Localization of Fertility Factor SP22 to Specific Cell Types Within the Anterior Pituitary Gland

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Sperm protein 22 (SP22) was recently identified in the anterior pituitary gland (AP) of male Golden Syrian hamsters using ion trap mass spectrometry. SP22 has been implicated in apoptosis, androgen receptor function, fertility, and ontogeny of early-onset Parkinson's disease. However, the role of SP22 in the pituitary has not been investigated. We cloned the cDNA for full-length SP22 from AP and posterior lobe (posterior pituitary and intermediate lobe) of the pituitary gland in adult male rats and Golden Syrian hamsters, confirming the presence of SP22 mRNA in the AP and posterior lobe. Because gonadal steroids are important regulators of AP function, and SP22 is associated with androgen receptor function, we used Western blots to compare SP22 in the AP of intact and orchidectomized male rats given placebo or a low or high dose of testosterone. SP22 did not differ with treatment, indicating that AP SP22 concentration was not regulated by testosterone. To localize SP22 to specific cells of the AP, mirror-image paraffin sections were labeled against SP22 and either luteinizing hormone (LH)β, thyroid-stimulating hormone (TSH)β, prolactin, adrenocorticotropic hormone (ACTH), or growth hormone (GH) using peroxidase-conjugated secondary antibody. Additional sections were colabeled with SP22 and one of the AP hormones using fluorescent secondary antibodies. SP22 colocalized in somatotropes and thyrotropes in rat and hamster. We identified SP22 in a small percentage of corticotropes, gonadotropes, and lactotropes. This is the first report that SP22 mRNA is present specifically in the AP, and SP22 is localized primarily in somatotropes and thyrotropes. SP22 may help regulate AP function and be particularly important for the control of GH and TSH secretion.
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During the conduction of studies investigating the proteome of the anterior pituitary gland (AP) in 2-month-old male Golden Syrian hamsters (*Mesocricetus auratus*), contraception associated protein 1 (CAP1) was identified in a cytosolic fraction of four pooled APs (1). Analyses using liquid chromatography tandem mass spectrometry and the database search software SEQUEST identified two peptides, consisting of 15 and 19 amino acids, respectively. These peptides were subsequently identified in a repeat study using seven pooled APs from 2-month-old male hamsters and a different extraction procedure (1). Together these two peptides form 18% of the covered sequence of the 189-amino-acid protein termed CAP1 protein in hamster (GenBank/EMBL accession number AJ431372) and CAP1 (GenBank/EMBL accession number AJ007291) or fertility protein SP22 in rat (GenBank/EMBL accession number NM057143). There is 93.1% homology (by amino acids) of the protein in the two species. Hamster CAP1 has 92.6% and rat SP22 has 91.5% homology (by amino acids) with human DJ-1 protein (GenBank/EMBL accession number D61380).

CAP1/SP22/DJ-1 was initially discovered as an oncogene that transformed mouse NIH3T3 cells (2) and later identified as a sperm or testis protein in rats and hamsters (3–6). Although the specific roles of this protein are unknown, it has been implicated in a diverse array of functions. It is correlated with fertility in rats and may play a role in sperm-egg interactions (5, 7). SP22 has also been shown to regulate androgen receptor (AR) interactions involved in gene transcription in a variety of cell lines including human 293T and monkey Cos1 cells (8, 9). It may be indicative of, or protective against, cellular oxidative stress and apoptosis (10–12). It also is mutated in a form of early-onset Parkinson's disease (13). In addition, DJ-1 was reported to be a circulating antigen in women with breast cancer (14). The mRNA for CAP1/SP22/DJ-1 was widespread in rat and

human tissues, including rat and human brain (4). Thus, it is not surprising that it was found in human pituitary gland that contains neural tissue (4). However, it was not localized specifically to the glandular AP.

In the present study we used reverse transcriptase–polymerase chain reaction (RT-PCR), immunohistochemistry, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to investigate CAP1/SP22/DJ-1 (hereafter referred to as SP22) in the pituitaries of adult male rats and hamsters. We report that SP22 is in the AP and posterior pituitary but not in the intermediate lobe. In the AP it is localized predominantly in somatotropes and thyrotropes, suggesting that it may be involved in GH and TSH secretion.

Materials and Methods

Reagents. General laboratory supplies and reagents including protease inhibitors were purchased from Fisher Scientific (Fairlawn, NJ) or Sigma Chemical Company (St. Louis, MO). Polymerase chain reaction (PCR) reagents were obtained from Applied Biosystems (Branchburg, NJ). TRIzol Reagent, TOPO TA Cloning kits, and synthesized primers were obtained from Gibco-Invitrogen (Carlsbad, CA). Protein Assay reagent was purchased from Bio-Rad Laboratories, Inc. (Hercules, CA). QIAprep Spin miniprep kits were obtained from Qiagen, Inc. (Valencia, CA). horseradish peroxidase (HRP), fluorescein isothiocyanate (FITC), and Texas Red conjugated secondary antibodies, SP22 primary antibody, and SP22 blocking peptide were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-SP22 was produced against a peptide mapping to the C terminal region of the human DJ-1 protein. DAB Plus kits were obtained from Zymed Laboratories (San Francisco, CA). Antirat hormones, luteinizing hormone (LH)β (AFP-2–11–27), thyroid-stimulating hormone (TSH)β (AFP-1–9–15), growth hormone (GH) (G3, and AFP-5672099), and prolactin (AFP-4283E) were obtained from the National Hormone and Pituitary Program, National Institutes of Health (Bethesda, MD). Anti-porcine adrenocorticotropic hormone (ACTH) was purchased from Immuno Nuclear Corp (Stillwater, MN). Anti-hamster prolactin (#42) was obtained from Dr. Frank Talamantes (University of California, Santa Cruz, CA).

