

# Identification of Mammosomatotrophs in the Turkey Hen Pituitary: Increased Abundance during Hyperprolactinemia\*

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## ABSTRACT

We have previously reported that the hyperprolactinemia in incubating turkey hens is associated with recruitment of lactotrophs in the pituitary gland. In this study we have used double immunofluorescence and *in situ* hybridization histochemistry to 1) identify mammosomatotrophs in the anterior pituitary gland of egg-laying turkey hens and incubating hens, and 2) verify PRL gene expression within mammosomatotrophs by colocalizing PRL messenger RNA in GH-immunoreactive (ir) cells. The pituitaries of laying and incubating turkey hens were collected, and the midsagittal sections were dual labeled for either PRL and GH or PRL messenger RNA and GH. The plasma PRL concentrations were higher in incubating hens ( $231 \pm 10.6$  ng/ml) than in laying hens ( $43 \pm 7.4$  ng/ml;  $P < 0.01$ ). In the

midsagittal pituitary sections, mammosomatotrophs were predominantly found scattered in the caudal lobe of the anterior pituitary gland, in the ventral half of the cephalic lobe, and at the junction of cephalic and caudal lobes. In incubating hens, the proportion of mammosomatotrophs was  $7.4 \pm 1.52\%$  (mean  $\pm$  SEM) of the total number of GH-ir and/or PRL-ir cells counted, which was significantly higher ( $P < 0.05$ ) than that found in laying hens ( $0.6 \pm 0.23\%$ ). Furthermore, PRL gene expression was observed in many GH-ir cells in the incubating hen pituitary gland. These data suggest that 1) mammosomatotrophs are present in the turkey pituitary gland, and 2) there is an increased abundance of mammosomatotrophs in the incubating turkey hen that may contribute to hyperprolactinemia. (*Endocrinology* 139: 781–786, 1998)

**M**AMMOSOMATOTROPHS are adeno-hypophysial cells that colocalize GH and PRL. The existence of mammosomatotrophs within the pituitary gland of several mammalian species has been reported previously (1). Mammosomatotrophs have been proposed as transitional cells in the bidirectional conversion of somatotrophs to lactotrophs (2).

Incubation behavior expressed by egg-laying turkey hens is associated with increased secretion of PRL from the pituitary gland that ultimately results in ovarian regression and loss of egg production (3). Therefore, a clear understanding of the mechanism of transdifferentiation to lactotrophs may lead to an insight into the prevention of hyperprolactinemia and the negative effects of incubation behavior on the reproductive system. Unlike the mammalian anterior pituitary gland, the turkey adeno-hypophysis consists of two distinct lobes, namely the cephalic and caudal lobes (4). In addition, the neurointermediate lobe, present in some mammalian species, is absent in birds. The distributions of lactotrophs and somatotrophs are anatomically separated in the turkey hen anterior pituitary gland. Lactotrophs are exclusively located in the cephalic lobe, whereas somatotrophs are distributed predominantly in the caudal lobe plus a few scat-

tered groups in the cephalic lobe of the laying turkey hen anterior pituitary gland.

Expression of incubation behavior by the turkey hens is accompanied by a 5- to 10-fold increase in the plasma PRL concentration (5). The hyperprolactinemia in the incubating turkey hen is associated with replacement of somatotrophs by lactotrophs in the anterior pituitary gland caudal lobe (4). As mammosomatotrophs are proposed as the transitional cells in the interconversion of somatotrophs and lactotrophs in rats (2), they should be present in the incubating turkey anterior pituitary gland if transdifferentiation plays an important role in the recruitment of lactotrophs and in augmenting PRL secretion. The existence of mammosomatotrophs, however, has not been reported in the turkey or any other avian anterior pituitary gland. Therefore, our objectives for the present study were to 1) identify mammosomatotrophs in the anterior pituitary gland of egg-laying turkey hens and incubating hens, and 2) verify PRL gene expression within mammosomatotrophs by colocalizing PRL messenger RNA (mRNA) in GH-immunoreactive (ir) cells.

## Materials and Methods

### Animals

Large white turkey hens (Nicholas strain) were housed in floor pens at the Beltsville Agricultural Research Center (Beltsville, MD). Feed and water were available at all times. Each floor pen was provided with trap nests for use by the hens to lay eggs. Egg-laying turkey hens visit the nest box just to lay eggs, whereas incubating hens prefer to stay in the nest box at all times and resist dislodging from the nest box. The turkey hens are free to move within the nest box and do not experience discomfort or stress as they are acclimated to the trap nesting over several weeks.

Received June 26, 1997.

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\* This work was supported in part by USDA Cooperative Agreement 58-1265-2-143.

