In Situ Hybridization Analysis of Anterior Pituitary Hormone Gene Expression During Fetal Mouse Development

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We used 35S-labeled oligonucleotides and cRNAs (riboprobes) to detect the temporal order and spatial pattern of anterior pituitary hormone gene expression in (B6CBF1 × B6CBF2)F2 fetal mice from embryonic Day 9.5 (E9.5) to postnatal Day 1 (P1). Pro-opiomelanocortin (POMC) mRNA was expressed in the basal diencephalon on Day E10.5, in the ventromedial zone of the pars distalis on Day E12.5, and in the pars intermedia on Day E14.5. The common α-glycoprotein subunit (α-GSU) mRNA first appeared in the anterior wall of Rathke's pouch on Day E11.5 and extended to the pars tuberalis and ventromedial zone of the pars distalis on Day E12.5. Thyroid-stimulating hormone-β (TSHβ) subunit mRNA was expressed initially in both the pars tuberalis and ventromedial pars distalis on Day E14.5, with an identical spatial distribution to α-GSU at the time. In contrast, luteinizing hormone-β (LHB) subunit and follicle-stimulating hormone β (FSHβ) subunit mRNAs were detected initially only in the ventromedial pars distalis on Days E16.5 and E17.5, respectively, in an identical distribution to each other. POMC-, α-GSU-, TSHβ-, LHB-, and FSHβ-positive cells within the pars distalis all increased in number and autoradiographic signal with differing degrees of spatial expansion posteriorly, laterally, and dorsally up to Day P1. POMC expression was typically the most intense and extended circumferentially to include the entire lateral and dorsal surfaces of the pars distalis. The expression of both growth hormone (GH) and prolactin (PRL) started coincidentally on Day E15.5. However PRL cells localized in the ventromedial pars distalis area similarly to POMC and the glycoprotein hormone subunits, whereas GH cells were found initially in a more lateral and central distribution within the lobes of the pars distalis. Somatotrophs increased dramatically in number and autoradiographic signal, extending throughout the pars distalis except for the most peripheral layer of cells on Day E17.5. Mammatrophs also increased in number but less abundantly than somatotrophs, and PRL expression remained more confined to central-medial and ventrolateral areas of the pars distalis up to Day P1. These data demonstrate distinctive patterns of expression for each of the major anterior pituitary hormone genes during development of the mouse pituitary gland and suggest that different groups of committed cells are the immediate precursors to the terminally differentiated hormone-secreting cell types. (J Histochem Cytochem 42:1117–1125, 1994)

KEY WORDS: In situ hybridization; Pituitary gland; Ontogeny; Mouse; Glycoprotein hormone α-subunit; Luteinizing hormone; Follicle-stimulating hormone; Pro-opiomelanocortin; Thyroid-stimulating hormone; Growth hormone; Prolactin.

Introduction

The anatomical development of the murine adenohypophysis has been described in detail (1). Briefly, an invagination of the stomodeal chyme and diencephalon. Cell proliferation in a particular spatial and temporal sequence establishes the three parts of the adenohypophysis: the pars tuberalis, pars distalis (anterior lobe), and pars intermedia (intermediate lobe) (4). Mitotic activity is highest in

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the densely packed epithelial cells of the dorsal region of Rathke’s pouch in juxtaposition to the neurohypophysis and diencephalon and is lowest in the anteroventral quadrant of Rathke’s pouch and the ventral areas of the pars distalis and the emerging pars tuberalis (4).

Many immunohistochemical studies have demonstrated a sequential pattern of cytodifferentiation during pituitary embryogenesis in different species (5). With the exception of rat (6), the pattern of expression of the pituitary cell-specific hormones at the mRNA level and its correlation with the immunohistochemical data have not been characterized completely. The mouse has become an increasingly important model for molecular analysis of mammalian pituitary development and differentiation owing to the existence of spontaneous mutant strains with altered pituitary development (7–9) and the recent technical advances in mouse molecular genetics, including nuclear microinjection and homologous recombination in embryonic stem cells. The latter techniques have produced transgenic mouse strains with pituitary-specific expression of reporter genes (10–14) or specifically engineered changes in the expression of hormones, growth factors, and the cascade of signaling molecules that control them (15–21). Therefore, the aim of this study was to obtain a detailed description of the temporal and spatial expression pattern of all the anterior pituitary hormones in fetal mouse to provide a solid basis for further ontogenic analysis.

