Embryonic Expression of the Luteinizing Hormone β Gene Appears to Be Coupled to the Transient Appearance of p8, a High Mobility Group-related Transcription Factor*

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The abbreviations used are: LH, luteinizing hormone; αGSU, α glycoprotein hormone subunit; β, embryonic day; NF-Y, nuclear factor-Y; PIPES, piperazine-N,N’-bis(2-ethanesulfonic acid); PBS, phosphate-buffered saline; CHAPS, 3-[[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; HMG, high mobility group; HMGA, high mobility group A; GnRH, gonadotropin-releasing hormone.

A comparison between two pituitary-derived cell lines (αT3-1 and LβT2) that represent gonadotropes at early and late stages of development, respectively, was performed to further elucidate the genomic repertoire required for gonadotropin specification and luteinizing hormone β (LHβ) gene expression. One isolated clone that displayed higher expression levels in LβT2 cells encodes p8, a high mobility group-like protein with mitogenic potential that is up-regulated in response to proapoptotic stimuli and in some developing tissues. To test the functional significance of this factor in developing gonadotropes, a knockdown of p8 in LβT2 cells was generated. The loss of p8 mRNA correlated with loss of endogenous LHβ mRNA and the loss of activity of a transfected LHβ promoter-driven reporter, even upon treatment with gonadotropin-releasing hormone. In addition, expression of p8 mRNA in developing mouse pituitary glands mirrored its expression in the gonadotrope-derived cell lines and coincided with the first detectable appearance of LHβ mRNA. In contrast, p8 mRNA was undetectable in the pituitary glands of normal adults. Taken together, our data indicate that p8 is a stage-specific component of the gonadotrope transcriptome that may play a functional role in the initiation of LHβ gene expression during embryonic cellular differentiation.

With its ability to stimulate gonadal steroidogenesis and gametogenesis, luteinizing hormone (LH) is essential for normal reproductive function in mammals (1, 2). Luteinizing hormone is a heterodimeric protein composed of an α glycoprotein hormone subunit (αGSU) common to all members of the glycoprotein family of hormones that is non-covalently linked to a unique LHβ subunit that confers its biological specificity (1, 2). Biosynthesis of LH depends on the coordinated expression of both the αGSU and LHβ subunit genes. In humans, the single copy αGSU gene resides on chromosome 6q12-q21 (Locus ID 1081) while the LHβ gene resides amid a cluster of six choric gonadotropin-β genes on chromosome 19q13.32 (Locus ID 1082). In addition to their different locations within the human genome, the pattern of expression of the genes encoding αGSU and LHβ are temporally and spatially distinct.

During development αGSU is seen throughout Rathke’s pouch as early as embryonic day (e) 9.5 in the mouse (3). By later stages of pituitary development and in adult mammals, αGSU expression is limited to thyrotropes and gonadotropes (4). Gonadotrope-specific expression of the αGSU gene is controlled by an array of regulatory elements, including the pituitary glycoprotein hormone basal element (5, 6), α basal elements (6), gonadotrope-specific element (7), and tandemly repeated cAMP response elements (8–10), as well as the intricate interplay between their cognate binding proteins (6).

In a fashion similar to the αGSU promoter, the LHβ gene is regulated by a combinatorial array of transcription factors and regulatory elements. In this case, however, the elements consist of those that bind early growth response 1 (Egr1) (11–16), steroidogenic factor 1 (SF-1) (11–20), Pitx1 (21–23), and an as yet unidentified Otx-related homeodomain protein (24), as well as proteins that bind elements in the distal domain, such as nuclear factor-Y (NF-Y) and Sp1 (16, 25–27). Unlike αGSU, which is a marker of early pituitary development, expression of the LHβ gene is one of the last steps that defines a mature gonadotrope.

As indicated above, many of the elements and proteins involved in regulation of LHβ gene expression and terminal differentiation of gonadotropes have been characterized; however, the full scope of factors required for differentiation to a mature cell that expresses all phenotypic markers of a gonadotrope, including LHβ, have yet to be identified. To that end, we compared the gene expression of cell lines that represent two distinct stages of gonadotrope development by differential display. αT3-1 cells represent early gonadotrope progenitors that express αGSU and gonadotropin-releasing hormone (GnRH) receptor but not the unique β-subunits of the glycoprotein hormones (28). The LβT2 cell line is characterized by expression of markers of fully differentiated gonadotropes, including the β-subunits of the gonadotropins (29, 30). Because these two cell lines represent phenotypic gonadotropes at embryonic days before (αT3-1) or after (LβT2) the ability to express LHβ, characterization of differentially expressed factors may uncover components that are essential for expression of the LHβ subunit gene and should refine our understanding of gonadotrope differentiation.