Nest boxes were checked four times a day between 0700–1300 h at 2-h intervals to 1) release the trapped hen and 2) check whether the hen had laid an egg. A record of daily nesting frequency and egg production was maintained for every hen. Egg-laying hens and incubating hens ( $n = 4$ ) were selected based on nesting frequency and egg production as follows: 1) hens that visited the nest box one or two times in a day and consistently laid an egg every 1–2 days were considered laying hens; and 2) hens that were found in the nest box at all nest checks without laying an egg for at least 10 days before death were classified as incubating hens.

### Collection of pituitaries

Pituitaries were collected from laying and incubating hens as previously described (6). Briefly, birds were anesthetized with sodium pentobarbital, and their heads were perfused with physiological saline followed by 4% paraformaldehyde solution. Brains and pituitaries were removed from the cranium and stored in 0.1 M sodium phosphate buffer (pH 7.2) at 4 C until processing. Pituitaries were dehydrated, cleared, and embedded in paraffin. Sagittal pituitary sections were cut at 4- $\mu$ m thickness using a Leica rotary microtome (Leica, Deerfield, IL), and serial sections were mounted on ribonuclease-free gelatin-coated microscopic slides.

### Double immunofluorescent staining

A polyclonal antibody raised in rabbit against turkey GH (7) and a mouse monoclonal antibody (VIAA2) raised against a synthetic peptide fragment of chicken PRL (8) were used for dual labeling. Tissue sections were deparaffinized, hydrated, and incubated for 1 h with 2.5% normal goat serum in 0.01 M Tris-HCl and 0.15 M sodium chloride (pH 7.4; TBS) containing 1% Triton X-100. Slides were then incubated with a mixture of anti-PRL antibody (1:5,000) and anti-GH antibody (1:15,000) for 36 h at 4 C in a humid chamber. After a series of washes in TBS, slides were incubated with the biotinylated antirabbit IgG (1:400; Vector Laboratories, Burlingame, CA) for 1 h at room temperature. Slides were then washed in TBS and incubated with a mixture of lissamine conjugated to antimouse IgG raised in goats (1:100; Jackson Immuno Research Laboratories, West Grove, PA) and avidin-fluorescein isothiocyanate (25  $\mu$ g/ml; Vector Laboratories) for 2 h in the dark at room temperature. After washing in TBS, coverslips were applied using Vectashield (Vector Laboratories). PRL-ir (red) and GH-ir (green) cells as well as mammosomatotrophs were studied using fluorescence microscopy (Axioplan microscope, Zeiss, New York, NY).

### Cell counts

The numbers of mammosomatotrophs, lactotrophs, and somatotrophs showing a complete cross-section of a cell with nucleus were counted under a microscope at  $\times 1000$  magnification in selected locations of midsagittal sections of the pituitary gland, where mammosomatotrophs were predominantly found in this study. The locations were the caudal lobe, the junction of cephalic and caudal lobes, and the ventral borders of cephalic lobe. The number of mammosomatotrophs as well as the total number of cells immunoreactive to GH and/or PRL counted from four midsagittal sections were summed for each hen. The ratio of mammosomatotrophs to the total number of immunoreactive cells that were positive for GH and/or PRL was expressed as a percentage and compared between laying and incubating hens by Student's *t* test using SAS (9).

### Dual in situ hybridization histochemistry and immunohistochemistry

To colocalize PRL mRNA and GH in pituitary cells, *in situ* hybridization histochemistry (ISHH) was carried out, followed by GH immunohistochemistry (IHC) on the same pituitary tissue sections.

**PRL mRNA probe preparation and digoxigenin labeling.** The full-length turkey PRL complementary DNA (cDNA; 890 bp) insert (10) was cloned into Bluescript KS<sup>+</sup> vector. The plasmid DNA containing the insert was extracted using the FlexiPrep kit (Pharmacia Biotech, Uppsala, Sweden), purified, and linearized using *Hind*III or *Bam*HI restriction enzymes

(Boehringer Mannheim, Indianapolis, IN). Antisense and sense strands of PRL cDNA insert were transcribed using T7 polymerase and T3 polymerase, respectively, and labeled with digoxigenin (Boehringer Mannheim).

