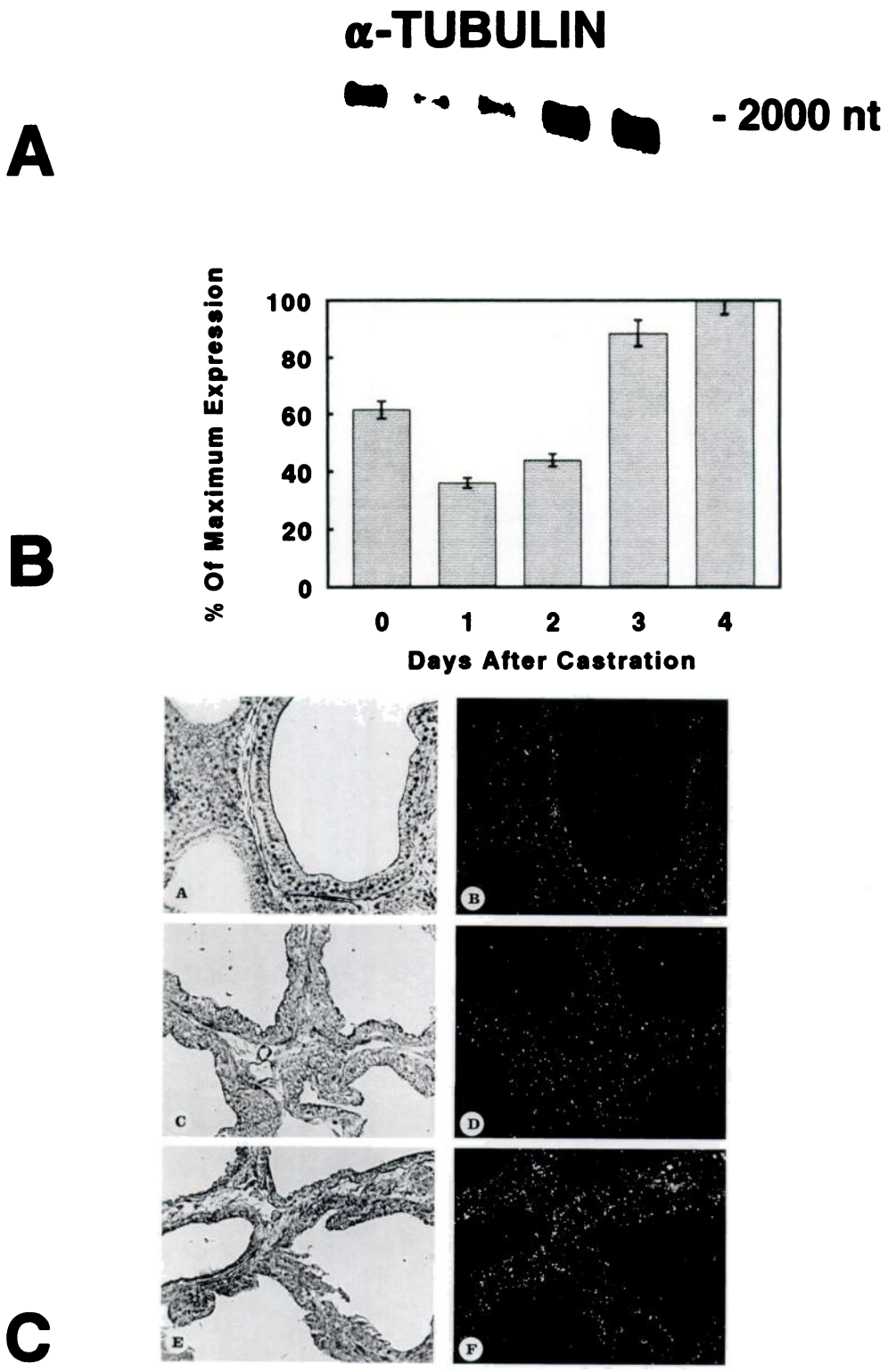


FIG. 2. Expression of α-tubulin mRNA in the ventral prostate after castration. Panel **A**: Northern analysis of α-tubulin steady-state mRNA levels. Poly(A)$^+$ RNA was extracted from the rat ventral prostate at different times after castration and 2 μg per lane was electrophoresed on formaldehyde gels, transferred to nylon membranes, and hybridized to ^{32}P-labeled cDNA specific for α-tubulin (1×10^7 cpm). The membranes were exposed for 18 hours. Panel **B**: Bar graph showing changes in steady-state levels of α-tubulin mRNA in the prostate after castration. The autoradiographs of the Northern blots were densitometrically scanned, and the values obtained for each point in the time course were compared to the day 0 control levels. The results represent the average of three independent scans for each day. Panel **C**: *In situ* hybridization of α-tubulin mRNA. Random sections from the rat ventral prostate excised at different times after castration were hybridized to ^{35}S-labeled cDNA insert specific for α-tubulin (1×10^6 cpm). The sections were exposed for 4 days. Panels **A**, **C**, and **E**—phase contrast images showing morphology of sections; Panels **B**, **D**, and **F**—darkfield illumination showing *in situ* hybridization; panels **A** and **B**—prostate from control animals; panels **C** and **D**—prostate 2 days after castration; panels **E** and **F**—prostate 4 days after castration. Magnification ×20.

