Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 4575–4580, April 1999
Medical Sciences

A 500-bp region, ≈40 kb upstream of the human *CYP19* (aromatase) gene, mediates placenta-specific expression in transgenic mice

AMRITA KAMAT*, KATHERINE H. GRAVES*, MARGARET E. SMITH*, JAMES A. RICHARDSON†, AND CAROLE R. MENDELSON*‡§

Departments of *Biochemistry, †Pathology, and ‡Obstetrics–Gynecology, The Cecil H. and Ida Green Center for Reproductive Biology Sciences, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75235-9038

Communicated by Jean D. Wilson, University of Texas Southwestern Medical Center, Dallas, TX, February 17, 1999 (received for review December 18, 1998)

ABSTRACT **In humans, aromatase P450 (product of *CYP19* gene), which catalyzes conversion of C_{19} steroids to estrogens, is expressed in a number of tissues, including ovary, adipose, and syncytiotrophoblast of the placenta. The 5′ untranslated regions of *CYP19* mRNA transcripts in these tissues are encoded by different tissue-specific first exons, which are spliced onto a common site just upstream of the translation initiation site in exon II. In placenta, the 5′ untranslated region of *CYP19* mRNA transcripts is encoded by exon I.1, which lies ≈40 kb upstream of exon II. To map genomic sequences required for placenta-specific *CYP19* expression, fusion genes containing 2,400 and 501 bp of placenta-specific exon I.1 5′ flanking DNA linked to the human growth hormone gene (hGH), as reporter, were introduced into transgenic mice. Expression of *CYP19*(I.1):hGH fusion genes containing as little as 501 bp of 5′ flanking DNA was placenta-specific and developmentally regulated. Furthermore, transgene expression occurred specifically in the labyrinthine trophoblast of the mouse placenta, which contains syncytial cells that may be analogous to the human syncytiotrophoblast. We show that a relatively small segment of DNA (≈500 bp) >40 kb upstream of the protein coding region of a human gene is able to direct expression in an appropriate tissue- and cell-specific manner in transgenic mice. These findings suggest that 5′ flanking DNA within 501 bp of exon I.1 of the human *CYP19* gene contains cis-acting elements that bind placenta-specific transcription factors that are conserved between humans and mice.**

Aromatase P450 (aromatase), the product of the *CYP19* gene, catalyzes the conversion of C_{19} steroids (androstenedione, testosterone) to C_{18} estrogens (estrone, estradiol), the committed step in estrogen biosynthesis (1). In most vertebrates, aromatase expression is restricted to the gonads and brain; however, in humans, aromatase also is expressed in adipose tissue of adults and in certain fetal tissues, including liver and the syncytiotrophoblast of the placenta, which expresses aromatase at very high levels (2, 3). The multinucleated syncytiotrophoblast is derived from the underlying mononuclear cytotrophoblasts, which do not express aromatase. Various ungulate species such as cows, pigs, and horses also exhibit placental formation of estrogens. By contrast, the placentae of rodents, such as rats and mice, do not have the ability to synthesize estrogens. At this time, little is known about the evolution or the physiological significance of estrogen biosynthesis in the placenta of humans. We have reasoned that characterization of the genomic regions that mediate expression of the human *CYP19* gene in placenta may provide insights into the molecular mechanisms of regulation of placenta-specific gene expression as well as those involved in syncytiotrophoblast differentiation.

Human *CYP19* is a single-copy gene that is composed of 10 exons; exons II-X encode the aromatase protein and 3′ untranslated region of the mRNA in all estrogen-producing tissues. On the other hand, alternative first exons encode unique 5′ untranslated regions of *CYP19* mRNAs that are expressed in different tissues. Therefore, *CYP19* mRNA transcripts in ovary, adipose tissue, and placenta contain different first exons that are alternatively spliced onto a common site just upstream of the translation initiation codon in exon II (4, 5). It is postulated that *CYP19* gene expression in these tissues is driven by tissue-specific promoters that lie upstream of these exons. The 5′ untranslated region of *CYP19* mRNA transcripts in ovarian tissue is encoded by an ovary-specific first exon that lies immediately upstream of exon II (6). By contrast, the adipose-specific first exon (I.4) lies ≈15 kb upstream of exon II (7), whereas the placenta-specific first exon (I.1) lies ≈40 kb upstream of exon II (6). Although no other cytochrome P450 has been found to utilize alternative promoters to regulate expression, prolactin and growth hormone-releasing hormone genes also appear to use an alternative distal promoter to regulate expression in placenta or uterine tissues (8, 9).