**ISHH.** Tissue sections were deparaffinized, hydrated, and treated with 0.2 N hydrochloric acid. Slides were rinsed in 0.1 M phosphate buffer (PBS) containing 0.3% Triton X-100 and treated with proteinase K (Sigma Chemical Co., St. Louis, MO; 10  $\mu$ g/ml) in 0.1 M Tris-HCl containing 50 mM EDTA for 20 min. The protein digestion was stopped using 1% glycine solution, and tissues were fixed in 4% paraformaldehyde. Tissue sections were then acetylated with 0.25% acetic anhydride and 0.1 M triethanolamine and incubated in prehybridization buffer [50% formamide in 2  $\times$  SSC (standard saline citrate)] at 37 C. Digoxigenin-labeled PRL riboprobe (50 ng/ml) was diluted in hybridization buffer (10 mM Tris-HCl, 12.5% Denhardt's solution, 50% formamide, 0.5% SDS, and 2  $\times$  SSC), applied to the slides, and incubated in a humid chamber at 45 C overnight. The slides were washed twice in 2  $\times$  SSC for 15 min each time at room temperature, twice in 0.1  $\times$  SSC for 20 min each time at 42 C, and once in 0.1  $\times$  SSC for 10 min at room temperature. The slides were then treated with 4% normal sheep serum in 0.1 M TBS containing 0.3% Triton X-100. Antidigoxigenin antibody conjugated to alkaline phosphatase (Boehringer Mannheim; 1:500) was applied to the tissue sections for 2 h. A color solution (450  $\mu$ g/ml 4-nitro blue tetrazolium chloride, 175  $\mu$ g/ml 5-bromo-4-chloro-3-indolyl phosphate, and 250  $\mu$ g/ml levamisole in 0.1 M TBS containing 50 mM MgCl<sub>2</sub>, pH 9.5) was applied until satisfactory blue color development was achieved. The color reaction was stopped using a solution of 50 mM EDTA in 0.1 M TBS containing 50 mM MgCl<sub>2</sub>.

**IHC.** Pituitary sections hybridized with PRL mRNA probe were then immunostained to locate GH using a standard immunoperoxidase method. A polyclonal antibody raised against turkey GH (7) was used at 1:15,000 dilution. Staining techniques, including controls, were performed as described previously (6). PRL mRNA (blue) and GH (brown) labeling in pituitary cells was studied using a Zeiss Axioplan microscope at a magnification of  $\times 1000$ .

### Controls

**Immunofluorescence.** PRL antibody and GH antibody were preadsorbed with their respective antigens (turkey PRL and/or turkey GH) for 48 h at 4 C and centrifuged at 100,000  $\times g$  for 20 min. The supernatant was used in place of PRL antibody and/or GH antibody as a control while immunostaining.

**ISHH.** The digoxigenin-labeled sense strand of turkey PRL cDNA was used for ISHH as the control.

### Plasma PRL analysis

The plasma PRL concentration (nanograms per ml) in egg-laying and incubating hens was measured by RIA (11), and data were compared by Student's *t* test using SAS (9).

## Results

### Plasma PRL concentrations

Plasma PRL concentrations in incubating turkey hens ( $231 \pm 10.6$  ng/ml) were significantly higher ( $P < 0.01$ ) than those in egg-laying hens ( $43 \pm 7.4$  ng/ml). At necropsy, egg-laying hens were found to have functional ovaries bearing a normal hierarchy of preovulatory follicles, whereas the incubating hens had completely regressed ovaries.

### Dual immunofluorescent labeling

The distribution of fluorescent-labeled PRL cells and GH cells within the midsagittal sections of the anterior pituitary gland (shown diagrammatically in Fig. 1) was identical to that described using an immunoperoxidase technique (4).

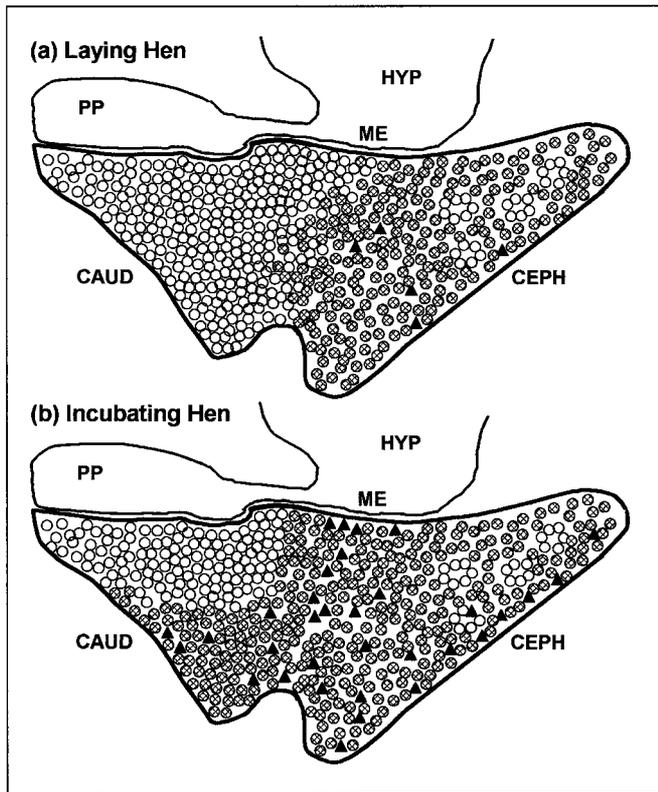


FIG. 1. Diagram of midsagittal anterior pituitary gland of a laying turkey hen (a) and an incubating turkey hen (b) showing the distribution of somatotrophs (open circle), lactotrophs (hatched circle), and mammosomatotrophs (triangle). HYP, Hypothalamus; PP, posterior pituitary; ME, median eminence; CEPH, cephalic lobe; CAUD, caudal lobe of the anterior pituitary gland. This diagram is not drawn to scale.