One factor that is more highly expressed in LβT2 cells than αT3-1 cells is the HMG-like nuclear phosphoprotein known as p8 or candidate of metastasis 1 (com1). The HMG class of proteins to which p8 is related (HMG-I/Y or HMG-A) function as architectural transcription factors that promote gene activa-
tion by relieving histone H1-mediated repression of transcription (31) and facilitating the formation of enhanceosomes as a consequence of both protein/DNA and protein/protein interactions (32). Like p8, these proteins, which have the capacity to bend, straighten, unwind, and induce loop or supercoil formation in linear DNA molecules in vitro, are at maximal levels of expression during embryonic development and in rapidly proliferating cells (32). While p8 lacks characteristic “A-T hook” DNA binding domains found in the INGA class of non-histone chromatin-binding proteins (32, 33), it does appear to bind DNA using a small guanine residue (34), and thus, p8 may perform a comparable role within cells. In the gonadotrope, p8 appears to be a stage-specific component of the cellular transcriptome that may play a functional role in the initiation of LHα gene expression. This potential is explored herein.

EXPERIMENTAL PROCEDURES

PCR Differential Display—Total RNA was extracted from confluent αT3-1 and LβT2 cells using the method of Chomczynski and Sacchi (35). Removal of chromosomal DNA contamination from samples, PCR differential display, and reamplification of DNA were performed as described in the Current Protocols in Molecular Biology (36). Each assay was performed using one of four degenerate anchored oligo(dT) primer sets (T7; M, N can be G, A, or C and K is G, A, T, and C) where each primer set is dictated by the 3’ base (N) with degeneracy in the penultimate (M) position by itself or each in combination with one of 26 decameric primers that were originally designed to allow for coverage of the expressed genome (37). Clones of interest were subcloned into the pCR®BLAST® vector (Invitrogen, Carlsbad, CA) using the standard protocol described by the manufacturer. The DNA sequence of each clone was found by dideoxynucleotide sequencing using Sp6 and/or T7 primers. Identification of clones was determined by comparing each sequence against the BLAST database (38).

Plasmid Vectors—All plasmid DNAs were prepared from overnight bacterial cultures using Qiagen DNA plasmid columns according to the protocol of the supplier (Qiagen, Chatsworth, CA). Murine p8-pcDNA3 was a gift from Juaniovana and colleagues (39). To produce the p8-antisense vector (p8AS-pcDNA3), p8-pcDNA3 was digested with BamHI and XhoI restriction endonucleases. The p8 insert was then blunt-ended and ligated into the EcoRI site of pcDNA3. Clones were dideoxynucleotide sequenced to verify the selection of a reverse orientation clone. The LβHα promoter-driven luciferase reporter vector (23) as well as the gSUSU promoter-driven luciferase reporter vector (6) have been described previously.

Cell Lines—The gonadotrope-derived cell lines, αT3-1 and LβT2, were maintained in high glucose Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and antibiotics (complete medium). To produce the control (C)-LβT2, p8-knockdown (KD)-LβT2, and p8-overexpressing (OE)-LβT2 cell lines, 24 × 10^4 LβT2 cells on a 100-mm culture dish were transfected with 16 µg of pcDNA3, 8 µg p8AS-pcDNA3, and 8 µg pcDNA3, respectively, using 80 µl of LipofectAMINE reagent (Invitrogen) and medium lacking serum and antibiotics. Stably transfected cells were selected by introducing 500 µg/ml G418 (Invitrogen) into the complete medium 3 days following the transfection. The cells utilized in these studies, which were maintained in the above-described complete medium supplemented with G418, represent pools of clones for each cell line derived from the parent LβT2 line.