Materials and Methods

Animals

Male Sprague-Dawley rats (250–300 g) were obtained from Charles River Inc. (Montréal, Québec, Canada). They were maintained in a controlled environment (14 hours light, 10 hours dark) and received Purina Rat Chow and water *ad libitum*. The animals were castrated via the scrotal route under light halothane anaesthesia. Animals were sacrificed by cervical dislocation at different days after castration, and the prostate glands were excised and processed immediately.

Materials

[α^{32}P]dCTP (3,000 Ci/mmol) and [α^{35}S]dCTP (>1,000 Ci/mmol) were obtained from Dupont Canada (Mississauga, Ontario, Canada). T7 quickprime labeling kits were obtained from Pharmacia (Baie d'Urfé, Québec, Canada). Geneclean® kits were purchased from Bio 101 (La Jolla, California). Vanadyl sulfate ribonucleoside complex and restriction enzymes were purchased from Bethesda Research Laboratories (Burlington, Ontario, Canada). Zetabind nylon membrane was purchased from Terochem Scientific (Toronto, Ontario, Canada). Restriction enzymes, oligo(dT)-cellulose, RNase A, yeast tRNA, and calf thymus DNA were ordered from Boehringer-Mannheim Canada (Dorval, Québec, Canada). 3-Aminopropyltriethoxysilane was purchased from Sigma Chemical Co. (St. Louis, Missouri). Cronex X-ray film and other photographic material were obtained from Henry's Photography (Toronto, Ontario, Canada). Nuclear track emulsion Type 2 was ordered from Kodak Canada Inc. (Toronto, Ontario, Canada). All other chemicals, of reagent grade, were purchased from Fisher Scientific Co. Ltd. (Ottawa, Ontario, Canada) or BDH (Toronto, Ontario, Canada).

Preparation of Radiolabeled Probes

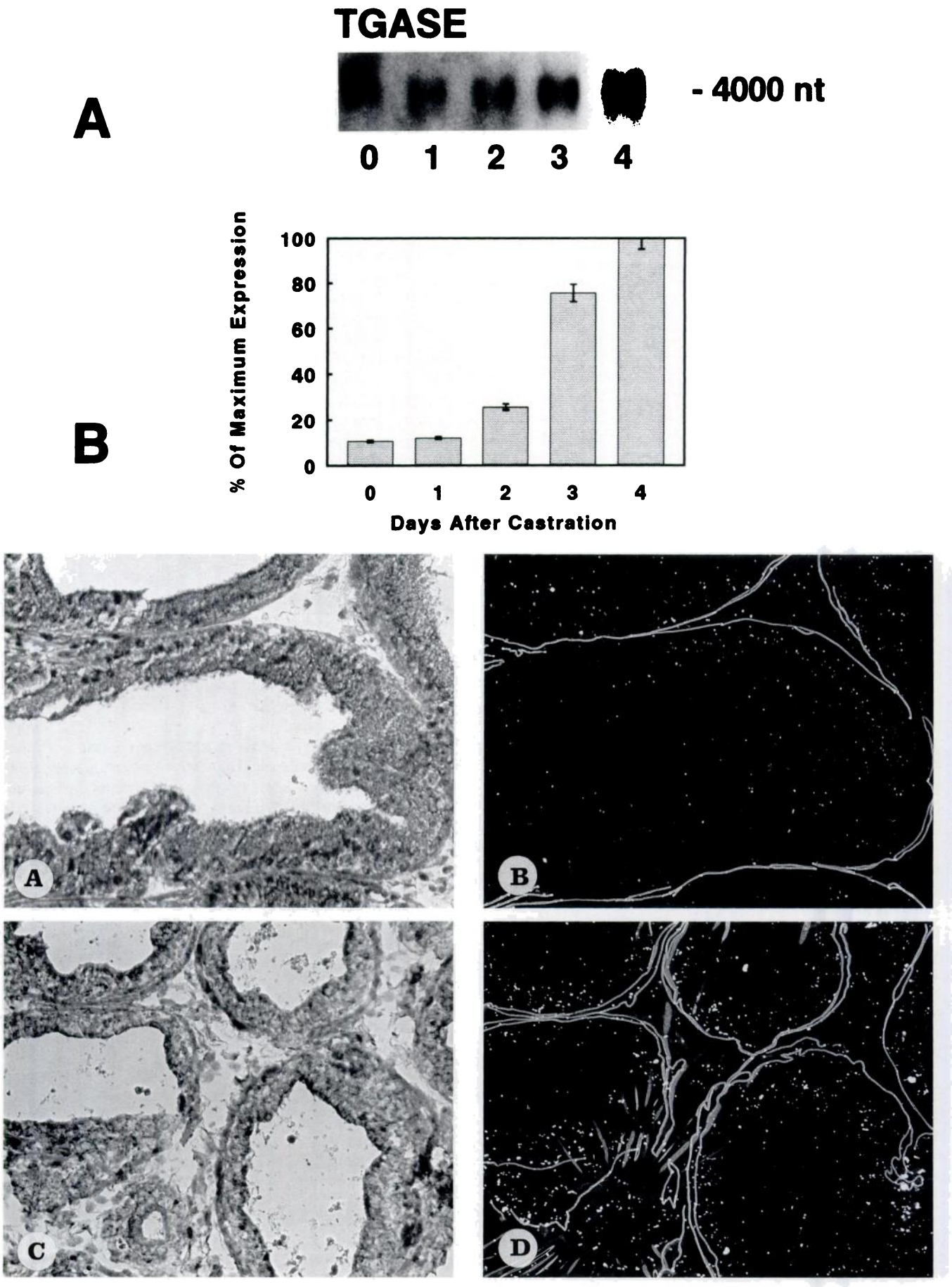
The TRPM-2, PSBP (specific for the C3 component of prostate steroid-binding protein), poly(ADP)ribose polymerase, α-tubulin, tissue transglutaminase, and Hsp27 cDNA inserts are contained in the plasmids pG17-H, pB44, pRADc, pMAT1.1, pG3Zf-3400, and pH8, respectively. Restriction digests (20 μg) of plasmid with the appropriate restriction enzyme were run on 1% preparative agarose gels. The gels were stained with ethidium bromide, insert bands were excised, and the insert DNA was purified using the Geneclean® kit (Bio 101). The cDNA inserts were labeled using the multiprime method with [α^{35}S]dCTP to a specific activity of approximately 4×10^8 cpm/μg or with [α^{32}P]dCTP using the T7 quickprime kit to a specific activity of 6×10^8 cpm/μg.