Briefly, PRL-ir cells were found only in the cephalic lobe in laying hens, but were abundantly found in both cephalic and caudal lobes of incubating hens. Mammosomatotrophs, which showed colocalization of PRL and GH, were found in the midsagittal anterior pituitary gland sections of incubating turkey hens. They were predominantly found scattered in the caudal lobe, in the ventral half of the cephalic lobe, and at the junction of cephalic and caudal lobes (Fig. 1, triangles). The bihormonal cells were very rare in egg-laying hens. Figure 2, A–C, shows photomicrographs of midsagittal sections of the anterior pituitary gland of incubating turkey hens, indicating lactotrophs (red), somatotrophs (green), and mammosomatotrophs (green and either red or yellow). The arrow in all figures indicates a mammosomatotroph. Islets of GH-ir cells and PRL-ir cells from the cephalic lobe are shown in Fig. 2A, in which at least one of the GH-ir cells also contained ir-PRL. A hypertrophied mammosomatotroph observed at the junction of the cephalic and caudal lobes contained ir-PRL adjacent to the nucleus and GH throughout the cytoplasm (Fig. 2B). Figure 2C shows a mammosomatotroph that appears to contain discrete secretory granules that colocalize PRL and GH (yellow fluorescence) as well as adjacent granules containing only PRL or GH. There was no immunofluorescence when PRL antibody and GH antibody were preadsorbed with PRL and GH and used in staining.

#### Mammosomatotroph cell count

In each laying and incubating hen, approximately 2000 cells were counted in each of four midsagittal pituitary sections at the locations described above. The proportion of mammosomatotrophs was  $0.6 \pm 0.23\%$  (mean  $\pm$  SEM;  $n = 4$ ) of the total number of immunoreactive cells that were positive for GH and/or PRL in laying hens, whereas the proportion was significantly increased ( $P < 0.05$ ) to  $7.4 \pm 1.52\%$  in incubating hens ( $n = 4$ ).

#### ISHH and IHC

A photomicrograph from a midsagittal pituitary section of an incubating turkey hen stained by ISHH and IHC is shown in Fig. 2D. The arrow in this figure points to a mammosomatotroph that colocalizes PRL mRNA (blue) and GH protein (brown) within the same cell. The PRL mRNA hybrids were always located in the cytoplasm. The ISHH procedures carried out on pituitary sections did not affect the subsequent GH immunostaining.

There was no ISHH staining in pituitary sections hybridized with the digoxigenin-labeled sense strand of turkey PRL cDNA or in regions of the caudal lobe of the anterior pituitary gland where there were no PRL-ir cells (data not shown). The specificity of PRL mRNA ISHH within the anterior pituitary gland was also evidenced by the hybridization signal occurring only in regions where there were PRL-ir cells in adjacent sections (data not shown).

#### Discussion

The present study documents the existence of mammosomatotrophs in the incubating turkey anterior pituitary gland. Such PRL- and GH-containing bihormonal cells have been identified in the human (12), bovine (13), goat (14), sheep (15), bat (16), rat (17), mouse (18), and musk shrew (19) anterior pituitary gland. To our knowledge, this is the first report of the existence of mammosomatotrophs within the avian pituitary gland.

In the present study, we first used immunofluorescent histochemistry to identify and quantitate mammosomatotrophs in the midsagittal pituitary gland tissue sections of laying and incubating turkey hens. Although we believe that localization of PRL and GH in discrete granules within clear cell boundaries using  $4\text{-}\mu\text{m}$  paraffin sections clearly demonstrates that these cells are mammosomatotrophs, we further confirmed the nature of these cells by demonstrating that *de novo* synthesis of PRL is occurring in GH-containing cells from incubating hens. We have previously shown that during the hyperprolactinemia that is characteristic of incubating hens, there is an increase in PRL-ir cells (4). In the present study, plasma PRL levels were 5.3-fold higher in incubating hens than in laying hens. During a shift to hyperprolactinemia, mammosomatotrophs should be characterized by the presence of PRL mRNA in the cytoplasm of GH-ir cells as the *de novo* synthesis of PRL occurs in these cells. Our data (Fig. 2D) clearly demonstrate that such cells occur adjacent to cells that contain only PRL mRNA (lactotrophs) or only GH (somatotrophs). Although gene expression for PRL has previously been demonstrated in laying