Animals and Collection of Pituitary Tissue. Adult male rats (CD) and Golden Syrian hamsters (LVG) were purchased from Charles River Laboratories, Inc. (Wilmington, MA). Animals were housed by species in rooms with controlled lighting (lights on 0700–1900 hr daily) and temperature (20°–22°C) and given Teklad Rodent Diet 8604 (Harlan, Madison, WI) and tap water *ad libitum*. Animals were maintained and used in accordance with the standards of the Institutional Animal Care and Use Committee of the University of South Carolina. They were handled three times per week for cage cleaning.

Animals were killed by decapitation between 1100 and 1400 hr. The time between picking up the animals and decapitation was <10 secs. After removing the cranial vault and brain to visualize the pituitary gland, the posterior lobe of the pituitary gland was separated from the AP *in situ* under magnification and with the use of fine forceps, and the AP was collected. Alternatively the entire pituitary gland was removed.

Cloning of Rat and Hamster Pituitary SP22. We investigated whether SP22 mRNA was present in AP and posterior lobe tissue. Five rats and six hamsters were purchased at 2 months of age and kept for 2–4 weeks before use. The APs and posterior lobes from all animals and a portion of testis from three rats were processed for RNA isolation. Only the lateral quarters of the AP were used to ensure there was no possible contamination with posterior lobe tissue.

Individual tissue samples were placed into sterile RNAase- and DNAase-free microfuge tubes containing 1 ml of Trizol reagent. Tissue was homogenized using minipestles, processed according to the manufacturer's protocol, and stored at –80°C until used for RT-PCR.

Total RNA (1 μg) from each AP, posterior lobe, and testis analyzed was reverse transcribed using MuLV reverse transcriptase and amplified using AmpliTaq DNA polymerase according to manufacturer's instructions using a GeneAmp PCR kit (Applied Biosystems). Conditions for PCR were 95°C for 2 mins, 35 cycles of 95°C 1 min and 64°C 1 min, 72°C for 7 mins, and a brief hold at 4°C. Forward (5′-ATGGCATCCAAAAGAGCTCTGGTC-3′) and reverse (5′-CTAGTCTTTGAGAACAAGCGGTGC-3′) primers were made using known homologous sequences for rat and hamster SP22 available in GenBank (accession numbers AJ431372, AF157511, AF157512, AJ007291, NM057143). Total RNA from rat testis was subjected to RT-PCR as a positive control. The negative control was sterile H_2O. Amplified cDNAs determined to be of the correct size on 1% agarose gels were ligated into pCRII TOPO vector (TOPO PCR cloning kit, Invitrogen) for subsequent DNA sequencing (University of Maine Sequencing Service, Orono, ME).

Immunohistochemistry. We used immunohistochemical techniques to determine which cell types contained SP22. Five rats and six hamsters were purchased at 3 months of age and kept for 1 week before use. Whole pituitary glands were placed in 5 ml of Bouin's solution for 48–72 hrs and then embedded in paraffin. Serial sections (6 μm) were cut through the entire gland and mounted on slides in a paired flip-flopped orientation as previously described (15), resulting in the exposed surfaces of each pair of sections being mirror images of one another.

Sections of pituitaries from all animals were stained for LHβ, TSHβ, ACTH, GH, and prolactin with a modification of procedures previously described (15) using peroxidase-labeled secondary antibody conjugates. The paired flip-flopped section for each antiserum was stained for SP22. Antibody dilutions were determined using serial dilutions of

antisera and were as follows: LHβ 1:20,000, TSHβ 1:20,000, GH 1:1,500 (G3) or 1:20,000 (AFP567209), ACTH 1:10,000, prolactin 1:1,000 for both antirat and antihamster sera, and SP22 1:150. Specificity of all primary antibodies was confirmed by an absence of staining in sections incubated with normal IgG or without primary antisera. Further specificity tests for SP22 were conducted by preincubating the antiserum with blocking peptide overnight at 4°C before use.

Sections were deparaffinized with xylenes, rehydrated with decreasing concentrations of ethanol followed by H_2O and PBS, and incubated with blocking agent (10% donkey or goat serum in PBS) for 1 hr at room temperature. Blocked sections were incubated with primary antibody overnight at 4°C. Slides were then washed with PBS and incubated for 1 hr at room temperature with a 1:600 dilution of HRP-conjugated secondary antibody (goat anti-rabbit for LHβ, TSHβ, ACTH, GH [AFP5672099] and both prolactins; goat anti-monkey for GH [G3]; donkey anti-goat for SP22). Sections were incubated with diaminobenzidine hydrochloride (DAB) to visualize peroxidase activity. They then were counterstained with hematoxylin. Sections were examined, and images captured, using an Axiophot microscope (Carl Zeiss, Inc.) and SPOT software (Holland, The Netherlands). Captured images were then aligned for comparison of cells observed in flip-flopped mirror images using Adobe Photoshop 6.0 software (Adobe Systems, San Jose, CA).