Extraction of RNA and Northern Hybridization

Total RNA was extracted from freshly excised prostate tissue using the LiCl/urea procedure (Auffray and Rougeon, 1979) with minor modifications (Tenniswood and Simpson, 1982). Polyadenylated RNA (poly[A]$^+$RNA) was isolated using oligo-(dT) cellulose chromatography (Aviv and Leder, 1972). Poly(A)$^+$RNA samples were electrophoresed on 1.5% denaturing formaldehyde agarose slab gels at 40 V for 20 hours. Total RNA was run in parallel as a size marker. The electrophoresed RNA samples were transferred to Zetabind nylon filters by capillary transfer and fixed to the membranes after transfer by UV crosslinking. Filters were prehybridized at 42°C for 2 hours in 50% formamide, 6× saline sodium phosphate ethylenediaminetetraacetic acid (SSPE) (1× SSPE = 0.15 M sodium chloride, 50 mM sodium phosphate and mMEDTA, pH 7.4), 5× Denhardt's solution (0.2% albumin, 0.2% Ficoll, and 0.2% polyvinylpyrrolidone), and 500 μg/ml single-stranded calf thymus DNA. The filters were hybridized by adding denatured [α^{32}P]dCTP-labeled probe (2 to 5×10^6 cpm/ml) to the prehybridization solution and incubating overnight at 42°C. The membranes were washed at 42°C in 2× saline sodium citrate (SSC) (1× SSC = 0.15 M sodium chloride and 0.015 M sodium citrate), 0.1% sodium dodecyl sulfate (SDS) for 10 minutes (4×), followed by 0.1× SSC for 30 minutes at 50°C. The membranes were sealed in plastic wrap and autoradiographed at −70°C between intensifying screens using Cronex X-ray film in Kodak X-Omatic cassettes. The autoradiograms were exposed for various periods of time to allow quantitative scanning using a Pharmacia LKB densitometer. Variations in loading were corrected by comparison to α- tubulin mRNA controls, and the relative levels for each mRNA were determined from several autoradiograms.

In Situ Hybridization

In situ hybridizations were performed as described by Lawrence and Singer (1986), with minor modifications (Rouleau et al, 1990). Freshly excised prostatic tissue was frozen in isopentane at −70°C, then sectioned to a thickness of 8 μm and placed on silane-treated slides (Rentrop et al, 1986). Frozen sections were fixed in 4% paraformaldehyde/phosphate-buffered saline (PBS) (0.14 M NaCl, 2.7 mM KCl, 10 mM Na_2PO_4, and 1.8 mM KH_2PO_4) and then rehydrated for 10 minutes in PBS and 5 mM $MgCl_2$. Sections to be treated with RNase were rinsed briefly in 2× SSC, whereas experimental slides were placed in fresh PBS and 5 mM $MgCl_2$ until ready for the acetylation step. Negative controls were treated for 1 hour at 37°C with 100 μl of RNase A (100 μg/ml). All slides (experimental and negative controls) were rinsed for 1 minute in 70% ethanol, three times for 2 minutes in PBS, twice for 5 minutes in 2× SSC, and then acetylated for 10 minutes at room temperature in 16 mM triethanolamine and 0.25% acetic anhydride, pH 8. The sections were rinsed five times for 2 minutes in PBS and 10 mM dithiothreitol (DTT), twice for 5 minutes in 2× SSC and 10 mM DTT, and prehybridized for 1 hour at room temperature in 40 μl of 50% formamide, 2× SSC, 0.4% bovine serum albumin (BSA), 30 mM DTT, 20 mM vanadyl ribonucleoside complex, 10 μg/ml yeast tRNA (heat denatured), and 200 μg/ml salmon testes DNA (heat denatured). Hybridizations were performed at 42°C overnight with 40 μl of the above mix supplemented with 10% dextran sulfate, and the labeled probe (1×10^6 cpm/slide). The sections were washed twice for 30 minutes in 50% formamide, 2× SSC, and 10 mM DTT at 50°C, three times for 20 minutes in 50% formamide, 1× SSC, and 10 mM DTT at 50°C, and three times for 20 minutes in 0.5× SSC and 10mM DTT at room temperature. The slides were then dehydrated twice for 10 minutes in 70% ethanol and once for 5 minutes in 100% ethanol. The slides were dipped in nuclear track photographic emulsion, exposed for 4–6 days at 4°C, and processed at 15°C