Transfection Assays—The day prior to transient transfection, C-LβT2 and p8-KD-LβT2 cells were plated at a density of ~2 × 10^6 cells/35-mm well. Transfections were carried out using medium lacking serum, antibiotics, and G418 selection medium with 10 µl of LipofectAMINE reagent, 2 µg each test vector, and 100 ng of pR-LCMV (Promega, Madison, WI), which was used to normalize data for transfection efficiency. Cell cultures were incubated with the transfection mixtures for ~18 h at 37 °C in a humidified atmosphere with 5% CO_2. Complete medium was then added to the cells, which where indicated, were also supplemented with 100 µg/ml G418. Twenty-four hours following the addition of fresh medium and hormonal treatments, cells were lysed in passive lysis buffer (Promega) and a dual-luciferase assay was performed on the cytosolic lysate as per the standard protocol (Promega). Transient transfections were performed a minimum of three times with at least two separate plasmid preparations for each construct that was transfected.

Northern Blot Analyses—For each Northern blot, 10 or 20 µg of total RNA were separated by electrophoresis in a 1% denaturing agarose gel and transferred to nylon membrane (Hybond-N+; Amersham Biosciences) by gravity and capillary action. After UV cross-linking to fix RNA to the nylon, the membrane was prehybridized for approximately 3 h and hybridized with the appropriate radiolabeled probe overnight at 45 °C in a roller-bot hybridization oven (Techne, Inc., Princeton, NJ). The hybridization solution consisted of 40% deionized formamide, 20% formamide hybridization buffer (800 µm NaCl, 200 µm dTDA, 4% SDS, 50 µm salmon sperm DNA). All probes were made by radiolabeling of cDNAs with [32P]deoxy-CTP or ATP (3000 Ci/mmol, PerkinElmer Life Sciences) using DECA-prime II kit as per the instructions of the manufacturer (Ambion, Austin, TX). The final washes following hybridization were in 0.5 × SSC, 0.5% SDS at 65 °C. The membranes were exposed to Biomax MR film (Eastman Kodak Co., Rochester, NY) for one or more days at −70 °C. In some cases, additional washes were performed using 0.5% sodium dodecyl sulfate (SDS)/20 °C (Amberson Biosciences) for 2–18 h followed by densitometric scanning and analysis using a Storm 820 PhosphorImager (Amersham Biosciences). Between hybridizations, each blot was stripped of radioactivity using the protocol enclosed with the nylon membrane. Northern blots were replicated at least three times, and densitometric scanning was performed on representative blots. Random-prime labeled DNA probes used in the Northern blot analyses consisted of the full murine p8 cDNA (39), murine LβHα cDNA representing bases 55 through 213 (40), murine cytokeskeleton β-actin cDNA representing bases 1210 through 1657 (41), a murine GnRH receptor cDNA representing bases 247 through 537 (42), and a cDNA encompassing the murine 18 S rRNA contained within primers included in the QuantumRNA 18 S Internal Standards kit (Ambion). In all cases a very small amount of total RNA from the multiple cloning sites of each vector was included when making probes.

In Situ Hybridization—Timed pregnancies were obtained by mating CF1 or FVB/N male × CF1 female. Noon on the day of copulatory plug detection was considered e0.5. Appropriately aged embryos were frozen in 2-methylbutane at approximately −30 °C and sectioned at 15 µm on a Hacker-Bright cryostat (Hacker Instruments and Industries, Fairfield, NJ). In situ hybridization was performed as described by Cushman et al. (43). Briefly, sections were warmed to room temperature for 30 min and fixed in 4% paraformaldehyde/phosphate-buffered saline (PBS) at 37 °C. Following proteinase K treatment (0.1 µg/ml), sections were fixed again in 4% paraformaldehyde/PBS and washed in PBS. Sections were acetylated using a 0.1 M triethanolamine, 0.25% acetic acid mixture and incubated with hybridization solution minus the probe (50% formamide, 5× SSC, 2% Boehringer blocking powder, 0.1% Triton X-100, 0.5% CHAPS, 1 mg/ml yeast RNA, 5 µg EDTA, 50 µg/ml heparin in diethyl pyrocarbonate-treated water) at 55 °C. Riboprobe was diluted in hybridization solution and allowed to hybridize overnight at 55 °C in a chamber humidified with 5 × SSC. The next morning, sections were washed in a 50% formamide buffer at 55 °C followed by a wash in 0.5× SSC at room temperature and blocked with the following solution in a chamber humidified with water: 10% heat-inactivated sheep serum; 2% BSA; 0.02% sodium azide in 50 µM Tris-CI (pH 7.5), 100 µM NaCl, 0.1% Triton X-100. Anti-digoxigenin Fab fragments (Roche Molecular Biochemicals) were diluted 1:1000 in blocking buffer, incubated on sections for 1 h at room temperature in a humidified chamber and washed in PBS. After equilibration in several washes of chromagen buffer (100 µm Tris-CI (pH 9.5), 100 µM NaCl, 50 µM MgCl2), sections were developed overnight in the chromagen buffer with 4.5 µM 4-nitro blue tetrazolium chloride and 3.5 µM 5-bromo-4-chloro-3-indolyl phosphate added as substrate for alkaline phosphatase activity. Sections were washed with PBS, fixed in 4% paraformaldehyde/PBS, washed, and mounted. In situ hybridization was repeated at least two times for each embryonic age.