Additional series of slides were colabeled with fluorescent secondary antibodies for SP22 along with one of the AP hormones and examined using a Zeiss LSM510 Meta confocal microscope (Carl Zeiss, Inc.). Texas Red–labeled cells (SP22) were visualized at 543 nm, and FITC-labeled cells (LH, TSH, GH, prolactin, ACTH) were visualized at 488 nm. Images were examined using Zeiss LSM 5 image browser. Primary and secondary antiserum concentrations were adjusted to produce a clear fluorescent signal above background. Primary antiserum concentrations were as follows: LHβ 1:15,000, TSHβ 1:15,000, GH 1:10,000 (AFP5672099), ACTH 1:1,000, prolactin 1:500 for both anti-rat and anti-hamster sera, and SP22 1:50. Texas Red–conjugated donkey anti-goat for SP22 and FITC-conjugated goat anti-rabbit for all AP hormone secondary antibodies were applied at 1:600 or 1:1200 for SP22, LHβ, TSHβ, prolactin, and ACTH and 1:2500 or 1:5000 for GH. Slides were processed as stated above, with the following modifications. Additional PBS washes (30 mins) were added before application of the blocking solution, before incubation with secondary antibody, and as a final wash. The blocking solution contained PBS, 10% donkey serum, 2.5% BSA, and 4 m*M* glycine. Secondary antisera were centrifuged (13,000 rpm; 4°C) for 50 mins before use. Coverslips were attached using 1,4-diazabicyclo[2,2,2]octane (DABCO) mounting medium (Sigma) to reduce bleaching of signal.

Microscope settings were adjusted separately for TR and FITC to minimize background fluorescence of a region of tissue on each slide devoid of specific staining (e.g., intermediate lobe). The same settings were then used to examine regions of the same slide that had cells that stained specifically for one of the AP hormones. Images were captured from scans using settings for both TR and FITC. Individual fluorescent-labeled cells could then be examined for dual label by splitting the images or by reducing contrast to eliminate one of the signals. Cells labeled with each AP hormone were classified as also labeled or not labeled with SP22.

Western Blot Analysis. Gonadal steroids can regulate AP function (16) and mRNA for AR (17), and SP22 has been implicated in AR function (8, 9). Therefore, we investigated the effects of testosterone on SP22 in the AP. Six intact and 16 orchidectomized male rats were purchased at 3 months of age and kept for 4 weeks before use. Groups consisted of intact rats (Control, $n = 6$), castrated rats given four empty 20-mm-long Silastic elastomer capsules (Castrate, $n = 5$), castrated rats given one capsule packed with crystalline testosterone (Cast + low T, $n = 6$), and castrated rats given four capsules filled with testosterone (Cast + high T, $n = 5$). Rats receiving implants were anesthetized with isoflurane. A high flank incision was made (1 cm), and capsules were inserted into a subcutaneous pocket as previously described (18). The incision was stapled closed, and the rats returned to normal housing after full recovery. When four capsules were implanted, a second incision was made on the other flank, and two capsules were inserted into each incision. Before insertion, capsules were soaked in four changes of sterile PBS for 48 hrs at room temperature to prevent a spike of testosterone release after implantation. Four hours before insertion, the PBS was changed to PBS containing penicillin (100 IU/ml) and streptomycin (100 μg/ml). Rats were killed 72 hrs after implants were inserted. The APs were collected and processed for Western blot analysis. Capsules were collected from all rats to confirm that they had remained in place and were undamaged. Trunk blood was collected from all rats, and serum was stored at –20°C until assayed in duplicate by coated-tube radioimmunoassay for total testosterone (Diagnostic Products Corp., Los Angeles, CA).

To isolate proteins from whole-cell extracts, freshly isolated individual APs were placed into microfuge tubes with 200 to 500 μl of cold lysis buffer (19) containing 50 m*M* Tris (pH 7.4), 1% NP-40, 0.25% deoxycholate, 150 m*M* NaCl, 1 m*M* each of EDTA, phenylmethylsulfonyl fluoride (PMSF), sodium vanadate, and sodium fluoride, and 1 μg/ml each of leupeptin, aprotinin, and pepstatin. Samples were homogenized using minipestles, incubated on ice for 30 mins, centrifuged for 20 mins at 13,000 *g*, and the supernatants were stored at –80°C. Protein concentration in the lysates was determined using Bio-Rad dye reagent. Proteins were also isolated from a portion of frontal cortex from three rats for use as positive controls.

Proteins isolated from rat individual AP or brain were separated (40–50 μg/lane) using 10% SDS-PAGE and transferred by semidry electroblotting to polyvinylidene