TGASE
A
- 4000 nt
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B
% Of Maximum Expression
100
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Days After Castration
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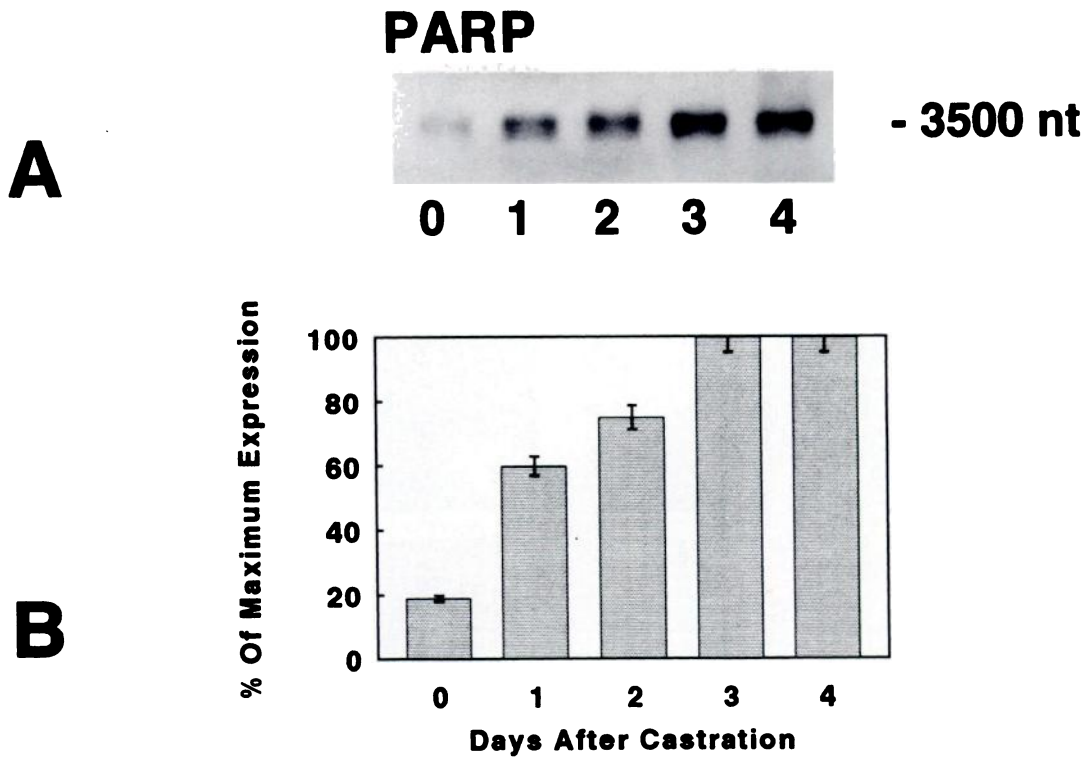

FIG. 4. Expression of PARP mRNA in the ventral prostate after castration. Panel **A**: Northern analysis of PARP steady-state mRNA levels. Poly(A)$^+$RNA was extracted from the rat ventral prostate at different times after castration and 2 μg per lane was electrophoresed on formaldehyde gels, transferred to nylon membranes, and hybridized to ^{32}P-labeled cDNA specific for PARP (1 × 10^7 cpm). The membranes were exposed for 4 hours. Panel **B**: Bar graph showing changes in steady-state levels of PARP mRNA in the prostate after castration. The autoradiographs of the Northern blots were densitometrically scanned, and the values obtained for each point in the time course were standardized by comparison to the α-tubulin mRNA levels (see Fig. 2).

as follows: 5 minutes in Kodak D-19 developer, 30 seconds in 2% acetic acid, 5 minutes in Kodafix, and 30 minutes in running water. The slides were lightly counterstained with hematoxylin and eosin and examined using a Zeiss Axioskop microscope. Photographs were taken using Kodak Tmax 100 film for phase contrast images and Ilford PANF 50 film for the darkfield images. For each probe sections shown are from the same labeling experiment, allowing direct comparison of the levels of expression at different times after castration.

To determine which cell type the genes are expressed, each section has been analyzed to identify individual stromal and epithelial compartments. The stromal regions were outlined with white ink. Between three and five sections for each probe were analyzed to determine the ratio of grains present over the epithelial cells versus the stromal cells. With all of the probes used, >90% of the grains were attributed to hybridization in the epithelial cells. However, it must be kept in mind that in all the sections there are ducts that have been sectioned at oblique angles, making it difficult to unequivocally assign the hybridization to one tissue compartment.