To study molecular events that culminate in and control expression of the *CYP19* gene in placenta, we have used human placental cells in primary culture and transgenic technology. In previous studies using primary cultures of human placental cells transfected with fusion genes composed of various amounts of DNA flanking the 5′ end of exon I.1 linked to human growth hormone (hGH), we observed that fusion gene expression increased in concert with syncytiotrophoblast differentiation, induction of aromatase activity, and *CYP19* gene expression. The results of deletion-mapping studies using transfected human trophoblast cells in primary culture suggested that exon I.1 5′ flanking sequences between −501 and −42 bp mediate trophoblast-specific expression of the *CYP19* gene (10). Transgenic mice, on the other hand, provide the most appropriate model system for defining gene-regulatory regions involved in tissue/cell-specific expression (11–13). Initially, we were uncertain as to whether the mouse would provide a suitable model to define regulatory regions of the human *CYP19* gene because mouse placenta does not express aromatase. However, results reported herein indicate that sequences between +103 and −501 bp upstream of *CYP19* exon I.1 contain the necessary cis-acting elements required for

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Abbreviations: aromatase, aromatase P450; hGH, human growth hormone; E, embryonic day; F_0, founder mice; F_1, first-generation mice.

§To whom reprint requests should be addressed at the * address. e-mail: cmende@biochem.swmed.edu.

cell-specific and developmentally regulated expression of the *CYP19* gene in the placenta of transgenic mice. These findings suggest that the transcription factors required for placenta-specific expression are conserved among humans and rodents, whereas the cis-acting genomic sequences that mediate the action of such transcription factors are not.

MATERIALS AND METHODS

Construction of *CYP19*(I.1):hGH Transgenes. Fusion genes containing either 2,400 or 501 bp of 5′ flanking DNA and 103 bp of untranslated exon I.1 of the human *CYP19* gene [*CYP19*(I.1)] were subcloned between the *Sal*I and *Bam*HI sites of the plasmid pACsk$_2$OGH to obtain recombinant *CYP19*(I.1)$_{-2400}$:hGH or *CYP19*(I.1)$_{-501}$:hGH fusion genes, respectively. The plasmid pACsk$_2$OGH contains the promoterless hGH structural gene (14, 15). In this manner, the first 103-bp segment of exon I.1 of the *CYP19* gene was fused to the first exon of the hGH structural gene. The plasmid then was digested with *Sal*I and *Xba*I to release the appropriate *CYP19*(I.1):hGH fusion gene from its vector sequences. The DNA then was isolated on agarose gels and purified (16) for microinjection.

Generation and Identification of Transgenic Mice Carrying *CYP19*(I.1):hGH Fusion Genes. Transgenic mice were generated by microinjection of the fusion gene DNA, described above, into the male pronucleus of fertilized F_1 hybrid mouse eggs (Institute of Cancer Research, ICR albino mice). The eggs were cultured to the two-cell stage, reimplanted into the oviducts of pseudopregnant mice (17), and allowed to develop until term or sacrificed on embryonic day (E) 10.5, E15.5, or E17.5. Transgene-positive animals then were identified, and the copy number of fusion genes per genome was ascertained by dot-blot analysis of tail DNA by using a ^{32}P-radiolabeled hGH cDNA probe as described previously (11). Transgenic founders (F_0), carrying *CYP19*(I.1)$_{-501}$:hGH fusion genes, were bred to establish at least five independent lines.

Northern Blot Analysis of hGH mRNA in Transgenic Mouse Tissues. For Northern blot analysis, various fetal F_0 mouse tissues carrying *CYP19*(I.1)$_{-2400}$:hGH fusion genes or fetal F_1, adult F_0, or F_1 mouse tissues carrying *CYP19*(I.1)$_{-501}$:hGH fusion genes were obtained. Total RNA was isolated from these tissues by homogenization in 4 M guanidinium isothiocyanate, followed by centrifugation through a cesium chloride gradient (5.7 M) (18). The pelleted RNA was resuspended in water, and ≈30 μg was electrophoresed in a formaldehyde-containing agarose gel. The RNA then was transferred to nitrocellulose and probed by using a ^{32}P-radiolabeled hGH cDNA fragment.

