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Expression and Regulation of the Pituitary- and Placenta-Specific Human Glycoprotein Hormone Alpha-Subunit Gene Is Restricted to the Pituitary in Transgenic Mice

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Expression of the glycoprotein hormone alpha subunit occurs in both the pituitary and placenta in humans. However, this study found that expression of this subunit is restricted to the pituitary in mice. An interspecies analysis of human alpha-subunit gene regulation was undertaken, using the transgenic-mouse approach. In mice transgenic for a genomic clone containing the complete human alpha-subunit gene and several kilobases of 5′- and 3′-flanking sequences, cell-type-specific expression and hormonal regulation of the human alpha-subunit transgene occurred in the mouse pituitary, whereas no expression of the transgene was detectable in the mouse placenta. These findings provide strong evidence that a common *trans*-acting factor (s) regulates glycoprotein hormone alpha-subunit gene expression in the human and mouse pituitaries; however, this factor(s) or a unique factor(s), though functional in the human placenta, is either nonfunctional or absent in the mouse placenta.

The human glycoprotein hormone alpha subunit is encoded by a single-copy gene (19) which has been mapped to chromosome 6 (37). The subunit encoded by this gene is shared by each member of the glycoprotein hormone family, which includes human chorionic gonadotropin (HCG), luteinizing hormone (LH), follice-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH). HCG, produced by placental trophoblast cells and LH, FSH, and TSH, produced by the pituitary gonadotrope (LH and FSH) and thyrotrope (TSH) cells, each consist of a glycosylated alpha subunit noncovalently linked with a glycosylated beta subunit unique to each hormone.

LH, FSH, and TSH are produced in the pituitaries of all mammalian species (43). The synthesis and secretion of these hormones is under complex hormonal control. Production of LH and FSH is stimulated by hypothalamic gonadotropin-releasing hormone and suppressed by gonadal steroids. TSH production is stimulated by hypothalamic thyrotropin-releasing hormone and suppressed by thyroid hormones. Steroid regulation of LH and FSH has been demonstrated in castrated animals, which have significantly increased levels of these gonadotropins in their pituitaries and sera (1, 2, 21, 33, 40). Moreover, these levels can be suppressed by replacement steroid therapy (2, 34, 39, 49). Similar studies in hypothyroid animals have demonstrated the importance of thyroid hormone in suppressing TSH production (12, 20, 23, 50, 51).

Whereas the pituitary glycoprotein hormones have been conserved throughout mammalian evolution, the evolution of placental chorionic gonadotropin (CG) appears to have occurred only recently. CG has been definitively identified only in primates (6, 9, 43) and horses (55, 62) and is not found in rats (7, 58, 63), mice (this report; N. Fox, unpublished data), or cows (38, 61). In humans, the beta subunit of HCG is encoded by multiple genes (4, 44, 45, 56) located on chromosome 19 (28, 37). These genes are tandemly linked to the highly homologous single LH beta-subunit gene, which coevolved with the HCG beta genes from an ancestral LH betalike gene (57). Similar CG gene complexes have not been found in the CG-negative species that have been examined (7, 58, 61), and these species do not express the common alpha-subunit gene in their placentas (7, 10, 38, 63; this report; Fox, unpublished data).

Evolution of the CG-LH beta-gene complex has apparently given rise to different modes of CG and LH beta-gene regulation; CG beta is expressed exclusively in the placenta, whereas LH beta is expressed exclusively in the pituitary (27, 38). The mechanism controlling the differential expression of these genes, however, has not been elucidated. Also unresolved is how the alpha- and beta-subunit genes (located on separate chromosomes) are coordinately expressed and how the alpha-subunit gene is activated in the placentas of CG-producing species but remains inactive in this tissue in CG nonproducers. Sequences conferring tissue-specific expression of the human alpha-subunit gene in malignant trophoblast cells in vitro have been localized to the 5′-flanking region of this gene and include a direct repeat of an 18-base-pair cyclic-AMP-responsive element (13–15, 25, 26, 52). These sequences may therefore be involved in the tissue-specific or hormonal regulation of the alpha-subunit gene in vivo.