Results

Expression of PSBP, TRPM-2, and α-Tubulin after Castration

After castration, the steady-state level of PSBP mRNA decreases steadily over the first 4 days (Fig. 1a) and becomes essentially undetectable by day 6 (results not shown). The steady-state level of TRPM-2 mRNA increases significantly over the same period, reaching a maximum on

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FIG. 3. Expression of TGase mRNA in the ventral prostate after castration. Panel **A**: Northern analysis of TGase steady-state mRNA levels. Poly(A)$^+$RNA was extracted from the rat ventral prostate at different times after castration and 2 μg per lane was electrophoresed on formaldehyde gels, transferred to nylon membranes, and hybridized to ^{32}P-labeled cDNA specific for TGase (1 × 10^7 cpm). The membranes were exposed for 7 days. Panel **B**: Bar graph showing changes in steady-state levels of TGase mRNA in the prostate after castration. The autoradiographs of the Northern blots were densitometrically scanned, and the values obtained for each point in the time course were standardized by comparison to the α-tubulin mRNA levels (see Fig. 2). Panel **C**: *In situ* hybridization of TGase mRNA. Random sections from the rat ventral prostate excised at different times after castration were hybridized to ^{35}S-labeled cDNA insert specific for TGase (1 × 10^6 cpm). The sections were exposed for 4 days. Panels **A** and **C**—phase contrast images showing morphology of sections; panels **B** and **D**—darkfield illumination showing *in situ* hybridization; panels **A** and **B**—prostate from control animals; panels **C** and **D**—prostate 4 days after castration. Magnification ×20.

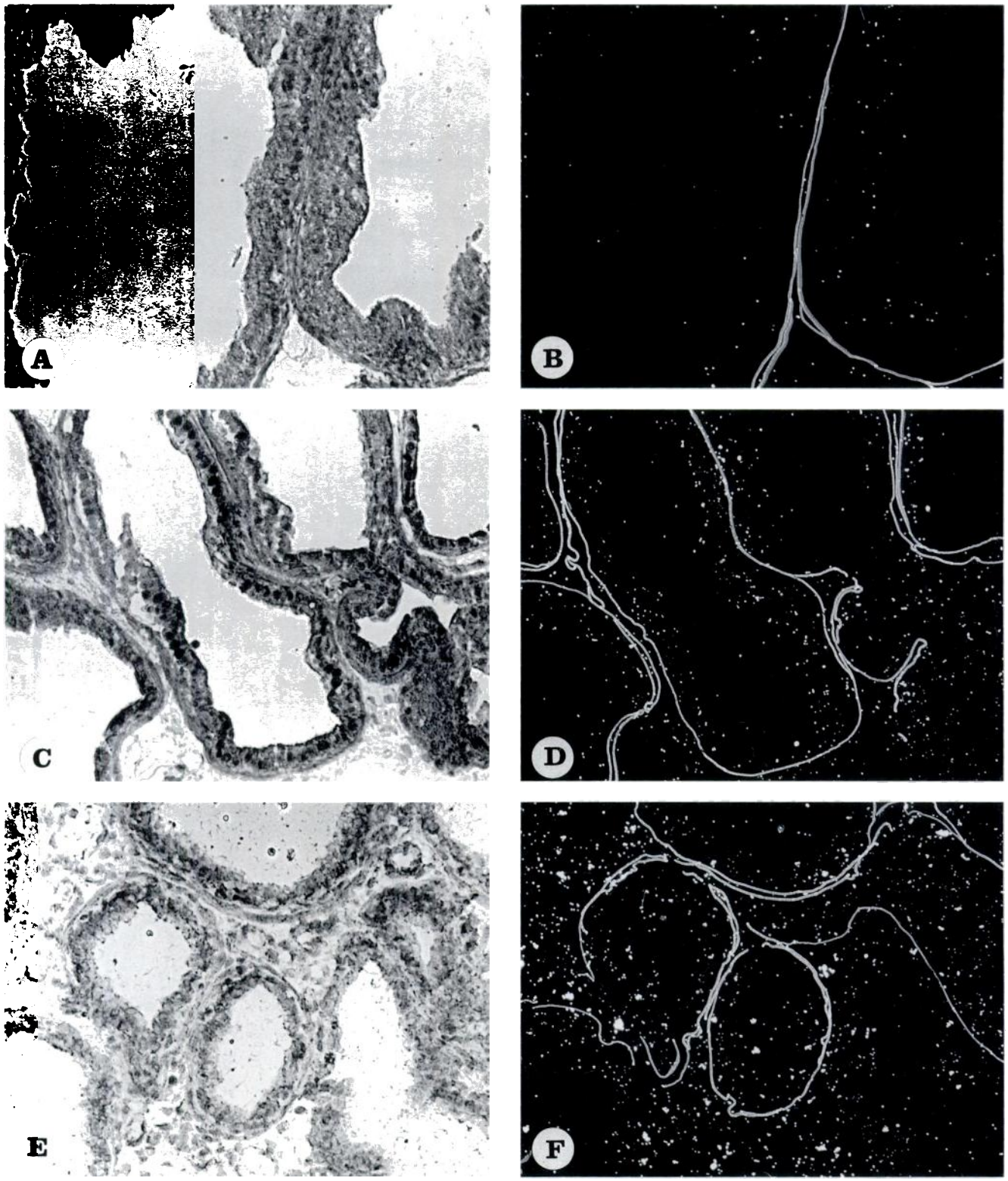

FIG. 4. Continued. Panel C: *In situ* hybridization of PARP mRNA. Random sections from the rat ventral prostate excised at different times after castration were hybridized to ^{35}S-labeled cDNA insert specific for PARP (1×10^6 cpm). The sections were exposed for 4 days. Panels A, C, and E—phase contrast images showing morphology of sections; panels B, D, F—darkfield illumination showing *in situ* hybridization; panels A and B—prostate from control animals; panels C and D—prostate 2 days after castration; panels E and F—prostate 4 days after castration. Magnification ×20.