***In Situ* Hybridization Analysis of hGH mRNA in Transgenic Fetal Mouse Placental Tissues.** E10.5 and E17.5 placental tissues were fixed for 16 h at 4°C in 4% paraformaldehyde-PBS (pH 7.4) and then rinsed and placed in cold saline. The tissues then were embedded in paraffin, sectioned, and adhered to vectabond-treated slides (Vector Laboratories). *In situ* hybridization analysis was performed on paraffin sections (19). To generate cRNA probes for hybridization of the sections, an hGH cDNA fragment (≈227 bp) was subcloned into pGEM7 plasmid (Promega). After linearization of the plasmid with appropriate restriction enzymes, ^{35}S-labeled sense and antisense hGH cRNA transcripts were synthesized by using a Maxiscript kit (Ambion, Austin, TX) and [^{35}S]UTP (1,250 Ci/mmol). After hybridization procedures, the slides were coated with K.5 nuclear emulsion (Ilford, U.K.) and exposed at 4°C for at least 7 days. The slides then were developed, counterstained with hematoxylin, and photographed by using bright- and dark-field optics.

Experimental Animals. Mice used in this research were treated in accordance with the guidelines set forth by the Animal Welfare Information Center and approved by the Institutional Animal Care and Use Committee at University of Texas Southwestern Medical Center at Dallas.

RESULTS

Placental Expression of the Transgene. To begin to define genomic regions responsible for placenta-specific expression of the human *CYP19* gene, we analyzed the expression of two fusion gene constructs containing either 2,400 bp [*CYP19*-(I.1)$_{-2400}$:hGH] or 501 bp [*CYP19*(I.1)$_{-501}$:hGH] of DNA flanking the 5′ end of the transcription initiation site and the first 103 bp of exon I.1 linked to the hGH structural gene, as reporter, in transgenic mice (Fig. 1).

Transgenic founder mice (F_0), carrying the *CYP19*-(I.1)$_{-2400}$:hGH fusion gene, were identified at E17.5, and placenta and other fetal tissues were analyzed for hGH mRNA expression. In three of the seven transgenic F_0 mice carrying the *CYP19*(I.1)$_{-2400}$:hGH transgene, expression was detected in the placenta (Table 1). In the case of the *CYP19*-(I.1)$_{-501}$:hGH fusion gene, F_0 mice carrying 1–25 integrated copies of the transgene were bred to establish 5 independent lines (F_1). Placental expression was detected in all five independent lines carrying the *CYP19*(I.1)$_{-501}$:hGH transgene (Table 1). No effects of hGH expression on reproductive function or fetal growth were evident in any of the transgenic animals analyzed.

Expression of hGH mRNA in Various Tissues of Transgenic Mice. To analyze tissue-specific expression of *CYP19*(I.1)$_{-2400}$:hGH fusion genes, fetal tissues from the two transgenic founder mice (A7-6 and A4-6) that expressed the *CYP19*(I.1)$_{-2400}$:hGH transgene in the placenta and from nontransgenic littermates were tested for the presence of hGH mRNA. In transgenic founder mice, transgene expression appeared to be placenta-specific; hGH mRNA was undetectable in brain, intestine, kidney, lung, and liver tissues (Fig. 2). In the nontransgenic littermates, hGH mRNA transcripts were undetectable in placenta as well as other tissues (data not shown).

In the case of the *CYP19*(I.1)$_{-501}$:hGH fusion gene construct, tissue samples from brain, testis, ovary, skin, adipose, adrenals, kidney, spleen, liver, lung, and heart tissues from either a pregnant F_0 female mouse (sacrificed on E17.5 to obtain placentae of F_1 mice) or an adult F_1 male mouse from a line that expressed the fusion gene in the placenta were tested for transgene expression. In three of the five independent transgenic mouse lines carrying *CYP19*(I.1)$_{-501}$:hGH fusion gene construct, high levels of hGH mRNA were present exclusively in the mouse placenta (Table 1 and Fig. 3 *Upper*). In two other transgenic lines, in addition to expression in placenta, there also were low levels of expression either in

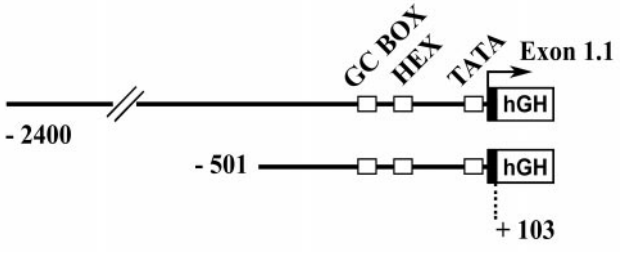

FIG. 1. Schematic diagram of human *CYP19*(I.1):hGH transgenes. The human *CYP19* DNA sequences encoding the first 103 bp of exon I.1 are shown as a solid box, whereas the 5′ flanking DNA is depicted as a solid line. The arrow indicates the position of the transcription initiation site and direction of transcription. The DNA encoding the hGH gene is represented by an open box. The positions of putative regulatory elements that we have found to be functionally important (10) are shown. These elements include the GC box (−233 bp), a hexameric (Hex, −183 bp) element, and the TATA box (−27 bp).