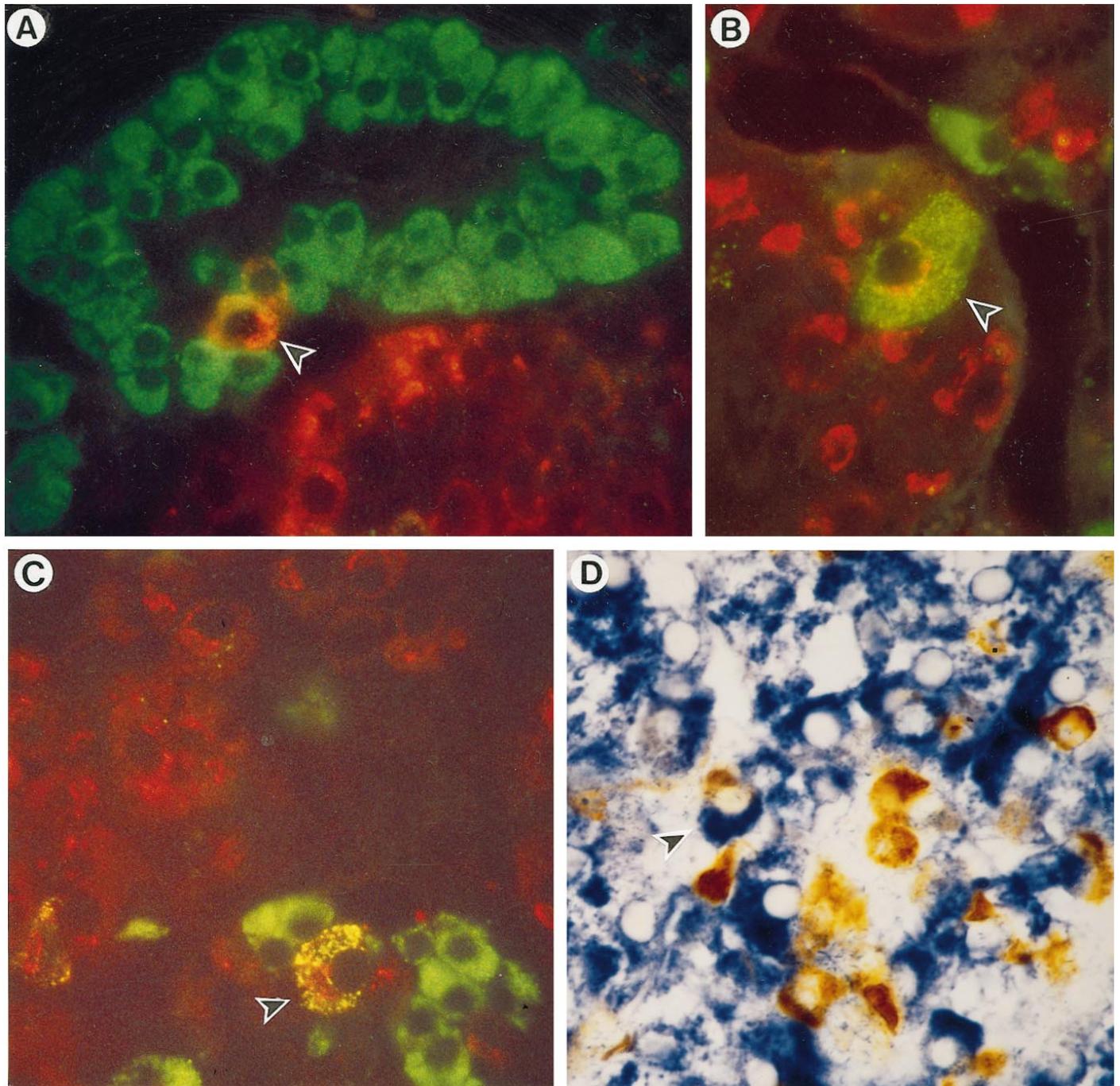


FIG. 2. A–C, Photomicrographs of midsagittal sections of the anterior pituitary gland of incubating turkey hens showing lactotrophs (red), somatotrophs (green), and mammosomatotrophs (green and red or yellow). Arrows in all panels indicate the mammosomatotroph. Original magnification,  $\times 1000$ . A, Islets of somatotrophs and lactotrophs in the pituitary cephalic lobe. B, Hypertrophied mammosomatotroph at the junction of the cephalic and caudal lobes, showing PRL molecules around the nucleus. C, Photomicrograph of the caudal lobe, showing mammosomatotrophs containing discrete granules that colocalize PRL and GH within the same secretory granules (yellow fluorescence). D, Photomicrograph of a midsagittal pituitary section of an incubating turkey hen stained by ISHH and IHC. The arrow indicates a mammosomatotroph, showing colocalization of PRL mRNA (blue) and GH (brown). Original magnification,  $\times 1000$ .