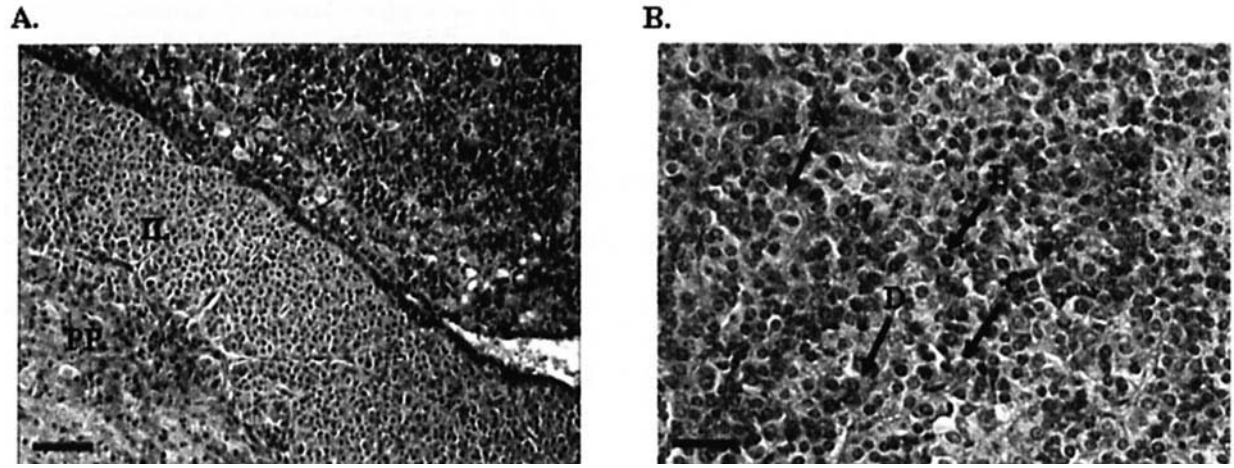


Figure 1. (A) Representative section of rat pituitary gland showing staining for SP22 in the anterior pituitary gland (AP) and posterior pituitary (PP) but not in the intermediate lobe (IL). Bar, 100 μm. (B) Representative section of hamster AP showing an abundance of cells that stained with antiserum against SP22. Four staining patterns were observed: A, no stain; B, predominantly nuclear stain; C, predominantly cytoplasmic stain; D, stain over both nucleus and cytoplasm. Similar patterns of staining were observed in rat and hamster. Bar, 50 μm.

difluoride (PVDF) membrane for detection of SP22. Membranes were stained with 0.5% Ponceau S to assess uniformity of gel loading and electrotransfer. Membranes were then destained with 20% acetonitrile, washed in Tris-buffered saline with 0.05% Tween (TTBS), and blocked with 5% nonfat dry milk in TTBS for 2 hrs at room temperature. Following blocking, membranes were washed with TTBS and incubated at 4°C overnight with SP22 antiserum 1:2000 in TTBS and 1% milk. Membranes were then thoroughly washed with TTBS and incubated with 1:4000 HRP-conjugated donkey anti-goat secondary antibody for 1 hr at room temperature. After thorough TTBS washes, specific bands were visualized using enhanced chemiluminescence. Membranes were stripped for 40 mins at 60°C, blocked, and probed using rabbit antiactin (1:200) for use as an unregulated protein to control for gel-to-gel variation as a result of differences in loading and transfer. Resulting films were digitized using UN-SCAN-IT gel automated digitizing system software (Silk Scientific Corporation, Orem, UT). Antibody specificity was determined by absence of staining after incubation of membranes with normal IgG or with primary antibody preabsorbed with blocking peptide.

Statistics. Duplicate serum testosterone concentrations were averaged. These average values were used to conduct statistics. Serum testosterone concentrations, percentage of cells colabeled with SP22 and each of the AP hormones, and SP22 in Western blots were compared by one-way ANOVA followed by Tukey's multiple comparison test. Values of $P < 0.05$ were considered to be statistically significant.

Results

Agarose gels of PCR products from all APs, posterior lobes, and testes revealed the presence of a transcript that was 570 bp. Direct sequencing confirmed SP22 cDNA cloned from both the AP and posterior lobe of the rat and hamster, indicating that SP22 is synthesized in both sites. The cDNA produced from AP, posterior lobe, and rat testis was identical to published sequences for hamster (GenBank accession no. AJ431372) and had greater than 99% homology to published sequences for rat (GenBank accession nos. AF157511, AF157512, AJ0072191). The inferred amino acid sequences were identical to published sequences.

Immunostaining for SP22 using HRP-labeled secondary antibody and hematoxylin counterstain revealed that SP22 is present in both species in numerous cells throughout the AP. In both species it also is present in the posterior pituitary but not in the intermediate lobe (Fig. 1). Within the AP, cells were found that had no staining, and other cells stained primarily in the nucleus or primarily in the cytoplasm, or both. Large gonadotropes were clearly observed that did not stain with SP22 antiserum in rat (Fig. 2) and in hamster (not shown). Pattern of staining and number and size of cells were consistent with an abundance of GH cells also staining for SP22 in rat (Fig. 3) and in hamster (not shown).

Confocal microscopy was used to determine the proportion of cells that labeled with LHβ, prolactin, ACTH, GH, or TSHβ and also labeled with SP22. Images were captured from three or more regions of each slide, and numbers of cells colabeled or not colabeled were recorded. Images of rat AP are shown in Figure 4. Similar images were observed for hamster AP (not shown). Because the relative abundance of AP cell types differs, total number of cells available to be counted varied. For example, thyrotropes typically represent about 2% of the cells of the male rat AP, but somatotropes represent over 22% of the cells (15). Total numbers of cells counted for rats ranged from 92 for corticotropes to 1615 for somatotropes. Total number of