Radiolabeled nucleic acid probes provide a sensitive and specific method for in situ detection of RNA transcripts in fetal tissues and obviate the conflicting results created in the last decades by some immunolocalization reports (22–24).

### Materials and Methods

**Tissue Samples.** (C57B116 X CBA)F1 male and female mice (Bantam and Kingman; Gilroy, CA) were housed in microisolator cages on a 0500–1900 hr light cycle. The presence of a vaginal plug on the morning after mating indicated embryonic Day 0.5 (at noon) (EO.5). Timed pregnant female mice were killed by cervical dislocation at 1-day intervals starting at Day E9.5 and the F2 embryos were removed, analyzed macroscopically for accurate staging according to Rugh (2), and then frozen embedded in OCT and stored at −70°C. Serial 10-μm cryostat sections were taken from fetal heads in both the sagittal and coronal axes and were mounted on Vectabond (Vector; Burlingame, CA) coated slides. Immediately before use, sections were fixed either in buffered 10% formalin (Fisher Scientific; Pittsburgh, PA) or in 4% paraformaldehyde in PBS, pH 7.4, for 30–60 min at room temperature (RT). Multiple sections at 70-μm intervals from two or more fetuses from two independent series of timed pregnancies at each age were hybridized to each of the seven probes. Studies were conducted in accordance with the Public Health Service Guide for the Care and Use of Laboratory Animals.

#### Probe Labeling.** Synthetic 30 BP anti-sense oligonucleotides homologous to mouse cDNA sequences were used to detect the more abundant pituitary hormone mRNAs. 35S-labeled oligonucleotide probes were obtained using 5 pmol of each 30 BP oligonucleotide, 50 pmol of α-[35S]-thio deoxiATP (1300 Ci/mmol) (New England Nuclear; Boston, MA) and 20 U of terminal deoxynucleotidyl transferase (Gibco BRL.; Gaithersburg, MD). After a 15-min incubation, the tailing reactions were stopped by phenol–chloroform extraction and ethanol precipitation and the probes used without further purification. The sequences of the oligonucleotides were: 5’ GCC TGA GCG ACT GGA GAA TCT CGG CAT 3’ for pro-opiomelanocortin (mPOMC) (25), 5’ GAA TAG GCC TGT CCC TCG GGA ATG TCA 3’ for growth hormone (mGH) (26), 5’ GAC CAT AAA CTC AGC GTC TTC ACC ATA CTG 3’ for prolactin (mPRL) (26), 5’ TGC CCT GTA GGA GAA GCA ACA GCC CAT ACT CTG 3’ for α-glycoprotein subunit (mGSU) (27), and 5’ ACA AAA GCA TGC TGC TTG CCC ACA AGC AAG 3’ for thyroid-stimulating hormone β subunit (mTSHβ) (28). Each of the chosen sequences was compared among themselves and with the GeneBank database to ensure that there was no significant homology to any other known genes. All the oligonucleotide probes specifically labeled cell populations in adult mouse pituitary glands with the expected distribution and number based on our previous studies of mouse pituitary by immunohistochemistry (13) and had no nonspecific hybridization to other tissues. In addition, each of the oligonucleotide probes hybridized to a unique, specific band on Northern blots identical to the reported sizes and then frozen embedded in OCT and stored at −70°C. Serial 10-μm cryostat sections were taken from fetal heads in both the sagittal and coronal axes and were mounted on Vectabond (Vector; Burlingame, CA) coated slides. Immediately before use, sections were fixed either in buffered 10% formalin (Fisher Scientific; Pittsburgh, PA) or in 4% paraformaldehyde in PBS, pH 7.4, for 30–60 min at room temperature (RT). Multiple sections at 70-μm intervals from two or more fetuses from two independent series of timed pregnancies at each age were hybridized to each of the seven probes. Studies were conducted in accordance with the Public Health Service Guide for the Care and Use of Laboratory Animals.

#### Table 1. Expression of anterior pituitary hormone genes during fetal mouse development

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<th>mRNA</th>
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<td>α-GSU</td>
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*a mRNA for each hormone subunit was detected by in situ hybridization using oligonucleotide probes for α-glycoprotein hormone subunit (α-GSU), pro-opiomelanocortin (POMC), thyroid-stimulating hormone-β subunit (TSHβ), growth hormone (GH), and prolactin (PRL), and riboprobes for lutetinizing hormone-β subunit (LHB) and follicle-stimulating hormone-β subunit (FSHβ). AL, anterior lobe; IL, intermediate lobe.