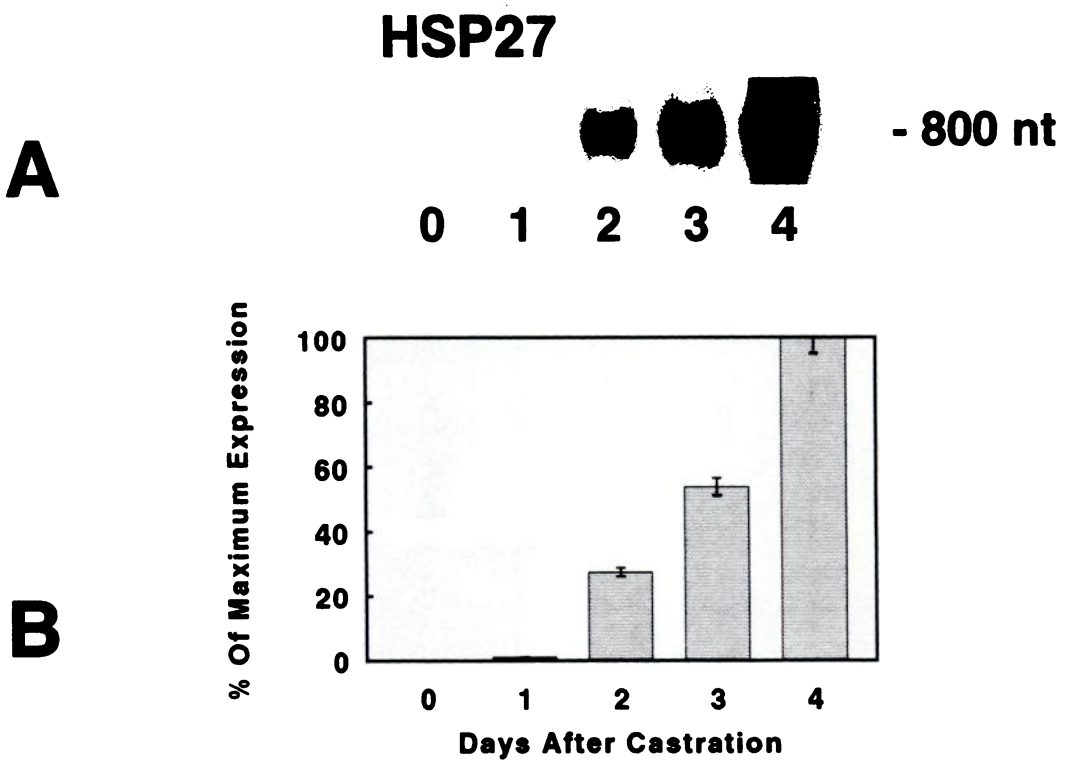

FIG. 5. Expression of Hsp27 mRNA in the ventral prostate after castration. Panel **A**: Northern analysis of Hsp27 steady-state mRNA levels. Poly(A)$^+$RNA was extracted from the rat ventral prostate at different times after castration and 2 μg per lane was electrophoresed on formaldehyde gels, transferred to nylon membranes, and hybridized to ^{32}P-labeled cDNA specific for Hsp27 (1×10^7 cpm). The membranes were exposed for 18 hours. Panel **B**: Bar graph showing changes in steady-state levels of Hsp27 mRNA in the prostate after castration. The autoradiographs of the Northern blots were densitometrically scanned, and the values obtained for each point in the time course were standardized by comparison to the α-tubulin mRNA levels (see Fig. 2).

day 4 (Fig. 1b) before declining to undetectable levels by day 8 after castration (results not shown), essentially in agreement with previous experiments (Heyns et al, 1977; Léger et al, 1987). The α-tubulin mRNA is expressed at a relatively low but easily detectable level in the rat ventral prostate; however, the steady-state levels do not change significantly after castration (Fig. 2a,b). The α-tubulin mRNA is expressed in the epithelial cells in the rat ventral prostate (Fig. 2c). Because the levels of α-tubulin do not appear to alter significantly during regression, and because the gene is expressed in the epithelial cells, we have standardized the expression of TGase, PARP, and Hsp27 mRNAs against the expression of α-tubulin mRNA as measured by hybridization to duplicate nylon filters to correct for variations in loading.

Expression of TGase, PARP, and Hsp27 after Castration

Tissue transglutaminase mRNA is detectable in the prostate prior to castration (Fig. 3a,b) and increases significantly between days 2 and 4 after castration. *In situ* hybridization confirms that the mRNA is expressed at low levels in the epithelial cells of the prostate prior to castration and that the increase in the expression of the mRNA is localized to the epithelial cells of the gland (Fig. 3c).