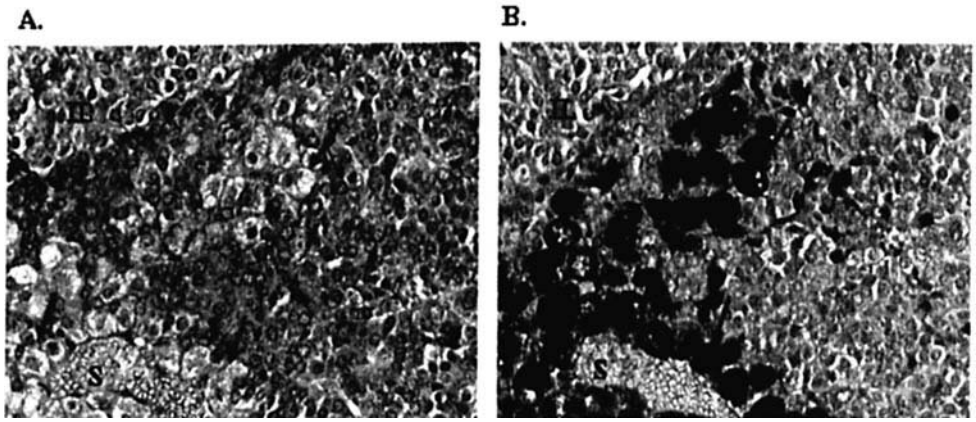


Figure 2. Section of rat AP stained with SP22 antiserum (A) and the flip-flopped mirror-image section stained with LHβ antiserum (B). Cells were observed that clearly stained with hormone antiserum but not with anti-SP22 (e.g., arrows). LHβ-positive cells were often clustered around a sinusoid (S). Cells in the intermediate lobe (IL) in the upper left of each panel did not stain for either SP22 or LHβ.

cells counted for hamsters ranged from 61 for thyrotropes to 529 for corticotropes. A greater percentage ($P < 0.01$) of somatotropes and thyrotropes were colabeled for SP22 than gonadotropes, lactotropes, or corticotropes (Fig. 5).

Mean (±SE) serum testosterone concentration in control rats with intact testes was 5.85 ± 1.44 ng/ml. It decreased in castrated rats (0.40 ± 0.32 ng/ml; $P < 0.05$) and increased with testosterone treatment to concentrations (3.74 ± 0.97 and 7.43 ± 1.51 in the low- and high-T groups, respectively) that did not differ significantly ($P > 0.05$) from control rats with intact testes.

Western analysis of AP (Fig. 6) and brain (not shown) whole-cell extracts revealed a specific band for SP22 at approximately 21,000 mol wt. Neither castration nor administration of testosterone to orchidectomized rats altered the ratio of SP22 to actin ($P > 0.05$) in AP.

Discussion

A recent proteomic study demonstrated the presence of SP22 in the AP of the Golden Syrian hamster (1). The mRNA for SP22 had previously been reported to be expressed in neural tissue (4, 20) and in human whole pituitary gland (4). Our cloning of SP22 DNA from individual rat and hamster AP and posterior lobe tissue demonstrates that SP22 mRNA is in both regions of the pituitary gland. Immunohistochemistry localized SP22 to the AP and the posterior pituitary but not to the intermediate lobe. Collectively, these findings indicate that SP22 is synthesized and localized in both AP and posterior pituitary.

More specifically, we have demonstrated that SP22 in the AP is localized primarily to the somatotropes and thyrotropes. In addition to the AP hormones, a number of other AP products have been localized in specific cell populations. For example, transforming growth factor β (TGFβ) has been reported in gonadotropes and lactotropes but not in other cell types. Other substances are produced by subpopulations of the main secretory cell types of the AP (21). For example, nerve growth factor is produced by a subpopulation of cells in all five cell types that secrete the six established AP hormones. Although we did not specifically examine the cells of the posterior pituitary, we observed nucleated cells that stained for SP22 in their cytoplasm and/or nuclei. In the frontal cortex SP22 was previously reported to be abundant in astrocytes and their processes (22). Thus, it is possible that at least some of the SP22-positive cells were pituicytes. Pituicytes are the most