and incubating bantam chicken pituitaries by ISHH (20), Fig. 2D extends that finding by demonstrating both PRL mRNA and GH within the same avian pituitary cell. Similarly, colocalization of PRL mRNA and GH within human adenohypophysial cells during pregnancy has been reported (21).

In the present study, mammosomatotrophs were scarce

(0.6%) in laying hen anterior pituitary glands. In contrast, mammosomatotrophs were relatively abundant within anterior pituitary glands of incubating hens (7.4%), and they were found localized in the ventral half of the cephalic lobe, in the caudal lobe, and at the junction of cephalic and caudal lobes (Fig. 1b). The mammosomatotroph in Fig. 2B appears

to be at an early transitional stage, with PRL-ir granules just appearing adjacent to the nucleus. Other mammosomatotrophs appeared to be in a later stage of transition, with yellow fluorescence indicating the mixing of PRL and GH in the same cytoplasmic area. PRL and GH appear to be located at different cytoplasmic locations within some mammosomatotrophs, suggesting that they may be stored in discrete secretory granules or compartments. Some mammosomatotrophs appeared to contain discrete granules that were immunoreactive for both hormones, suggesting that GH and PRL may either be segregated in separate granules or be colocalized within the same granule. In mammalian mammosomatotrophs, GH and PRL were reported to occur both inside the same secretory granules (12) and in separate granules (22). In previous immunohistochemical studies, the occurrence of mammosomatotrophs was reported to be rare in the lactating goat (14), whereas in sheep, mammosomatotrophs comprised 0.15% of all pituitary cells (15). Mammosomatotrophs formed 5.6–9.6% of all pituitary cells obtained from virgin, late lactating, and postweaning rats (2) in reverse hemolytic plaque assays, and their relative proportions did not significantly change at the above physiological states. The proportions of PRL and GH dual secretors (mammosomatotrophs) in cell cultures obtained from bovine pituitaries at early luteal, midluteal, late luteal, and follicular phases varied significantly between 4–16% of all pituitary cells (23). The 12.3-fold increase in the proportion of mammosomatotrophs among GH- and PRL-ir cells with incubation behavior in the turkey hens observed in the present study is much greater than the relative change in the proportion of mammosomatotrophs reported in other species. Thus, the turkey hen may offer an excellent model for studying somatotroph and lactotroph transdifferentiation because of the rapid and dramatic changes in PRL secretion that occur when the hen shifts from egg production to incubation behavior, and because the anatomical distribution of lactotrophs and somatotrophs changes with respect to the animal's physiological state.

Besides PRL and GH colocalization, pituitary cells have been shown to contain or express more than one hormone such as ACTH and FSH (24), PRL and FSH (25), and GH and LH $\beta$  or FSH $\beta$  mRNA (26). It is thus evident that adult differentiated pituitary cells retain their functional plasticity.

The physiological significance of the mammosomatotroph is not conclusively known in all of the mammalian species studied. Porter *et al.* (2, 27) proposed that in female rats, mammosomatotrophs may represent a group of transitional cells in the interconversion of somatotrophs to lactotrophs during gestation and lactation. Results from our earlier studies as well as those of others (1) suggest that there is recruitment of PRL-producing cells in the pituitaries of vertebrate animals under certain physiological conditions. The hyperprolactinemia in incubating bantam chicken hens has been associated with an increase in pituitary PRL secretors (28). Similarly, PRL-containing cells were increased in the pregnant human pituitary, with a concomitant decrease in GH-containing cells (29). Considering the above, mammosomatotrophs observed in incubating turkey hens may be viewed as pituicytes, which support increased PRL production and maintenance of hyperprolactinemia. In addition, they may

also be regarded as transitional cells in the transdifferentiation of mature somatotrophs to lactotrophs.