One means of studying the tissue and species specificity of gene expression is through the development of transgenic animals in which cloned genes from one species are introduced into the germ line of another (8, 22, 29, 53, 59). Thus, the human α-1 antitrypsin gene and the human Thy-1 gene in mice are each regulated according to their patterns of expression in humans, which differ from the patterns of endogenous α-1 antitrypsin and Thy-1 gene expression in mice (22, 29). Similarly, the pattern of rat α_{2u} globulin gene expression in transgenic mice recapitulates the pattern of expression seen in rats (53). In these models, differences in the expression of the exogenous transgenes and their endogenous mouse homologs have been attributed to the divergent evolution of their *cis*-acting regulatory sequences (22, 29, 53). Evolutionary changes in the expression of *trans*-acting

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regulatory factors may also occur and have been proposed to account for the expression of the fetal $^{3}\gamma$-globin gene in embryonic erythroid cells in transgenic mice (8).

In this study, we propose that the species-specific expression of the glycoprotein hormone alpha-subunit gene in humans and mice results from the divergent evolution of the *trans*-acting factors which regulate this gene. We found that expression of the human alpha-subunit gene in transgenic mice is restricted to the same tissue, i.e., the pituitary, in which the endogenous mouse gene is expressed. Transgene expression in the pituitary occurs within the appropriate cell types and is hormonally responsive.

MATERIALS AND METHODS

Transgenic mice. Mouse zygotes were harvested from the oviducts of C57BL/6 mice. DNA solution containing 10 μg of pGα per ml was introduced into the male pronucleus by using a finely pulled micropipette and a Lietz micromanipulator. Microinjection pipettes were prepared from Kwik-Fil capillary tubes (1B100F-4, WPI) on a micropipette puller (model 700C; David Kopf Instruments). The tips of finely pulled micropipettes were briefly dipped in 10% hydrofluoric acid, rinsed in methanol, and then dipped in siliconizing agent (Glass Treet; Alltech Associates). After being rinsed in methanol, the pipettes were heated to 80°C for 1 h and left at room temperature overnight or longer before use. Hydrofluoric acid treatment ensured a patent tip in the pipettes. Microinjected embryos were cultured overnight to the two-cell stage and transferred to the oviducts of pseudopregnant outbred CD-1 female mice on day 1 (day of the vaginal plug) of pseudopregnancy. This was accomplished by inserting a sharpened embryo transfer pipette through the wall of the oviduct between the infundibulum and the ampulla and expelling the embryos into the oviduct lumen. Mice born were tested for retention of the microinjected DNA by Southern blotting (54) of endonuclease-restricted tail DNA isolated essentially as described by Hogan et al. (24).

Dot blotting. To isolate tissue RNA, small pieces of tissue were dissected at autopsy and homogenized in buffer containing 100 mM sodium acetate, 1 mM EDTA, and 0.5% sodium dodecyl sulfate (pH 5.2), using a Brinkman polytron. Individual pituitaries were homogenized in a 0.5-ml Dounce tissue grinder. The homogenate was extracted three times with hot (60°C) phenol equilibrated with buffer containing 100 mM sodium acetate and 1 mM EDTA (pH 5.2) and then extracted two times with chloroform. RNA was precipitated by the addition of 1/10 volume of 3 M sodium acetate (pH 5.2) and 2 volumes of absolute ethanol and stored at −70°C until use.

For dot blotting, precipitated RNA samples were suspended in H_2O and 10 μl (0.1 to 10 μg, depending on the experiment) was mixed with an equal volume of 20× SSC (1× SSC is 0.15 NaCl plus 0.015 sodium citrate) and dotted onto nitrocellulose by using a dot blotting apparatus (Schleicher & Schuell, Inc., Keene, N.H.). Genomic DNA, included on some dot blots, was prepared by mixing DNA samples (5 μg of DNA in 100 mM Tris–1 mM EDTA) with an equal volume of 20× SSC, boiling the samples for 10 min to denature the DNA, and then quenching the samples on ice. Samples were then loaded onto the dot blot apparatus as above. After baking for 2 h at 80°C, blots were prehybridized overnight in buffer containing 50% formamide, 5× Denhart solution, 3× SSC, and 100 μg of denatured salmon sperm DNA per ml. Fresh buffer containing 10% dextran sulfate and 10^7 cpm of probe was then added, and the samples were left to hybridize for 16 h. Probes were prepared either from purified cDNA inserts (human and mouse alpha-subunit cDNA probes) or from plasmid-plus-cDNA insert (mouse actin probe). Each probe was labeled to the same specific activity (2×10^8 to 3×10^8/μg) with [^{32}P]dCTP by nick translation. After hybridization, blots were washed two times for 30 min each in 0.1% sodium dodecyl sulfate–0.1× SSC at 60°C, air dried, and exposed to Fuji XR film. Probes were removed from blots to be rehybridized by submersing the blots in boiling water for 3 min.