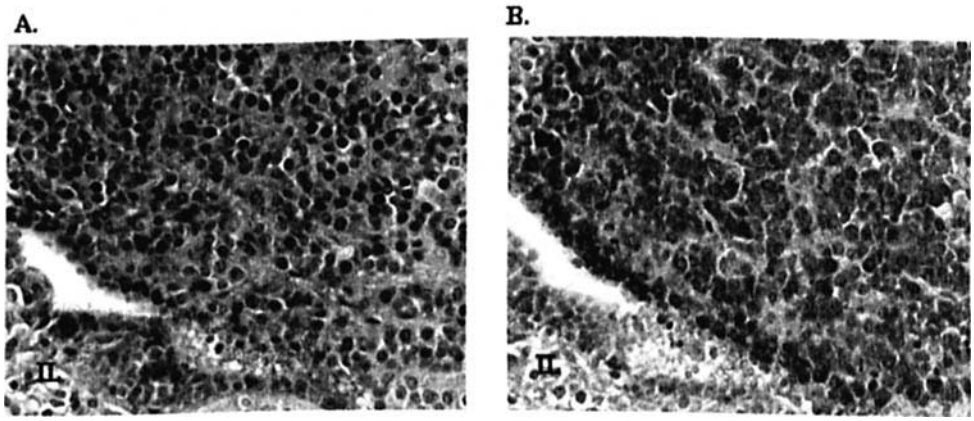
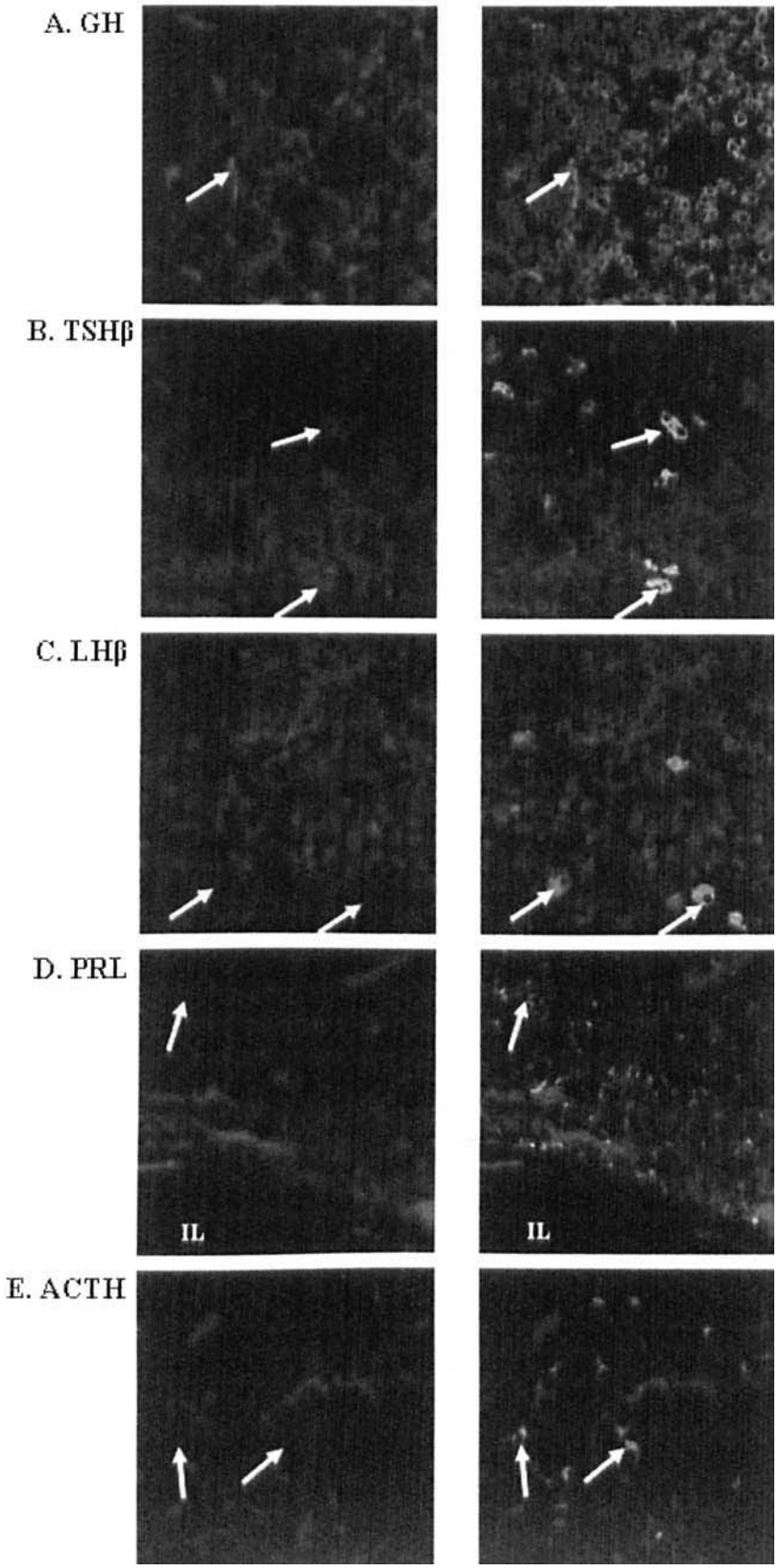


Figure 3. Section of rat AP stained with SP22 antiserum (A) and the flip-flopped mirror image section stained with GH (G3) antiserum (B). Cells in the intermediate lobe (IL) did not stain.


A. GH
B. TSHβ
C. LHβ
D. PRL
IL
IL
E. ACTH

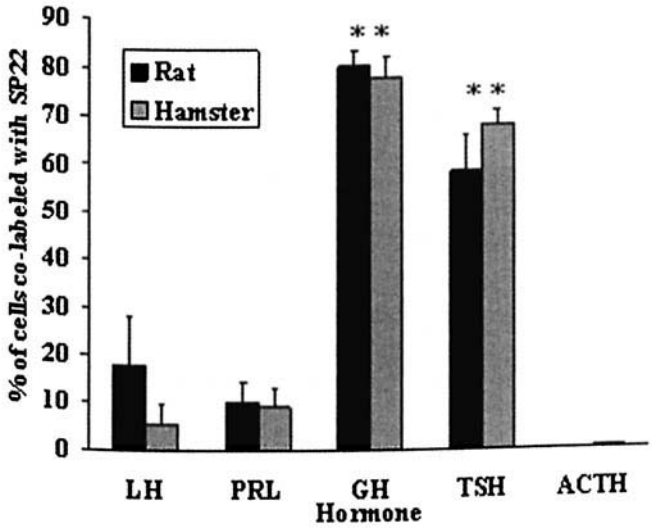


Figure 5. Percentage of AP cells that stained with antisera against LHβ, prolactin (PRL), GH, TSHβ, or ACTH that also stained with antisera against SP22 in male rats and hamsters. *Percentages for GH and TSH were greater ($P < 0.01$) than those for the other hormones.