Probes Used for in Situ Hybridization—Plasmids were linearized as follows to generate sense and antisense probes: 435-bp gSUSU cDNA (GenBankTM accession number NM00989389; bp numbers 181 through 616 of gSUSU) in pHOGEMOE22 was linearized with BamHI for antisense (pHOGEMOE22) or LβHα cDNA (pHOGEMOE22 from amino acid numbers 5008497, bp numbers 12 through 2450) in BSK was linearized with HindIII for antisense and BamHI for sense probes; and full murine p8 cDNA in pcDNA3 was linearized with EcoRI for antisense and ApaI for sense probes. Digoxigenin-labeled riboprobe was generated with in vitro transcription using 10× DIG RNA labeling mix (using MOL4001 Molecular Biochemicals) and purified with the RNeasy minikit (Qiagen).

Experimental Animals—All animal studies were conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee of Case Western Reserve University.
RESULTS

p8 mRNA Is Up-regulated in Cells That Represent Mature Gonadotropes—To identify factors that may be essential for maturation of gonadotropes, differential display was employed to compare the mRNA populations from gonadotrope-derived cells that represent distinct developmental stages, namely αT3-1 and LβT2. Because over 300 differentially expressed cDNAs were isolated using this method, reverse Northern blots were performed to verify the expression seen by differential display (data not shown). After reamplification and nucleotide sequencing of several clones, we focused our attention on clone 100 (Fig. 1A), which was identified as the 3’-untranslated region of p8 using the BLAST database available through the National Center for Biotechnology Information (38). p8 shows both sequence and structural similarities with factors that have proven vital for embryonic development (34, 44). To confirm the differential expression of p8 in αT3-1 and LβT2 cells, we examined total RNA from each cell line by Northern blot analysis and found that mRNA encoding p8 was significantly up-regulated in LβT2 compared with αT3-1 cells (Fig. 1, B and C).

Silencing of Endogenous p8 in LβT2 Cells—To determine the functional significance of p8 mRNA, a plasmid vector driving expression of p8-antisense was used to stably transfect LβT2 cells (p8-KD-LβT2). We anticipated that this aberrant message would form double-stranded RNA with the endogenous p8 transcript and either inhibit its translation or target it for degradation, thus allowing for sequence-specific, post-transcriptional silencing of the p8 gene. Our data support the latter possibility as no detectable levels of p8 mRNA were observed in p8-KD-LβT2 cells (Fig. 2). In contrast, in LβT2 cells that were stably transfected with a plasmid encoding the sense strand of p8 (p8-OE-LβT2), a 579-nucleotide p8 mRNA representing the transcription product from the transfected vector was identified. Importantly, endogenous p8 transcript (639 nucleotides) was detected in total RNA from untransfected LβT2 cells as well as cells that have been stably transfected with the empty, negative control vector (C-LβT2). Thus, stable transfection of the p8 antisense vector effectively removed p8 mRNA from LβT2 cells.

It is known that double-stranded RNA in the cytoplasm of mammalian cells can trigger profound effects, including a global suppression of translation and mRNA degradation in a sequence-nonspecific manner as part of an interferon response (45), as well as apoptosis (46). To provide evidence that the loss of endogenous p8 was due to sequence-specific rather than nonspecific gene silencing, Northern blot hybridization with a radiolabeled β-actin probe was performed (Fig. 2). Levels of detectable β-actin mRNA were comparable across RNA samples from p8-KD-LβT2 cells, p8-OE-LβT2 cells, as well as C-LβT2 and normal, untransfected LβT2s, indicating that the product of this architectural gene, and likely other transcripts, was not affected by the presence of double-stranded RNA in the p8 knockdown cell line.