Antisera. Rabbit anti-human FSH alpha-subunit antiserum (FP98437719) and rabbit anti-rat LH alpha-subunit antiserum (AFP7264B; cross-reactive with mouse alpha subunit) (provided by Albert F. Parlow, Pituitary Hormones and Antisera Center, Harbo-UCLA Medical Center, Torrance, Calif.), were used to detect the transgenic and endogenous alpha subunits, respectively. Each antiserum was tested in preliminary immunohistochemical studies to determine its cross-reactivities. Anti-human alpha antiserum did not react with normal mouse pituitary sections at dilutions of 1:50 or higher but reacted strongly at dilutions of 1:10 to 1:1,000 with transgenic mouse pituitaries or cultures of Jar human choriocarcinoma cells, which synthesize the alpha subunit (48). Anti-rat alpha antiserum reacted with normal and transgenic mouse pituitary sections at dilutions of 1:10 to 1:200 but did not react with human alpha subunit in choriocarcinoma cells at these dilutions. Reactivity of the anti-human and anti-rat alpha antisera was abolished by preabsorption with purified human and rat alpha-subunit preparations, respectively (provided by A. W. Parlow). Both antisera were used in our experiments at a dilution of 1:50.

Indirect immunofluorescence. For indirect immunofluorescence, mouse pituitaries were fixed in cold 2% paraformaldehyde for 16 h, dehydrated in graded alcohols (4°C), cleared in xylene (4°C), and embedded in Paraplast. Sections of 5 μm were mounted on slides, deparaffinized, rehydrated, and incubated with rabbit anti-human or anti-rat alpha antiserum for 1 h. After three washes (5 min each) in phosphate-buffered saline, sections were incubated for 1 h with fluorescein isothiocyanate-labeled goat anti-rabbit immunoglobulin G (Organon Teknika, Malvern, Pa.) diluted 1:20. After three rinses in phosphate-buffered saline sections were covered with cover slips and photographed under an epifluorescence microscope (Leitz/Opto-Metric Div. of E. Leitz Inc., Rockleigh, N.J.). As controls, normal rabbit serum or anti-alpha antiserum preabsorbed with human or rat alpha-subunit preparations was substituted for the first antibody on adjacent serial sections.

Antibody was eluted from immunolabeled sections by incubating antibody-labeled sections in acidic potassium permanganate (60) for 20 s, rinsing the samples in running tap water for 2 min, and washing the samples in phosphate-buffered saline for 5 min. After a check for residual fluorescence under the fluorescence microscope, incubations with primary and secondary antibody were repeated as described above. Some eluted sections were incubated with fluorescein isothiocyanate-labeled second antibody alone to confirm the complete removal of the first primary antibodies.

Castrations. Hemizygous male mice from transgenic mouse lines 41 and 46 were mated with nontransgenic C57BL/6 female mice. Litters born within 1 week of each other were weaned and analyzed for the presence of the transgenes (transmitted to 50% of offspring). Half of the transgenic and nontransgenic mice were surgically orchectomized, and 3 months later RNA was extracted from the pituitaries of both the orchectomized mice and their nonor-