*b The intensity of the hybridization signal and number of cells labeled were estimated qualitatively and indicated as follows: − , no specific hybridization signal; + , signal above background in a small number of cells; + + , moderate signal in an increased number of cells; ++ ++ , strong signal in an increased number of cells; + + + + + , signal similar to adult pituitary.
Figure 1. Brightfield photomicrographs of POMC mRNA in cryostat sections of mouse fetal pituitary detected by in situ hybridization with a 35S-labeled oligonucleotide probe at Days (a) E10.5, (b) E11.5, (c,d) E12.5, (e) E13.5, (f) E14.5, (g) E15.5, and (h) E17.5. Sections a-c are sagittal and sections d-h are coronal. Anterior is to the left on the sagittal sections. Arrows in c and d point to individual corticotrophs on the ventral surface of the anterior lobe. Arrow in f points to melanotrophs in the intermediate lobe. AL, anterior lobe; BD, basal diencephalon; IL, intermediate lobe; NL, neural lobe; RP, Rathke's pouch; SC, sphenoid cartilage; III, third ventricle; IV, fourth ventricle. Bars = 300 μm.

macia, Piscataway, NJ). The specificity of these probes was confirmed by Northern blot hybridization analysis of mouse pituitary RNA extracted from intact and gonadectomized mice. The FSHP probe hybridizes to a single 1700 bp mRNA and the LHP probe hybridizes to a 700 bp mRNA.

In Situ Hybridization with Oligonucleotide Probes. After fixation, sections were washed in PBS (10 min at room temperature (RT), three changes), followed by 2 × SSC (10 min, RT, three changes) (1 × SSC is 0.15 M NaCl, 0.015 M Na citrate, pH 7.2). Slides were then drained and incubated under parafilm coverslips with 40 μl of a pre-hybridization solution containing 50% formamide, 4 × SSC, 1 × Denhardt's solution, 250 mg/ml yeast tRNA, 500 mg/ml denatured salmon sperm DNA, 10 mM dithiothreitol (DTT), and 10% dextran sulfate at 37°C for 1 hr in a humid chamber. Slides were drained again and incubated with 40 μl of pre-hybridization solution plus 20,000 dpm/ml of probe for 14 hr at 37°C. Post-hybridization washes were as follows: 1 × SSC, 1 mM DTT (15 min, RT, four changes), 2 × SSC, 50% formamide (15 min, 37°C, four changes), and 1 × SSC (30 min, RT, three changes). After a quick rinse in deionized water, slides were dehydrated in ethanol (70 and 95%), dried at RT, dipped in Kodak NTB3 emulsion, and exposed for 10-14 days. After developing, slides were lightly counterstained with hematoxylin, dehydrated, and mounted in DPX.

In Situ Hybridization with Riboprobes. Fixed sections were washed in PBS (5 min, RT, twice), treated with 0.0025% acetic anhydride in 0.1 M
triethanolamine at pH 8.0 (10 min, RT), rinsed briefly in 2 × SSC, and quickly dehydrated in ascending ethanols. Sections were drained, desiccated under vacuum, and incubated under coverslips with a hybridization solution consisting of 50% formamide, 0.3 M NaCl, 1 × Denhardt’s solution, 20 mM Tris, pH 8.0, 1 mM EDTA, pH 8.0, 10% dextran sulfate, 500 mg/ml tRNA, 2 mM DTT, and 20,000 dpm/ml of probe. Coverslips were sealed with DPX and slides placed in an incubator at 55-60°C for 20-24 hr. After incubation, DPX was removed and slides immersed in 4 × SSC to allow coverslips to slide off. Sections were rinsed in four changes of 4 × SSC (5 min, RT) and treated with 20 mg/ml RNAse A for 30 min at 37°C. Finally, slides were washed in 2 × SSC, 1 mM DTT (5 min, RT, twice), 1 × SSC, 1 mM DTT (10 min, RT), 0.5 × SSC, 1 mM DTT (10 min, RT), and 0.1 × SSC, 1 mM DTT (30 min, 65°C), dehydrated in ethanol (50, 75, 95, and 100% containing 0.1 × SSC and 1 mM DTT) and exposed for autoradiography as described for the oligonucleotide probes.