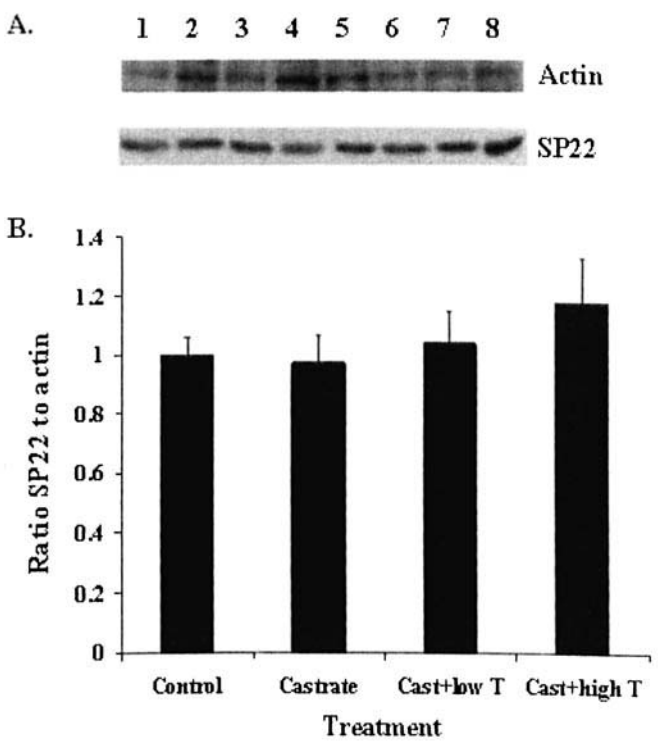


Figure 6. (A) Representative Western blot of AP from intact male rats (Lanes 1, 2) or castrated rats given empty capsules (Lanes 3, 4), one capsule containing testosterone (Lanes 5, 6), or four capsules containing testosterone (lanes 7, 8). (B) Ratio of SP22 to actin (mean ± SE for 5–6 rats in each group) in whole-cell lysates from intact rats (Control) or castrated rats given four empty capsules (Castrate), one testosterone capsule (Cast + low T), or four testosterone capsules (Cast + high T). Treatments did not differ ($P > 0.05$).

abundant nucleated cell of the posterior pituitary and are considered to be modified astrocytes.

Western blots of rat whole-cell extracts revealed a specific band for SP22 of approximately 21,000 mol wt. This size is in keeping with reported sizes of SP22, which range from 19,000 to 28,000 mol wt. The discrepancy in reported molecular weights may reflect species differences or differences in physiological state. As many as five isoforms of SP22 have been reported (5, 14, 22, 23). Variations in molecular weight may also result from differences in N-glycosylation (6). In addition, Welch and coinvestigators reported two different sizes of mRNA transcripts for SP22 (5). Western blot analyses in the present study did not suggest a change in the concentration of SP22 in the AP in response to orchidectomy or testosterone replacement in orchidectomized rats. Our results do not rule out the possibility that a shift occurred either in the intracellular location of SP22 (i.e., from cytoplasm to nucleus) or in the ratio of SP22 isoforms. Such changes would not be revealed by Western analysis.

The role of SP22 in the AP is presently unknown. We observed immunohistochemical staining for SP22 demonstrating nuclear and/or cytoplasmic localization. Both nuclear and cytoplasmic SP22 were reported in Hela cells, and it was suggested that SP22 may be translocated to the nucleus, depending on stage of the cell cycle (12). Thus, nuclear staining could reflect cells that have entered into the S phase of the cell cycle. Nuclear localization of SP22 may also be indicative of proliferation or transdifferentiation of cells. Nagakubo and coinvestigators observed a proliferative effect of SP22 on Hela cells (2). In addition, proliferation of cells in prostate and breast cancers is associated with enhanced expression of SP22 (11, 14). In the AP, changes in cell size and number of cells cosecreting hormones occur under several physiological conditions. For example, lactotropes proliferate in response to estradiol and a number of paracrine factors (24, 25), and the proportion of

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Figure 4. Sections of rat AP dual labeled with Texas Red for SP22 and FITC for hormone. The left panels show the scanned images for the Texas Red channel only, showing cells in red that contain SP22. Images showing both channels are in the right panels. AP hormones labeled the cells a bright green. Cells that contained both SP22 and AP hormone were red in the image on the left and yellow-green to orange on the right. Row A, Most of the cells that stained for GH (yellow-green to orange cells in the right panel) colabeled with SP22 (red cells in the left panel). Sinusoids containing red blood cells autofluoresced with Texas Red (arrow). Row B, Most of the cells that stained for TSHβ (yellow-green to orange cells in the right panel) colabeled with SP22 (red cells in the left panel). Arrows depict two cells that colabeled. Row C, Section stained for SP22 and LHβ. Arrows depict two cells that labeled for LHβ (green cells in right panel) that did not contain SP22. Row D, Section stained for SP22 and prolactin. Arrows depict a cell that labeled for prolactin (green cell in right panel) that did not contain SP22. Row E, Section stained for SP22 and ACTH. Arrows depict two cells that labeled for ACTH (green cells in right panel) that did not contain SP22. Minimal background fluorescence is seen in the intermediate lobe (IL) shown at the bottom left of the prolactin images (row D).