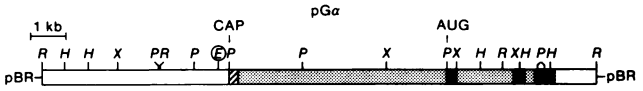

FIG. 1. Genomic clone of the human glycoprotein hormone alpha-subunit gene. The 17-kb genomic clone used in these experiments was isolated by Fiddes and Goodman (19) and contains the 9.4-kb human glycoprotein hormone alpha-subunit gene and 5.7 and 1.9 kb of 5′- and 3′-flanking sequences, respectively. Exons are indicated by solid regions, introns are indicated by lightly shaded regions, and flanking sequences are indicated by open boxes. The first exon (hatched region) encodes the 5′ untranslated region and is separated from the rest of the gene by a long 6.4-kb intervening sequence. The location of the two directly repeated 18-base-pair enhancers is indicated (circled E). The entire clone was microinjected into the nuclei of mouse zygotes as supercoiled plasmid (vector pBR322). Restriction sites shown: R, *Eco*RI; H, *Hin*dIII; X, *Xba*I; P, *Pst*I.

chectomized littermates. Pituitary RNA samples from three mice (orchectomized or nonorchectomized) were pooled and dot blotted at three different concentrations.

RESULTS

Production of transgenic mice. The genomic clone used in our experiments for the production of transgenic mice contains the 9.4-kilobase (kb) human glycoprotein hormone alpha-subunit gene plus 5.7 and 1.9 kb of 5′- and 3′-flanking sequences, respectively (pGα, provided by John Fiddes; Fig. 1). Included in this clone are the promoter region and previously identified 18-base-pair tissue-specific–cyclic-AMP-responsive enhancer sequences (position shown in Fig. 1). Mouse zygotes were microinjected with pGα and transferred to the oviducts of pseudopregnant foster mothers as described in Materials and Methods. Of 30 mice born from transplanted embryos, 6 contained integrated copies of pGα as determined by Southern blotting (not shown). Four of these founder mice were successfully bred to produce stable lines of transgenic mice containing approximately 4 (line 17), 40 (line 38), 80 (line 41), and 8 (line 46) copies of the alpha transgene. Two founders died before being extensively analyzed or bred. Transmission of the transgene occurred in 50% of the progeny of each founder, consistent with the stable integration of the transgenes at a single locus.

Expression of pGα in transgenic mouse tissues and placenta. The distribution of pGα expression in various tissues was examined in offspring from each line by RNA dot blot analysis, using a nick-translated human alpha cDNA probe (18). Transgenic alpha mRNA was detected exclusively in the pituitary in three of the lines, whereas expression occurred in both the pituitary and the testis of line 17 (Fig. 2A). Consecutive rehybridization of this blot with a mouse glycoprotein hormone alpha-subunit cDNA probe (11) and a mouse actin cDNA probe (3) confirmed the tissue specificity of endogenous mouse alpha expression in the pituitary (Fig. 2B) and verified the presence of RNA in each dot (Fig. 2C). No cross-hybridization of the human cDNA probe with mouse alpha mRNA or of the mouse probe with human alpha mRNA occurred under the hybridization conditions used (see Materials and Methods).

To examine expression in the placenta, nontransgenic female mice were mated with hemizygous transgenic male mice and sacrificed on either day 12 or day 17 of pregnancy. Fetuses and their placentas were then isolated and screened for the presence of the transgene DNA (fetuses) and RNA