Hyperprolactinemia has been associated with ovarian regression and loss of egg production in turkey hens that result in a huge economic loss to the turkey industry (3). Furthermore, hyperprolactinemia in turkey hens brings about a significant decrease in body weight by affecting feed and water intake. The metabolic effects due to hyperprolactinemia in turkey hens include hemoconcentration, hypoglycemia, hypothermia, and increased blood ketone concentrations (30). Some of the hyperprolactinemic turkey hens die due to the above metabolic effects if left untreated (our unpublished data). In men and women, hyperprolactinemia causes dysfunction of the hypothalamic-pituitary axis, the gonads, and the adrenal cortex (31). In women, hyperprolactinemia causes menstrual disturbances, galactorrhea, infertility, and hirsutism, whereas in men, impotence and oligospermia result. Furthermore, hyperprolactinemia has been reported to cause glucose intolerance, hyperinsulinemia, and insulin resistance in rats and humans (32, 33). One of the mechanisms underlying the maintenance of hyperprolactinemia is the overabundance of mammosomatotrophs observed in incubating hen pituitary glands. Mammosomatotroph adenoma of the pituitary gland has been reported to cause both hyperprolactinemia and elevation of basal GH secretion, resulting in gigantism (34, 35).

In conclusion, we have shown that mammosomatotrophs exist in the avian pituitary gland. In addition, mammosomatotrophs were found in greater abundance when the turkey hens expressed incubation behavior. Further studies on the transdifferentiation mechanisms involving somatotrophs and lactotrophs may help to explain the increased PRL production that results in incubation behavior and ovarian regression in turkey hens.

### Acknowledgments

We thank Dr. Luc Berghman for providing PRL antibody, Dr. E. A. Wong for gift of turkey PRL cDNA, and Ms. Shirley Moore for the PRL RIA.

### References

1. Frawley LS, Boockfor FR 1991 Mammosomatotrophs: presence and functions in normal and neoplastic pituitary tissue. *Endocr Rev* 12:337–355
2. Porter TE, Wiles CD, Frawley LS 1991 Evidence for bidirectional interconversion of mammotropes and somatotropes: rapid reversion of acidophilic cell types to pregestational proportions after weaning. *Endocrinology* 129:1215–1220
3. El Halawani ME, Rozenboim I 1993 The ontogeny and control of incubation behavior in turkeys. *Poult Sci* 72:906–911
4. Ramesh R, Proudman JA, Kuenzel WJ 1996 Changes in pituitary somatotroph and lactotroph distribution in laying and incubating turkey hens. *Gen Comp Endocrinol* 104:67–75
5. Proudman JA 1995 The biology of egg production and fertility. In: Bakst MR, Wishart GJ (eds) *Proceedings of the First International Symposium on Artificial Insemination of Poultry*. Poultry Science Association, Savoy, pp 128–148
6. Ramesh R, Proudman JA, Kuenzel WJ 1995 Changes in pituitary somatotrophs and lactotrophs associated with ovarian regression in the turkey hen (*Meleagris gallopavo*). *Comp Biochem Physiol* 112C:327–334
7. Bakst M, Haddick S, Proudman J, Maruyama K 1993 Immunocytochemical localization of some turkey pituitary hormones using antisera to human hormones. *Poult Sci* 72:1127–1131
8. Berghman LR, Grilles L, Vanhamme L, Proudman JA, Foidart A, Balthazart J, Vandesande F 1992 Immunocytochemistry and immunoblotting of avian prolactin using polyclonal and monoclonal antibodies toward a synthetic fragment of chicken prolactin. *Gen Comp Endocrinol* 85:346–357