somatomammotropes varies with changes in the steroid milieu (26, 27). Size and number of somatotropes are altered during aging (28). The proportion of gonadotropes secreting LHβ, FSHβ, or both also varies with stage of the estrous cycle (29, 30). We observed SP22 in up to 9% of the lactotropes and 18% of the gonadotropes in rats and hamsters. These percentages compare favorably with the percentage of lactotropes that cosecrete GH (27, 31) and the percentage of gonadotropes that cosecrete GH (29, 32). Thus, it is possible that the gonadotropes and lactotropes expressing SP22 reflect those that are cosecreting GH. Pit-1 has been implicated in a similar role where localization of Pit-1 to gonadotrope nuclei was indicative of their transdifferentiation to lactotropes by estrogen (33). It is possible that the presence of SP22 in the nucleus, or its presence in a subpopulation of cells, is indicative of those cells that are either proliferating or converting to secretion of a different hormone. Alternatively, SP22 synthesis may be increased in response to hormonal stimulation to specific cells. For example, the human SP22 promoter was activated by Sp1 (34). In turn, Sp1 has been implicated in the GnRH-induced stimulation of the LHβ gene promoter (35, 36).

SP22 has been linked to nuclear AR function. Androgen receptors and AR mRNA have been reported in mixed AP cell populations (37–39) and have been localized in gonadotropes (40, 41) and some somatotropes (41). In addition, AR mRNA in the AP is increased following castration in rats and decreased by administration of dihydrotestosterone (17). In the present study, SP22 concentration did not change in response to orchidectomy with or without testosterone replacement. However, changes in intracellular distribution of SP22 or in SP22 isoforms could alter its ability to regulate AR function. Taira and coinvestigators (42) reported that colocalization of SP22 and AR was important to AR activity. The AR is normally repressed by binding of PIASxα (protein inhibitor of activated STAT [signal transducer and activator of transcription]; Ref. 8). SP22 appears to regulate AR activity by binding to PIASxα and preventing its binding to AR. A 570-amino-acid SP22 binding protein has also been reported to play a similar role in the testis (9) by interacting with histone deacetylase complex. PIAS proteins interact with STAT, inhibiting their binding to DNA. Evidence suggests that PIAS works with the SUMO (small ubiquitin modifier) pathway, and the activity of AR-dependent genes may be a combined effect of PIAS protein-stimulated SUMOylation of both receptors and cofactors (43). In the testis, PIAS, AR, and SP22 are all associated with the regulation of spermatogenesis (7, 22, 44, 45), but a direct connection has not yet been established.

Cell viability may be affected by SP22. SP22 has been implicated as a possible modulator of cell responses to oxidative stress and as a result is able to modify cell death and apoptosis. Taira and coinvestigators reported that SP22 was able to neutralize the effects of hydrogen peroxide (H_2O_2) and dopaminergic neurotoxins on SH-SY5Y cells by oxidizing, resulting in a shift in SP22 to a more acidic pI and preventing cell death (12). It was suggested that mutation of SP22 reduces this protective effect and may play a role in the death of tuberoinfundibular neurons in some forms of early-onset Parkinson's disease. In peritoneal macrophages and J774 cells as well as lung tissue, an increase in the acidic form (pI 5.8) of SP22 was indicative of oxidative stress during inflammatory responses to lipopolysaccharides (10). In prostate cells, SP22 was stimulated by H_2O_2 before the onset of apoptosis, and it was suggested that a high level of SP22 was protective against cell death (11). In the pituitary, steroids and other factors are able to alter AP apoptosis and in some cases are linked to production of reactive oxygen species (46, 47). In primary AP cell cultures, nitric oxide was observed to stimulate or protect against cell death depending on factors such as concentration and duration of exposure (48, 49). The potential role for SP22 in the control of AP cell death has not yet been investigated.

Most notably, the predominance of SP22-positive somatotropes and thyrotropes raises the possibility that SP22 may play a role in GH and TSH secretion. The precise mechanisms controlling the proliferation of and secretion by these cells has not been fully elucidated. Somatotropes are largely regulated by the combined effects of GH-releasing hormone (GHRH), ghrelin, and somatostatin, but secretion of GH is also modulated by gonadal steroids and diurnal rhythms (50–52). Androgen receptors have been reported in somatotropes, suggesting one possible interaction with SP22. Second messenger systems that have been implicated in GH secretion include cAMP/Ca^{2+}, tyrosine kinases, and NO/cAMP (50, 53). The list of possible regulators of GH gene transcription includes Sp1, which has been linked to SP22 (34, 54). Secretion of TSH from thyrotropes is primarily under stimulatory control of thyrotropin-releasing hormone (TRH) and inhibitory control of thyroid hormones (55). At present, a regulator of the TSHβ gene that is specific to the thyrotrope has not been identified.

SP22 mRNA was previously shown by dot blot analysis to be present in whole pituitaries (4). More recently, SP22 was identified specifically in the AP using ion trap mass spectrometry (1). The present study, however, is the first to demonstrate that the AP and posterior lobe both contain SP22 mRNA, and the posterior pituitary contains SP22. We also demonstrate that SP22 in the AP is localized primarily in somatotropes and thyrotropes. Although a specific role for SP22 in the AP has not been defined, it may have several important roles including (i) proliferation and/or transdifferentiation of AP cells, (ii) protection of AP cells against oxidative stress, accumulation of reactive oxygen species, and apoptosis, and (iii) regulation of cell functions mediated through AR. The abundance of somatotropes and thyrotropes that contain SP22 suggests that it may be important in the regulation of GH and TSH secretion.

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