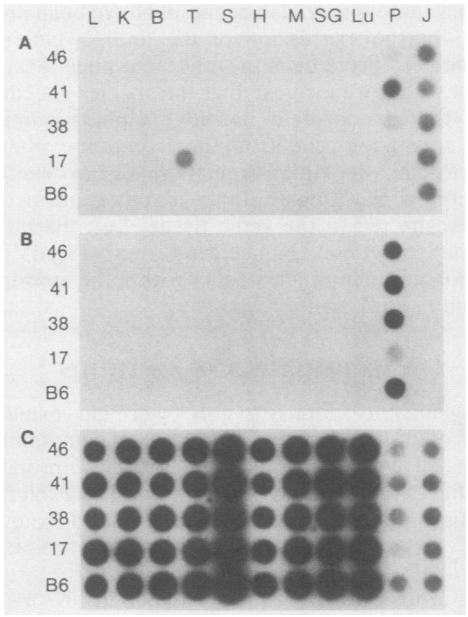

FIG. 2. RNA dot blot analysis of transgenic mouse tissues. Total RNA was extracted from tissues obtained from transgenic mice belonging to lines 46, 41, 38, and 17 and from a nontransgenic C57BL/6 mouse (B6). Total RNA from human choriocarcinoma cells was isolated from the cultured cell line Jar (J). Tissues tested: liver (L), kidney (K), brain (B), testis (T), spleen (S), heart (H), muscle (M), salivary gland (SG), lung (Lu), and pituitary (P). Dots in column P contain 1 μg of pituitary RNA isolated from one mouse from each line. Dots in column J contain 1 μg of Jar RNA. All remaining dots contain 10 μg of tissue RNA. (A) Dot blot hybridized with human alpha-subunit cDNA probe. Hybridization to Jar RNA, line 17 testis RNA, and all transgenic mouse pituitary RNAs is seen. No hybridization to endogenous mouse alpha-subunit mRNA (B6, P) or other tissue RNAs is seen. (B) Same blot as in panel A, rehybridized with the mouse alpha-subunit cDNA probe. Hybridization is seen only in the dots containing pituitary RNA. (C) Same blot as in panels A and B, rehybridized with the mouse actin probe. All dots hybridize, demonstrating the presence of RNA in each dot.

(placentas) by DNA-RNA dot blotting (Fig. 3). Six fetuses and placentas (each having a 50% chance of being transgenic) were randomly selected from each transgenic line for study. No transgene-derived or endogenous alpha-subunit transcripts were detected on either day of development (Fig. 3A and B). Rehybridization of this blot with an actin probe verified the presence of RNA in each dot (Fig. 3C). To rule out the possibility of preferential loss of the transgene in the trophoblast cell lineage, some placentas of transgenic fetuses were analyzed for the presence of the transgene; all were found to be positive for transgene DNA (data not shown).

Analysis of alpha transgene expression in the pituitary. To localize cells within the pituitary in which expression of the alpha transgene occurred, indirect immunofluorescence was performed on sections of transgenic mouse pituitaries. To distinguish the transgenic (human) alpha subunit from the endogenous (mouse) alpha subunit in pituitary sections, an antiserum produced against the human alpha subunit was used which is approximately 1,000-fold more reactive with human alpha subunit than with rodent alpha subunit (Parlow, personal communication; Fox, unpublished data). No reactivity with nontransgenic mouse pituitaries was observed when this antiserum was used in indirect immunofluorescence studies at a dilution of 1:50 or higher (Fig. 4A). By

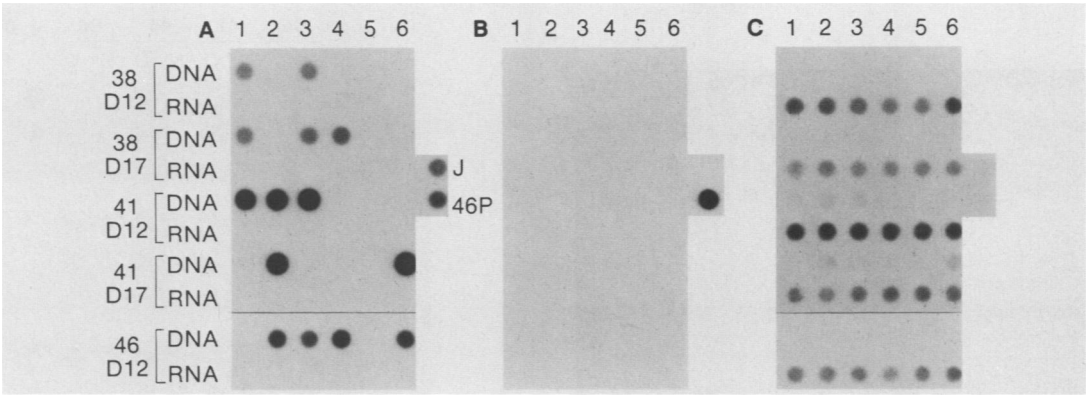

FIG. 3. DNA-RNA dot blot analysis of transgenic mouse placentas. Hemizygous male mice from lines 38, 41, and 46 were mated to nontransgenic C57BL/6 female mice. On day 12 or 17 of pregnancy, fetal DNA and placental RNA from six fetal or placental units were isolated and dotted (5 μg of DNA per 10 μg of RNA) onto nitrocellulose. (A) Hybridization to human alpha-subunit cDNA probe. Positive signals in DNA samples indicate which fetuses or placentas are transgenic. No hybridization to placental RNAs is seen, but hybridization is detected in samples containing 1 μg of Jar (J) or 1 μg of line 46 pituitary (46 P) RNA. (B) Same blot as in panel A, rehybridized with the mouse alpha-subunit cDNA probe. Only the pituitary sample (46P) is positive. (C) Same blot as in panels A and B, rehybridized to the mouse actin cDNA probe. All placental RNA samples hybridize, verifying the presence of RNA in each dot.