9. SAS 1987 SAS/STAT Guide for Personal Computers, version 6. SAS Institute, Cary
10. Wong EA, Ferrin NH, Silsby JL, El Halawani ME 1991 Cloning of a turkey prolactin cDNA: expression of prolactin mRNA throughout the reproductive cycle of the domestic turkey (*Meleagris gallopavo*). *Gen Comp Endocrinol* 83:18–26
11. Proudman JA, Opel H 1981 Turkey prolactin: validation of a radioimmunoassay of changes associated with broodiness. *Biol Reprod* 25:573–580
12. Zurschmiede C, Landolt AM 1987 Distribution of growth hormone and prolactin in secretory granules of the normal and neoplastic human adenohypophysis. *Virchows Arch B Cell Pathol* 53:308–315
13. Fumagalli G, Zanini A 1985 In cow anterior pituitary, growth hormone and prolactin can be packed in separate granules of the same cell. *J Cell Biol* 100:2019–2024
14. Sanchez J, Navarro JA, Bernabe A, Gomez S, Garcés B, Gomez MA 1994 Identification of somatomammotroph cells in lactating goats (*Capra hircus*) by fluorescence and immunogold techniques. *Acta Anat* 149:39–45
15. Thorpe JR, Ray KP, Wallis M 1990 Occurrence of rare somatomammotrophs in ovine anterior pituitary tissue studied by immunogold labeling and electron microscopy. *J Endocrinol* 124:67–73
16. Ishibashi T, Shiino M 1989 Subcellular localization of prolactin in the anterior pituitary cells of the Japanese house bat, *Pipistrellus abramus*. *Endocrinology* 124:1056–1063
17. Nikitovitch-Winer MB, Atkin J, Maley BE 1987 Colocalization of prolactin and growth hormone within specific adenohypophyseal cells in male, female, and lactating female rats. *Endocrinology* 121:625–630
18. Sasaki F, Iwama Y 1988 Sex difference in prolactin and growth hormone cells in mouse adenohypophysis: stereological, morphometric, and immunohistochemical studies by light and electron microscopy. *Endocrinology* 123:905–912
19. Ishibashi T, Shiino M 1989 Co-localization pattern of growth hormone (GH) and prolactin (PRL) within the anterior pituitary cells in the female rat and female musk shrew. *Anat Rec* 223:185–193
20. Talbot RT, Hanks MC, Sterling RJ, Sang HM, Sharp PJ 1991 Pituitary prolactin messenger ribonucleic acid levels in incubating and laying hens: effects of manipulating plasma levels of vasoactive intestinal polypeptide. *Endocrinology* 129:496–502
21. Stefaneanu L, Kovacs K, Lloyd RV, Scheithauer BW, Young Jr WF, Sano T, Jin L 1992 Pituitary lactotrophs and somatotrophs in pregnancy: a correlative *in situ* hybridization and immunocytochemical study. *Virchows Arch B Cell Pathol* 62:291–296
22. Hashimoto S, Fumagalli G, Zanini A, Meldolesi J 1987 Sorting of three secretory proteins to distinct secretory granules in acidophilic cell of cow anterior pituitary. *J Cell Biol* 105:1579–1586
23. Kineman RD, Henricks DM, Faught WJ, Frawley LS 1991 Fluctuations in the proportions of growth hormone- and prolactin-secreting cells during the bovine estrous cycle. *Endocrinology* 129:1221–1225
24. Moriarty GC, Garner LL 1977 Immunocytochemical studies of cells in the rat adenohypophysis containing both ACTH and FSH. *Nature* 265:356–358
25. Loinski NE, Horvath E, Kovacs K 1989 Double-labeling immunogold electron-microscopic study of hormonal colocalization in nontumorous and adenomatous rat pituitaries. *Am J Anat* 185:236–243
26. Childs GV, Unabia G, Rougeau 1994 Cells that express luteinizing hormone (LH) and follicle-stimulating hormone (FSH)  $\beta$ -subunit messenger ribonucleic acids during the estrous cycle: the major contributors contain LH $\beta$ , FSH $\beta$ , and/or growth hormone. *Endocrinology* 134:990–997
27. Porter TE, Hill JB, Wiles CD, Frawley LS 1990 Is the mammosomatotrope a transitional cell for the functional interconversion of growth hormone- and prolactin-secreting cells? Suggestive evidence from virgin, gestating, and lactating rats. *Endocrinology* 127:2789–2794
28. Lopez ME, Gregory CC, Porter TE 1996 Cellular basis for elevated prolactin secretion during incubation behavior in bantam chickens: analysis by reverse hemolytic plaque assay. *Biol Reprod* 54:826–833
29. Scheithauer BW, Sano T, Kovacs KT, Young WF, Ryan N, Randall RV 1990 The pituitary gland in pregnancy: a clinicopathologic and immunohistochemical study of 69 cases. *Mayo Clin Proc* 65:461–474
30. Zadworny D, Walton JS, Etches RJ 1984 The relationship between plasma concentrations of prolactin and consumption of feed and water during the reproductive cycle of the domestic turkey. *Poult Sci* 64:401–410
31. Kirby RW, Kotchen TA, Rees ED 1979 Hyperprolactinemia: a review of recent clinical advances. *Arch Intern Med* 139:1415–1419
32. Foss MC, Paula FJ, Paccola GM, Piccinato CE 1995 Peripheral glucose metabolism in human hyperprolactinemia. *Clin Endocrinol (Oxf)* 43:721–726
33. Reis FM, Reis AM, Coimbra CC 1997 Effects of hyperprolactinemia on glucose tolerance and insulin release in male and female rats. *J Endocrinol* 153:423–428
34. Felix IA, Horvath E, Kovacs K, Smyth HS, Killinger, DW, Vale, J 1986 Mammosomatotroph adenoma of the pituitary associated with gigantism and hyperprolactinemia. A morphological study including immunoelectron microscopy. *Acta Neuropathol (Berl)* 71:76–82
35. Dubuis JM, Deal CL, Drews RT, Goodyer CG, Lagace G, Asa SL, Van Vliet G, Collu R 1995 Mammosomatotroph adenoma causing gigantism in an 8-year old boy: a possible pathogenetic mechanism. *Clin Endocrinol (Oxf)* 42:539–549