Results

A summary of the results is shown in Table 1. The onset of mRNA expression and relative strength of the hybridization signals are indicated. The spatial pattern of each pituitary hormone mRNA is described below.

Corticotrophs and Melanotrophs

Cells expressing the POMC gene were first seen in the basal diencephalon on Day E10.5 (Figure 1a). By Day E11.5 Rathke's pouch was narrowed ventrally and POMC expression was still confined to a discrete population of cells in the adjacent diencephalon (Figure 1b). In the pituitary gland, scattered cells were observed on E12.5 in the ventromedial zone of the anterior lobe but not in the pars tuberalis (Figures 1c and 1d). Increasing numbers of POMC-expressing cells were gradually identified posteriorly and laterally in the ventral area of the anterior lobe on Day E13.5. On Day E14.5,
Figure 4. Brightfield photomicrographs of (a,b,d,f) LHβ-subunit and (c,e,g) FSHβ-subunit mRNA in cryostat sections of mouse fetal pituitary detected by in situ hybridization with a 35S-labeled riboprobe at Days (a) E16.5, (b,c) E17.5, (d,e) E18.5, and (f,g) P1. Sections a-f, and g are sagittal and sections d and e are coronal. Anterior is to the left on the sagittal sections. Arrows in a and c point to faintly hybridizing gonadotrophs on the earliest days of LHβ and FSHβ gene expression, respectively. AL, anterior lobe; IL, intermediate lobe; NL, neural lobe; PT, pars tuberalis; SC, sphenoid cartilage. Bars = 300 μm.

POMC-expressing cells first appeared in the intermediate lobe and extended circumferentially along the entire lateral borders of the anterior lobe in a pattern identical to that produced in an adrenalectomized adult mouse (12) (Figure 1). The pattern of expression at Days E15.5 and E17.5 (Figures 1g and 1h) was similar to that at birth, with gradually increasing expression in melanotrophs of the intermediate lobe. A thin rim of anterior lobe cells immediately ventral to the intermediate lobe was always devoid of POMC expression, and this zone is maintained into adulthood.

Thyrotrophs and Gonadotrophs

Glycoprotein hormones expressed by these cell types (TSH, LH, and FSH) are heterodimers consisting of a common α-subunit and a hormone-specific β-subunit. The α-GSU was first detected on Day E11.5 in a cluster of cells present in the anteroventral aspect of Rathke's pouch (Figure 2a). One day later the level of expression increased and remained confined to the pars tuberalis, the rostral extension of the anterior lobe (Figure 2b). The localization of α-GSU
Figure 5. Brightfield photomicrographs of (a–c) prolactin and (d–f) growth hormone mRNA in cryostat sections of mouse fetal pituitary detected by in situ hybridization with 35S-labeled oligonucleotide probes at Days (a,d) E15.5, (b,e) E16.5, and (c,f) E17.5. Sections a–f are coronal. Arrows in a and d point to faintly hybridizing lactotrophs and somatotrophs on the earliest days of prolactin and GH gene expression, respectively. AL, anterior lobe; IL, intermediate lobe; NL, neural lobe; SC, sphenoid cartilage. Bars = 300 μm.

on Day E12.5 was clearly different from the initial pituitary expression of POMC demonstrated in a serial sagittal section (Figure 1c). Although many of the α-GSU-expressing cells remained in the pars tuberalis, a dorsal and lateral gradient of α-GSU expression in cells of the anterior lobe was seen with increasing age (Figures 2c–2e).

The earliest expression of TSHβ subunit was observed on Day E14.5 in the pars tuberalis and in a few cells of the basal margin of the anterior lobe (Figure 3a). Thyrotrophs increased in number throughout the anterior lobe, retaining the dual sites of expression in the pars tuberalis and the anterior lobe as shown on Day E17.5 (Figure 3b) up to birth. As expected, thyrotrophs were the least abundant cell type at birth.