The LHβ Promoter Is Non-functional When p8 Is Knocked Down in LβT2 Cells—Expression of the p8 gene appears to follow a pattern that corresponds to LHβ expression in gonadotrope-derived cell lines. To determine the potential impact of p8 on regulation of the LHβ gene, p8-KD-LβT2 and C-LβT2 cells were tested for their ability to support activity of a LHβ promoter-driven luciferase reporter after transient transfection. As expected, the C-LβT2 cell line displayed levels of luciferase activity ~4-fold higher than that from cell lines transfected with a promoterless reporter vector (Fig. 3A), levels that are comparable with normal LβT2 cells (data not shown). In cells lacking p8 (p8-KD-LβT2), the LHβ promoter was non-functional, even in the presence of 100 nM GnRH (Fig. 3B). To confirm that the effect of p8 knockdown on the LHβ promoter was not due to an overall hindrance of cellular processes, an αGSU promoter-driven construct was also tested. This vector was shown to be functional in p8-KD-LβT2 cells as well as C-LβT2 cells. Thus, p8 appears to be crucial for expression of LHβ in LβT2 cells. However, overexpression of p8 does not allow for activation of LHβ-driven luciferase expression in heterologous cells (COS7 and αT3-1 cells) or enhanced activation in homologous cells (LβT2 cells) (data not shown). While there are many potential explanations for the lack of an increase in LHβ gene expression upon overexpression of p8, the simplest may be that endogenous p8 in LβT2 cells may already be exerting a maximal effect on the LHβ gene. In the heterologous cells it is possible that other factors vital to the impact of p8 on
LHβ promoter activity may be absent. In other words, p8 may be necessary but not sufficient for expression of the LHβ gene, and the mechanism by which p8 regulates LHβ gene expression may be very complex and/or quite indirect.

**LHβ mRNA Is Down-regulated in p8-KD-LβT2 Cells Even in the Presence of GnRH**—To determine the importance of p8 on activation of endogenous genes in gonadotrope-derived cells, we extended our investigation to include Northern blot analysis of the LHβ gene. As shown in Fig. 4, there is a lack of LHβ gene expression in cells that stably express the p8 antisense strand (p8-KD-LβT2). In contrast, low levels of LHβ gene expression were detected in normal, untreated LβT2 cells, C-LβT2 cells and p8-OE-LβT2 cells. To further characterize the impact of p8 removal on LHβ activity, the various LβT2 cell lines were treated with 100 nM GnRH for 6 h prior to harvest and collection of RNA for analysis by Northern blot. While this treatment paradigm increased LHβ gene expression in normal LβT2, C-LβT2, and p8-OE-LβT2 cells, it could not rescue expression of LHβ in the p8-KD-LβT2 cells. This provides further functional evidence for the importance of the p8 transcription factor in LHβ gene expression in LβT2 cells.

Unlike LHβ, the GnRH receptor gene is expressed in the LβT2 cells lacking p8 (Fig. 4). In the absence of GnRH stimulation, this level of gene expression is higher in p8-KD-LβT2s than p8-OE-LβT2, C-LβT2, or normal LβT2 cells. However, upon treatment of each cell line with 100 nM GnRH for 6 h, GnRH receptor gene expression is lowest in the p8-KD-LβT2 cells. While a modest increase in expression was observed in the other cell lines, the GnRH receptor gene does not appear to be GnRH responsive in p8-KD-LβT2 cells. In addition, other genes (αGSU, β-actin, and Egr-1) that are GnRH-responsive in gonadotropes, normal LβT2 cells, and C-LβT2 cells do not show an increase in gene expression upon GnRH stimulation in p8-KD-LβT2 cells (data not shown), opening the intriguing possibility that p8 impacts the GnRH signaling cascade in LβT2 cells.