contrast, strong immunofluorescence labeling of cells was detected in transgenic mouse pituitaries (Fig. 4B) at antibody dilutions of 1:50 to 1:1,000 (the highest dilution tested). In each case, immunofluorescent labeling was limited to subpopulations of cells within the anterior pituitary. No labeling of cells in the intermediate lobe (Fig. 4) or neurohypophysis (not shown) was seen.

To determine whether this reactivity was confined to the gonadotrope and thyrotrope cells, which make up approximately 10 to 15% of the cells in the anterior pituitary and normally synthesize the alpha subunit, a second antiserum specific for rodent alpha was used to detect endogenous mouse alpha subunits. Sections reacted with the anti-human antiserum were treated with acidic potassium permanganate to remove the antibody complexes present and then reacted with the anti-rodent alpha antiserum as described in Materials and Methods. Both antisera labeled identical cells within the anterior pituitary (Fig. 4C), which indicated that alpha transgene expression occurred within the gonadotrope and thyrotrope cells.

Hormonal regulation of the alpha transgene in vivo. Significant increases in the steady-state levels of alpha-subunit and LH beta-subunit mRNAs are seen in the pituitaries of orchectomized male rats (1, 40). To determine whether gonadectomy has a similar effect on the expression of the endogenous mouse and introduced human alpha-subunit genes in transgenic mice, groups of male transgenic and nontransgenic control animals were orchectomized and sacrificed 1 month later to examine the levels of endogenous and transgenic alpha mRNA in their pituitaries. As determined by densitometric scanning of the blot in Fig. 5, the levels of both the transgenic (Fig. 5A) and the endogenous (Fig. 5B) alpha mRNAs were increased three- to fourfold over control levels. Rehybridization of this blot with the mouse actin probe confirmed that equal amounts of RNA were loaded in dots being compared (Fig. 5C). Thus, hormonal regulation of the transgene parallels that of the endogenous gene.

DISCUSSION

Our data show that the pituitary- and placenta-specific human glycoprotein hormone alpha subunit is expressed exclusively in the gonadotrope and thyrotrope cells of the anterior pituitary in transgenic mice. In one transgenic line, inappropriate expression has been found to occur in the testis; however, Northern (RNA) blot analysis and preliminary immunohistochemical evidence suggest that these transcripts are aberrantly large and are not translated (Fox, unpublished data). Since these transcripts were not detected in any other tissue, they are apparently regulated by a testis-specific factor(s) and may be generated by readthrough transcription from a flanking cellular promoter or from a rearranged transgene copy (or copies).

By indirect immunofluorescence, we have shown that human alpha subunits are synthesized in the transgenic mouse pituitary. Whether these subunits are secreted or combine with endogenous mouse LH, FSH, or TSH beta subunits remains to be tested, although no physiologic abnormalities (e.g., infertility, precocious puberty, hyperthyroidism, or hypothyroidism) have been observed in our transgenic mice and all appear reproductively normal. Functional interspecies hybrid molecules have been produced between human and bovine glycoprotein hormone subunits (42, 47) but have not been reported for human and mouse subunits. If functional hybrid molecules are produced in our transgenic mice, the absence of abnormalities may not be unusual given that the alpha subunit is normally synthesized in excess of the glycoprotein hormone beta subunits in the pituitary (16, 30, 31, 40, 46). Beta-subunit synthesis is thus believed to be rate limiting in the production of functional hormone (17, 21, 43).