Table 1. Expression of *CYP19*(I.1)$_{-2400}$:hGH and CYP19(I.1)$_{-501}$:hGH fusion genes in transgenic mice

				Placenta		Nonplacental tissues	
Transgene	Sex (F_0)	Line	Copy no.	Generation	Expression	Generation	Expression
−2,400	NA	8-1	3	F_0	−	ND	ND
	NA	9-1	2	F_0	−	ND	ND
	NA	3-4	3	F_0	−	ND	ND
	NA	5-4	5	F_0	+	F_0	−
	NA	A4-6	12-25	F_0	+	F_0	−
	NA	A7-6	6-12	F_0	+	F_0	−
	NA	A8-10	12-25	F_0	−	ND	ND
−501	Male	2218	12-25	F_1	+	F_1	Ovary (low)
	Male	2219	25	F_1	+	F_1	−
	Male	2222	12-25	F_1	+	F_1	−
	Female	2146	1-3	F_1	+	F_0	Brain (low)
	Female	2152	6-12	F_1	+	F_0	−

NA, not applicable; ND, not determined.

ovary (Fig. 3 *Lower*) or brain (data not shown). It should be noted that ovary and brain express the endogenous aromatase gene, albeit from different tissue-specific promoters. These results, therefore, suggest that sequences within 501 bp upstream of the transcription start site within exon I.1 contain cis-acting elements that mediate selective expression of *CYP19* in placenta.

Developmental Regulation of *CYP19*(I.1)$_{-501}$:hGH Fusion Gene Expression in Transgenic Mouse Placenta. Because we observed that 501 bp of exon I.1 5′ flanking sequences direct placenta-specific expression, it was of interest to analyze relative levels of expression in placenta at different stages of fetal development. Shown in Fig. 4 is a Northern blot of RNA from placentae of fetal mice from the same transgenic line at E10.5, E15.5, and E17.5. As can be seen, *CYP19*(I.1)$_{-501}$:hGH transgene expression was detected as early as E10.5 and increased as a function of gestational age.

Cellular Localization of *CYP19*(I.1)$_{-501}$:hGH Transgene Expression in Mouse Placenta. The distribution of hGH expression in the placenta of transgenic mice was analyzed by *in situ* hybridization, using an antisense hGH probe. As shown in Fig. 5 *A* (dark field) and *B* (bright field), *CYP19*(I.1)$_{-501}$:hGH transgene expression was detected as early as E10.5 in the mouse placental labyrinthine trophoblast layer. Reporter gene expression was absent in the spongiotrophoblast and trophoblast giant cells. Fig. 5 *C* and *D* shows a low-power view of *in situ* hybridization of hGH mRNA in E17.5 placenta from a transgenic mouse carrying the *CYP19*(I.1)$_{-501}$:hGH transgene and from a nontransgenic littermate, respectively. Once again, the transgene was expressed specifically in the labyrinthine layer of the transgenic mouse placenta; hybridization signal was absent in the wild-type placenta. Similar to the results of Northern blot analysis, lack of hybridization of the cRNA probe in the placenta of the nontransgenic mouse indicates that the hGH cRNA does not crossreact with endogenous mouse placental hormones. Hybridization signal using the sense hGH cRNA probe was absent in E10.5 or E17.5 mouse placenta carrying the *CYP19*(I.1)$_{-501}$:hGH transgene (data not shown). These results, therefore, suggest that sequences between +103 bp and −501 bp flanking the 5′ end of exon I.1 of the human *CYP19* gene mediate labyrinthine trophoblast-specific expression of promoter I.1 of the human *CYP19* gene.

DISCUSSION

In the present study, we have used transgenic mouse technology to define the regulatory regions surrounding the human *CYP19* gene that mediate placenta-specific expression. Our

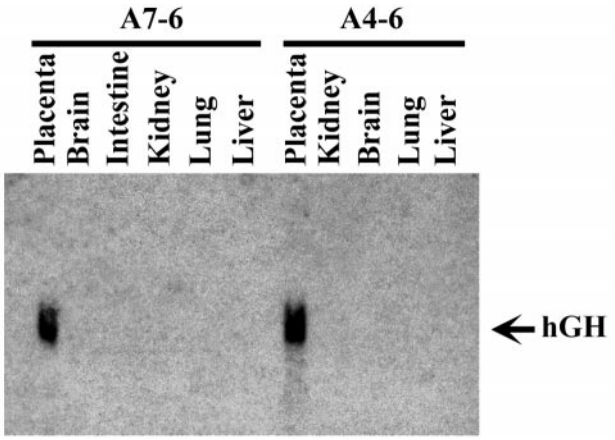

FIG. 2. Expression of the *CYP19*(I.1)$_{-2400}$:hGH transgene is placenta-specific in two independent E17.5 founders. Total RNA (30 μg) isolated from various tissues of two E17.5 founder mice (A7-6 and A4-6) was analyzed for hGH mRNA by Northern blotting by using a full-length ^{32}P-labeled hGH cDNA as probe as described in *Materials and Methods*.