**Temporal Expression of p8 Gene in Vivo Mirrors That of the Gonadotrope-derived Cells**—Expression of the p8 gene is very low in gonadotrope-derived precursor cells (αT3-1), while higher levels of expression are seen in cells that represent a later stage of gonadotrope differentiation (LβT2). To determine whether a similar pattern of temporal expression of p8 mRNA occurs in vivo, in situ hybridization was performed in murine tissues at distinct developmental time points (Fig. 5). Whereas p8 was found to be undetectable in the developing pituitary tissues at distinct developmental time points (Fig. 5). Whereas p8 was found to be undetectable in the developing pituitary...
the gonadotrope-derived cells. In situ
expression of the p8 gene in the developing pituitary gland is transient, as it was again undetectable in the pituitary glands of normal adult mice (bar = 300 μm).

As expected, αGSU mRNA was detectable throughout the pituitary gland at embryonic day 16.5 (prior to detection of LHβ), e17.5 (gonadotropes at this stage have a phenotype similar to that of LβT2 cells), and e18.5, indicating that expression of the p8 gene in the developing pituitary gland is stage-specific and transient, as it was again undetectable in the pituitary glands of normal adults. Thus, p8 follows a transient pattern of expression, similar to other factors vital to pituitary development (47, 48).

As expected, αGSU mRNA was detectable throughout the pituitary gland at each embryonic day tested as well as in presumptive gonadotropes and thyrrotropes of the adult. In addition, while LHβ mRNA was observed in the pituitary glands of adult mice and at later stages of embryonic development (e17.5 and e18.5), we were unable to detect the transcript at earlier time points, such as e16.5. While others have been able to detect very low levels of LHβ gene expression in pituitary glands at e16.5 by use of 35S-labeled oligonucleotides (49), we were unable to do so using digoxigenin-labeled cRNA. However, we were able to detect p8 expression using the same type of probe at this developmental age. Thus, it appears that expression of the p8 gene precedes LHβ gene expression in the developing pituitary gland.

**DISCUSSION**

Like Egr1, SF-1, and Pitx1, which have all been described as playing a role in terminal differentiation of gonadotropes (11–14, 18–20), the p8 nuclear phosphoprotein may play a functional role in LHβ gene expression as well as a potential role in cellular differentiation. However, unlike Egr1, SF-1, and Pitx1, p8 expression in the developing pituitary gland is transient. High levels of expression can be detected during later organogenesis, while no p8 is detectable in the pituitary glands of normal adult mice. Thus, p8 may represent a true embryonic factor vital to gonadotrope development and LHβ gene expression.

Activation of LHβ gene expression likely involves transcription factors that bind directly to DNA regulatory elements as well as additional transcriptional regulators that influence the binding or activity of these proteins, some of which are also likely to affect the chromatin mechanics of the gene. Clearly, p8, a new member of the HMG family of chromatin-binding proteins, plays a role in expression of the LHβ gene as its absence in LβT2 cells corresponds to an absence of LHβ gene expression. What is not known, however, is how p8 functions to direct this expression.

In addition to its ability to relieve histone H1-mediated repression of transcription, the HMGA class of proteins are also known for their ability to specifically interact with numerous transcription factors to form stereospecific multiprotein enhanceosome complexes (50–52). This allows the architectural transcription factors considerable flexibility in regulating the expression of a large number of genes (33, 53). p8 has been shown to enhance the activity of other transcription factors (54) as well as interact directly with both factors and coactivators of transcription (55). Thus, p8 may function in a similar fashion to activate the LHβ promoter, forming an enhanceosome with factors known to be important for basal expression of LHβ. In fact, one of the numerous factors that HMGA has been shown to interact with is NF-Y (NF-YA subunit) (56), a factor that is known to be important for activation of LHβ (27) and FSHβ (57) gene expression. In this regard, determining the protein partners of p8 in LβT2 cells may provide insight toward its function in LHβ gene expression.

Alternatively, or perhaps concurrently, p8 may be playing a role in the remodeling of chromatin during development, moving the gene from transcriptionally inactive heterochromatic regions to euchromatin, allowing for the induction of LHβ gene expression. This may explain why p8 is not necessary for expression of the LHβ gene in adult mice and why overexpression of p8 in LβT2 cells does not enhance LHβ promoter activity or endogenous LHβ gene expression. In addition, with its mitogenic potential (44, 58) and possible anti-apoptotic role (59), p8 may be involved in gonadotrope cellular proliferation during development. Its absence of expression in the adult may be critical to maintaining/limiting the gonadotrope population within the anterior pituitary gland as p8 has been shown to be up-regulated in proliferating and tumorigenic tissues (44, 59–62). A similar temporal pattern of expression has been observed with other factors vital to developmental processes (59–62), including other members of the HMGA family (53).