A three- to fourfold increase in the endogenous and transgenic alpha mRNA levels was observed in castrated transgenic mice. Castration has previously been reported to increase the gonadotropin subunit mRNA levels in rats (1, 40), and replacement sex steroid therapy has been shown to suppress pituitary gonadotropin mRNA levels in castrated sheep (2, 33, 34, 39). These studies have thus implicated estrogens and androgens in the pretranslational regulation of gonadotropin synthesis. The hormonal regulation of mouse or human pituitary gonadotropins has not been examined at the molecular level in previous studies. Thus, our findings are the first to suggest that gonadal steroids may suppress gonadotropin subunit mRNA levels in the mouse pituitary. Since the levels of biologically active gonadotropin may

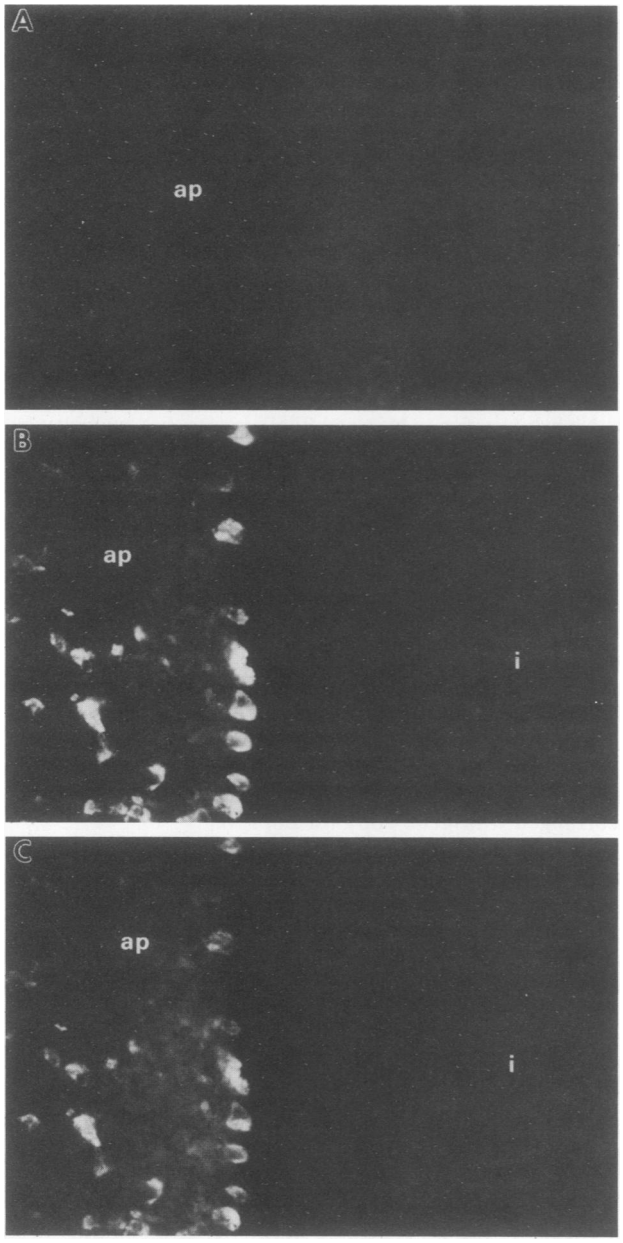

FIG. 4. Indirect immunofluorescence on transgenic mouse pituitaries. Sections of nontransgenic and transgenic mouse pituitaries were reacted with antiserum specific for human or rodent alpha subunits and fluorescein-conjugated secondary antibody. (A) Nontransgenic C57BL/6 mouse pituitary reacted with anti-human alpha antiserum diluted 1:50. No cross-reactivity of the human alpha-subunit-specific antiserum with endogenous alpha subunits in the anterior pituitary (ap) is seen. (B) Transgenic mouse pituitary reacted with the anti-human alpha-subunit antiserum. Strong reactivity is seen in subpopulations of cells within the anterior pituitary (ap). No reactivity is seen in the intermediate (i) lobe. (C) Same slide as in panel B after elution of the antibody complexes and sequential immunolabeling with anti-rodent alpha antiserum and fluorescent secondary antibody. Reactivity is restricted to the same subpopulations of cells as reacted in panel B. Magnification: (A) ×400; (B and C) ×250.