LHβ and FSHβ expression occurred at Days E16.5 and E17.5, respectively, in the anteroventral area of the pars distalis (Figures 4a and 4c). No gonadotrophs were detected in the pars tuberalis at any time. A steady increase in LHβ-expressing cells was noted up to Day P1, extending posteriorly and laterally (Figures 4b, 4d, and 4f). FSHβ was expressed at lower levels than LHβ at all time points, with no remarkable increase until P1 (Figures 4c, 4e, and 4g). The localization of the two gonadotropin-β subunits was identical in serial sections at all time points, suggesting that they were expressed in common bihormonal cells as seen in the adult mouse (13,31). However, our study does not exclude the possibility that monohormonal gonadotrophs also exist in the developing mouse pituitary gland. Male and female fetuses were not studied separately in this series.

Somatotrophs and Mammatrophs

The expression of both GH and PRL started coincidentally at Day E15.5 but whereas PRL-expressing cells localized in the ventromedial area of the pars distalis (Figure 5a), clusters of GH-expressing cells were found more laterally in adjacent serial sections (Figure 5d). The number of somatotrophs increased dramatically, extending to central and lateral areas of the anterior lobe and sparing only the most peripheral margin and giving a very intense autoradiographic signal on Day E17.5 (Figures 5e and 5f). Mammatrophs showed a parallel but smaller increase in cell number and silver grain density per cell by Day E17.5 and remained localized to a more restricted medial ventral zone and a strip of cells immediately opposed to the ventral surface of the intermediate lobe (Figures 5b and 5c).
Discussion

The five distinct cell types of the adenohypophysis show a characteristic ontogenetic pattern of hormone expression following defined spatial and temporal sequences in different species. In the present study these parameters for the expression of the anterior pituitary hormone genes are described in the fetal mouse.

A central question raised by the study of fetal pituitary gland differentiation is whether a pool of common pluripotential stem cells differentiates directly to each of the mature phenotypes or indirectly through a series of programmed commitment steps. The expression of the α-GSU in rat, detected as early as Day E11 in the hypophyseal placode before the formation of Rathke's pouch, represents one of the first molecular markers known in the anterior pituitary (6). The facts that even at these earliest stages α-GSU expression follows an anteroposterior gradient and that the proliferative activity varies in different areas of Rathke's pouch (4) indicate that cells in the immediate pituitary precursor, or even earlier, are already a heterogeneous population with committed fates. The restricted localization of the different cell types in the developing gland also suggests that distinct clonal populations exist in the committed Rathke's pouch which will proliferate in response to certain factors. In mouse we have not detected α-GSU transcripts before the formation of a constricted Rathke's pouch on Day E11.5, comparable to E13 in rat, but later its distribution parallels that described in rat (6). This discrepancy could be due to the use of an oligonucleotide probe in this work and a riboprobe in the previous study, a true interspecies difference, or a very rapid induction and termination of transcription of mouse α-GSU in the placode and posterior wall of Rathke's pouch that was not detected in our series. In our study the first terminally differentiated pituitary cell type is the corticotroph. As described in a previous report in mouse (32), the POMC gene is activated on E12.5 in the anterior lobe corticotrophs present in the anterior margins of the pars distalis, in the area most closely related to the basal diencephalon where POMC transcripts are detected 2 days earlier. Similarly, immunoreactive corticotrophs are the first and most abundant cell type to differentiate in primordial organ cultures, suggesting the capacity of the fetal pituitary for self-differentiation (33). Some POMC peptides are known to be mitogenic and to play important roles during neurogenesis (34,35). As the first classical pituitary hormones produced in the fetal pituitary gland, before the establishment of a definitive vascular network, POMC peptides should be considered as a possible source of autocrine or paracrine signals. Conversely, coculture experiments have also suggested that some stimulus from Rathke's pouch is necessary for normal development of POMC-expressing cells in the diencephalon (36).

The thyrotrphs identified by TSHβ subunit gene expression, second in temporal sequence, are found on Day E14.5 in a distribution similar to that of α-GSU. A previous immunohistochemical report described the earliest appearance of thyrotrphs later in development on Day E16.5 in mouse (37). In contrast to another immunohistochemical study in rat (24), our data using highly hormone-specific radiolabeled probes indicate that the pars tuberalis is specialized to express only α-GSU and TSHβ during mouse pituitary development. Studies in rat using in situ hybridization also support the assertion that the pars tuberalis is associated only with the expression of α-GSU, TSHβ, and a transcription factor termed thyrotrph embryonic factor (TEF) that is presumptively involved in regulating TSHβ expression in development (6,38).