The expression of PARP mRNA in the prostate prior to castration is very limited, but the steady-state levels increase substantially by day 1 after castration, and the steady-state level of the mRNA remains elevated for the next 3 days after castration (Fig. 4a,b). As shown in Figure 4c, the gene is expressed in the epithelial cell compartment of the prostate after castration.

Hsp27 mRNA is not detectable in the prostate prior to castration or on day 1 after castration. The expression of the mRNA increases dramatically between days 2 and 4 after castration (Fig. 5a,b). *In situ* hybridization confirms that the gene is induced in the prostate 4 days after castration, in the epithelial cells of the gland (Fig. 5c).

Discussion

The regression of the rat ventral prostate after castration is known to occur as a result of apoptotic cell death, which occurs in approximately 90% of the epithelial cells of the gland within a 6- to 8-day period following castration

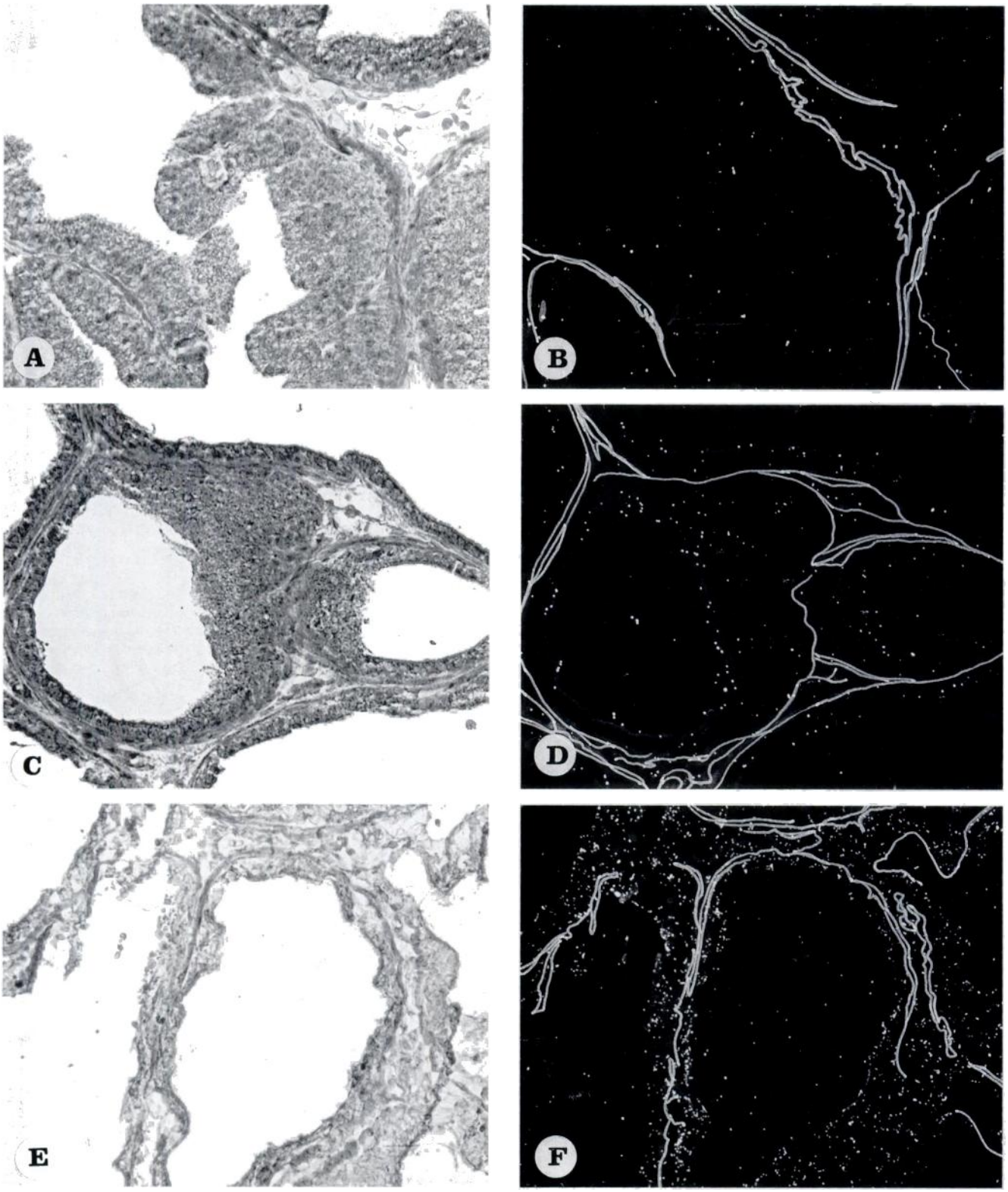

FIG. 5. Continued. Panel C: *In situ* hybridization of Hsp27 mRNA. Random sections from the rat ventral prostate excised at different times after castration were hybridized to ^{35}S-labeled cDNA insert specific for Hsp27 (1×10^6 cpm). The sections were exposed for 4 days. Panels A, C, and E—phase contrast images showing morphology of sections; panels B, D, and F—darkfield illumination showing *in situ* hybridization; panels A and B—prostate from control animals; panels C and D—prostate 2 days after castration; panels E and F—prostate 4 days after castration. Magnification ×20.