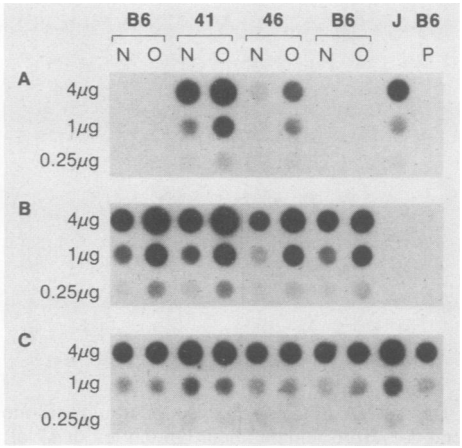

FIG. 5. Dot blot analysis of pituitary RNAs from orchectomized and nonorchectomized transgenic mice. Pituitary RNA from orchectomized and nonorchectomized transgenic mice (lines 41 and 46) and C57BL/6 (B6) mice were dotted onto nitrocellulose in the amounts shown. Jar (J) and C57BL/6 placental (B6, P) RNAs were included as controls for probe specificity. (A) Blot hybridized with the human alpha-subunit cDNA probe. Hybridization signals are stronger in 41 and 46 dots containing pituitary RNA from orchectomized (O) transgenic mice than in dots containing RNA from their nonorchectomized (N) transgenic littermates. No hybridization of orchectomized or nonorchectomized C57BL/6 mouse pituitary (B6, N and O) or placental (B6, P) RNA is seen. (B) Same blot as in panel A, rehybridized with the mouse alpha-subunit cDNA probe. Signals are stronger in pituitary RNA dots from all orchectomized mice than in dots from nonorchectomized mice. No hybridization to Jar RNA or mouse placental RNA is seen. (C) Same blot as in panels A and B, rehybridized with mouse actin probe. Equivalent hybridization signals are seen in pituitary RNA samples from orchectomized and nonorchectomized mice, which indicates that equal amounts of RNA were loaded.

actually be depressed in castrated mice (41), the production of functional dimeric hormone in the pituitary is likely regulated at other levels of subunit synthesis, assembly, and secretion as well (1). Since gonadal steroids also influence gonadotropin production in humans (5, 32, 35, 36), our results provide indirect evidence that the human alpha-subunit gene may also be directly or indirectly regulated by these steroids.

Our observation that endogenous alpha-subunit gene expression is not detected in the mouse placenta is consistent with findings in rats. No gonadotropin alpha-subunit or beta-subunit gene expression has been detected in the rat placenta, and no beta CG gene(s) has been found in the rat genome (7, 58, 63). Thus, expression of the alpha-subunit gene in rats and mice is apparently restricted to the pituitary. Although detailed structural or functional analyses of the rat or mouse alpha-subunit gene have not been performed, sequences conferring tissue-specific expression of the human alpha-subunit gene in transfected human trophoblastic tumor cells in culture have been localized to the human alpha-subunit gene 5′-flanking region (13–15, 25, 26, 52). These sequences include two copies of an 18-base-pair cyclic-AMP-responsive element, the duplication of which has been implicated in the evolution of alpha-subunit gene expression in human and primate placentas (14). Whether such an element is present in the mouse alpha-subunit gene is unknown; however, our results suggest that the presence of this direct repeat in the human alpha-subunit gene is not sufficient to confer tissue-specific expression of this gene in mouse placental trophoblast cells.

The absence of detectable human alpha-subunit transgene expression in the mouse placenta suggests that rodent (mouse and rat) placental trophoblast cells lack the regulatory factors necessary for expressing this gene. This finding also suggests that evolution of such regulatory factors or their controlling genes, rather than, or in conjunction with, evolutionary changes in the *cis*-acting regulatory sequences within or surrounding the alpha-subunit gene itself, are responsible for expression of the alpha-subunit gene in human placental trophoblast cells. Such regulatory factors or their controlling genes may have coevolved with the HCG-LH beta-gene complex and might even lie in close proximity to this complex on chromosome 19. Whether these factors and the sequences with which they interact are the same as or different from those regulating gonadotropin expression in the pituitary is unknown; however, our results indicate that the enhancer or repressor sequences required for pituitary expression of the human alpha-subunit gene must be contained within the cloned gene used here and must be able to respond to *trans*-acting factors in the mouse pituitary.

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