Determination of pituitary cell types during organogenesis occurs in response to a series of extrinsic and intrinsic signaling molecules. In Rathke’s pouch, multiple molecular gradients help to establish distinct patterns of transcription factors that allow for commitment, positional determination, and differentiation of pituitary cell types. For example, Rosenfeld and colleagues have shown that a ventral to dorsal gradient of BMP2 expression in Rathke’s pouch induces a ventral to dorsal gradient of GATA2 expression (63, 64). The high levels of GATA2 in the most ventral aspect of the developing gland restrict Pit-1 gene expression in presumptive gonadotropes, allowing for induction of the transcription factors that are critical for differentiation of the cell type, including SF-1 (64). In fact, in vivo expression of a dominant-negative GATA2 inhibited terminal differentiation of gonadotropes while extended expression of GATA2 dorsally expanded the gonadotrope population. Perhaps like SF-1, expression of the p8 gene is regulated by GATA2. In this regard, several potential GATA-binding sites are located in the 5′ flanking region of the murine p8 gene (39).
that have yet to be characterized.

While p8 is important for LHβ gene expression in vitro, a definitive answer to the significance of p8 for gonadotrope maturation and initiation of LHβ gene expression in vivo will lie in studies involving temporally disregulated expression or targeted disruption of the p8 gene in mice. In regards to the latter, Iovanna and colleagues have recently developed knock-out mice to study the role of p8 on cell growth, apoptosis, and tumor development (65, 66). No abnormalities were found in organs of these mice by histological analysis, including liver, lung, intestine, pancreas, testis, brain, kidney, and heart (65). To this point, the p8 knock-out mice have yet to be assessed for defects in fertility or gonadotrope development. To determine whether p8 is a dominant member of a signal transduction pathway or serves as a component of a complex mechanism (perhaps even redundant or compensatory) for initiation of LHβ gene expression, a close examination of the pituitary glands, reproductive fitness, and reproductive system organ development in these mice will be necessary. In addition, the identification and characterization of p8-responsive genes may better elucidate the role of p8 in LHβ gene expression and the development of gonadotropes from committed precursors to differentiated cells.

Of the factors that appear to be essential for terminal differentiation of gonadotropes and expression of LHβ, p8 is unique among them because of the transient component to its expression. p8 gene expression is detectable at e16.5 in pituitary cells, one developmental day prior to detection of LHβ mRNA in our system. By its temporal pattern of expression, p8 appears to be a marker of differentiating gonadotropes that acquire the ability to express LHβ. However, p8 appears to be more than simply a marker of the developing gonadotrope as the knock-down of p8 in LfT2 cells corresponds with an inability of these cells to express the LHβ gene. Thus, our data indicate that p8 is a stage-specific component of the gonadotrope transcriptome that may play a functional role in LHβ gene expression during embryonic cellular differentiation.

Acknowledgments—We thank past and present members of the Nilson Laboratory for invaluable assistance, especially Danielle Grove-Strawser, David Peck, Kristina Treanor, and Allison Atwood. We are also grateful to Dr. Juan Iovanna and members of his laboratory for the p8-pcDNA3 plasmid. Drs. Sally Camper and Hoonkyo Suh, as well as the latter, Iovanna and colleagues have recently developed knock-out mice to study the role of p8 on cell growth, apoptosis, and tumor development (65, 66). No abnormalities were found in organs of these mice by histological analysis, including liver, lung, intestine, pancreas, testis, brain, kidney, and heart (65). To this point, the p8 knock-out mice have yet to be assessed for defects in fertility or gonadotrope development. To determine whether p8 is a dominant member of a signal transduction pathway or serves as a component of a complex mechanism (perhaps even redundant or compensatory) for initiation of LHβ gene expression, a close examination of the pituitary glands, reproductive fitness, and reproductive system organ development in these mice will be necessary. In addition, the identification and characterization of p8-responsive genes may better elucidate the role of p8 in LHβ gene expression and the development of gonadotropes from committed precursors to differentiated cells.

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