(Sandford et al, 1984; English et al, 1985). It is now well established that the activity of a number of proteins increases during the regression of the gland, including cathepsin D, urokinase and tissue type plasminogen activators, and RNase (Lee, 1981; Freeman et al, 1990; Sensibar et al., 1990). In addition, a number of castration-induced mRNA have been identified in the regressing prostate (Montpetit et al, 1986; Lee and Sensibar, 1987), and a number of these have now been cloned, including TRPM-2 (clusterin, SGP-2) (Léger et al, 1987; Wong et al, 1993), RVP-1 (Briehl and Miesfeld, 1991), and the Yb subunit of glutathione-S-transferase (Chang et al, 1987). The increase in the steady-state levels of these genes and a number of others, including p53, Rb, fos, and Hsp70, has led to the suggestion that they are involved in the control of ACD (Buttyan et al, 1988; Colombel et al, 1992). However, as has been recently pointed out, increases in the steady-state levels of mRNA in the regressing tissue, even if the expression is localized to the dying epithelium, need to be interpreted with caution, because genes may be induced (or their steady-state levels increased) for a variety of reasons that are not associated with the apoptotic process (Tenniswood et al, 1994). For example, genes may be induced in the dying cells as part of a futile stress response or as part of a survival mechanism in cells that do not undergo ACD. Indeed, before genes can be categorized as primary or secondary thanatogens, corroborative evidence needs be obtained in other tissues, and a functional role in the apoptotic process needs to be firmly established. In our current experiments we have investigated the induction of three potential thanatogens in the rat ventral prostate after castration. Tissue transglutaminase has been implicated in ACD in the liver (Fesus and Thomazy, 1988) and thymocytes (Fesus et al, 1991b), and the product of the TGase activity, the ϵ-(γ-glutamyl)lysine bonds, have been specifically identified in apoptotic bodies, suggesting that TGase activity is required to ensure the increased rigidity of the apoptotic bodies (Fesus et al, 1989, 1991a,b). In the prostate, TGase mRNA is induced in the epithelial cells with kinetics that are very similar to those seen for TRPM-2 mRNA induction. Thus TGase appears to be required for the normal completion of ACD, but it is unlikely to be involved in the induction of the process, and as such represents a secondary thanatogen.

The induction of poly(ADP)ribose polymerase mRNA in the epithelial cells of the prostate after hormone ablation suggests that PARP is also a secondary thanatogen. However, in glucocorticoid- or calcium ionophore-treated thymocytes (McConkey et al, 1989; Hoshino et al, 1992; Ucker et al, 1992), PARP induction appears to be a futile attempt to repair the double-strand DNA breaks introduced by the Ca^{2+}/Mg^{2+}-dependent endonuclease (Cleaver and Morgan, 1991). However, the role of the poly(ADP)ribose polymerase is not firmly resolved because the induction of poly(ADP)ribose polymerase in the liver appears to be required for endonuclease activation (Jones et al, 1989), whereas in activated T-lymphocytes ADP-ribosylation of the endonuclease appears to suppress endonuclease and topoisomerase activity (Rice et al, 1992; Ferro et al, 1983). Because the PARP enzyme is activated by DNA strand breaks and is known to modify several of the enzymes involved in DNA repair, it is likely that the induction of PARP is an attempt by the dying cell to repair the damage caused by nuclease activation (Ohashi et al, 1983; Ueda and Hayaishi, 1985).

The induction of Hsp27 mRNA in the epithelial cells of the prostate following hormone ablation is particularly interesting because this mRNA is induced with kinetics that are distinctly different from those seen for TRPM-2, TGase, and PARP mRNAs. Hsp27 mRNA is not detectable in the prostate before castration nor for the first 24 hours after castration. The gene is highly induced in the epithelial cells of the prostate between 24 and 48 hours and is expressed at elevated levels by day 4 after castration. This distinct time course of expression appears to represent a different response to castration, and it may be either a futile response or part of a survival strategy of a subset of the epithelial cells in the gland. Because random sections were used for *in situ* hybridization, it is not possible to determine whether the expression of the Hsp27 gene is restricted to a particular subset of epithelial cells in the gland. However, it is of interest that the Hsp27 mRNA is not expressed in the lactating mammary gland after weaning (Guenette et al, 1994), suggesting that the expression of the gene may not occur in all tissues undergoing ACD. The secretory epithelium of the lactating mammary gland is presumably programmed to undergo ACD in response to the decline in the levels of trophic hormones that accompany weaning. Because the fluctuations in circulating androgen levels in the male are not profound, it is probable that the prostatic epithelium is not programmed to respond to massive changes in the trophic hormone in the same way, in which case the changes in Hsp27 mRNA levels may represent a futile stress response. It will be necessary to examine the expression of Hsp27 in other models of ACD to establish the relevance of this response in ACD.

The results presented here demonstrate that in addition to TRPM-2, several other genes are expressed in the prostatic epithelial cells that undergo ACD after castration. The role of the gene products in ACD remains to be elucidated. Although it is likely that tissue transglutaminase is a secondary thanatogen that is required for the completion of the apoptotic process, it is quite probable that Hsp27 and poly(ADP)ribose polymerase represent either stress responses or futile responses to androgen ablation.

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