In a detailed in situ hybridization study, Dollfø et al. (39) reported the expression of GH mRNA on Day E15.5 in mouse coincidently with detectable protein levels of the Pit-1 homeodomain transcription factor in the same cells. In a previous report, Slabaugh et al. (40) detected GH also at Day E16 by using two-dimensional PAGE of mouse pituitary protein extracts. PRL mRNA expression was detectable on E16 and hormone expression on P8, respectively, in the mentioned reports. Our results are in agreement with those reported by Dollfø et al. (39), although we did not observe the reported decrease in PRL mRNA levels on E17. Transgenic pituitary cell ablation studies have suggested the existence of a population of common somatomammotrophs that then differentiate into either GH or PRL cells (41,42). These two studies differ in their conclusions concerning a second lineage of PRL cells that arise independently of GH expression, however. We have noted the existence of two apparently distinct populations of somatotrophs and mammotrophs in different locations at their earliest stages, based on our observations in serial pituitary sections, consistent with the multiple lineage hypothesis. Dollfø et al. (39) also described a distinct GHF-1-negative PRL cell population in the pituitaries of dwarf mice transgenic for a GH–dipheria toxin fusion gene.

The gonadotrophs are the last cell type to differentiate, with LHβ and FSHβ subunit expression detected at Days E16.5 and E17.5, respectively. Previously, the earliest localization of LHβ using immunohistochemistry was reported on E17.5, with no sex differences until postnatal stages in the mouse (37). No previous studies of fetal mouse FSHβ expression have been published. However, an electron microscopic study of mouse pituitary reported the existence of LH and FSH gonadotrophs at Day E17 based on morphological criteria (43). As in adult mouse (44) (Kumar RT and Low MJ, unpublished data), LHβ was expressed more abundantly than FSHβ at all fetal ages and the distribution of the two β-subunits overlapped, consistent with the presence of a high proportion of dual hormonal gonadotrophs. The similar timing and pattern of expression of LHβ and FSHβ suggest that a common regulatory factor or signal may be responsible for initiating transcription of these two genes in development.

The existence of restricted gene expression patterns throughout pituitary embryogenesis and the partial capacity for self-differentiation in explanted organ cultures (33) suggest that anterior pituitary cell types are likely to influence each other by cell-to-cell contact and/or by paracrine signals during the processes of proliferation and differentiation. An example of possible interactions is the reported effect of α-GSU on the proliferation of PRL cells in organ cultures (45). A second example is the production by gonadotrophs of basic fibroblast growth factor, which has both autocrine and paracrine mitogenic effects (46). Differentiation of pituitary cells is unlikely to be influenced by distantly produced growth factors because a functional vascular supply consisting of portal vessels, arterioles, and venous drainage is not established until E17 in mouse (43), later than the onset of gene transcription for all the pituitary hormones except, possibly, FSHβ.

Genetic approaches provide a means of unraveling the nature and regulation of these developmental interactions. The analysis of dwarf mice lacking GH, PRL, and TSH production due to natural mutations of the Pit-1 gene established the basis for the study.
of regulatory transcriptional events (7,8). Further evidence from transgenic mice expressing a fusion gene consisting of distal 5' flanking sequences from the Pit-1 gene ligated to Simian virus 40 large T-antigen-coding sequences suggests that transcriptional regulation of Pit-1 in pituitary ontogenesis is mediated by distinct, temporally ordered signaling events (47). In rat, the timing and pattern of α-GSU expression in the development of the pituitary primordium led to the viewpoint that terminally differentiated cells in the anterior pituitary might be derived from common α-GSU-expressing progenitor cells. However, an α-GSU–diphtheria toxin transgene ablated all gonadotrophs and reduced the number of mammotrophs, leaving the other cell types, including thyrotrophs, unaffected (48). Similarly, α-GSU–SV40 T-antigen transgenic mice develop pituitary tumors of gonadotroph but not thyrotroph origin (49). Therefore, different cis-acting elements and/or transcription factors seem to be required for transcription in these primordial α-GSU cells, compared with the later developing thyrotrophs and gonadotrophs that express α-GSU together with a β-subunit (50).

In summary, the mammalian pituitary gland is a valuable model for studying the processes of development and terminal differentiation from a primordial ectodermal placode. Our data demonstrate the temporal sequence and topography of gene expression for each of the major hormone products of mouse pituitary and will be useful for future investigations of the molecular mechanisms that control pituitary cell ontology.

Acknowledgments

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