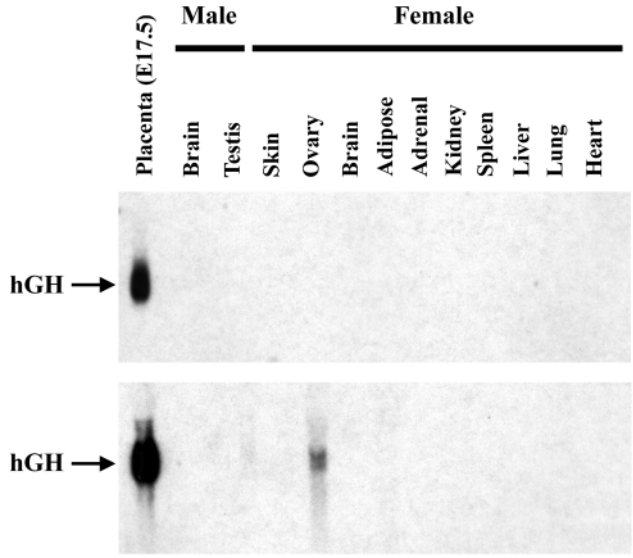

FIG. 3. CYP19(I.1)$_{-501}$:hGH fusion gene is expressed in placenta of transgenic mice. Aliquots of total RNA (30 μg) isolated from placenta of an E17.5 F_1 mouse or from various tissues of an adult F_1 male or female mouse (line 2219, *Upper*, or line 2218, *Lower*) carrying the *CYP19*(I.1)$_{-501}$:hGH transgene were analyzed by Northern blotting by using a ^{32}P-labeled hGH cDNA probe.

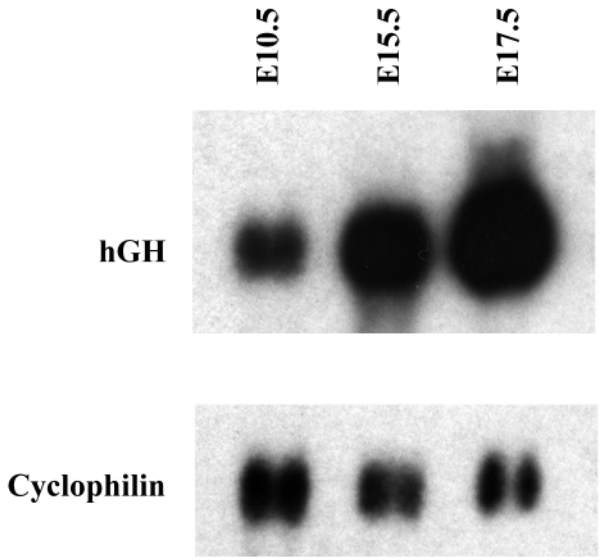

FIG. 4. Expression of a *CYP19*(I.1)$_{-501}$:hGH fusion gene is detectable in transgenic mouse placenta as early as E10.5 and is developmentally regulated. Placentae at E10.5, E15.5, and E17.5 were obtained from a single line of transgenic mice carrying the *CYP19*(I.1)$_{-501}$:hGH fusion gene. Total RNA was isolated and analyzed by Northern blotting for hGH expression by using a ^{32}P-labeled hGH cDNA probe. To evaluate loading efficiency, the blot was stripped and reprobed with a ^{32}P-labeled cyclophilin cDNA.

findings indicate that as little as 501 bp of *CYP19* exon I.1 5′ flanking DNA are able to direct placenta-specific expression in transgenic mice. Transgene expression was detectable as early as E10.5 and confined to the labyrinthine trophoblast layer.

In normal human pregnancy, estrogen production by the placenta increases progressively to very high levels near term (50–150 mg/day of estriol) (20). Aromatase, the enzyme complex that catalyzes the conversion of C_{19} steroids to estrogens, is expressed specifically in the syncytiotrophoblast layer of the human placenta. At present, the physiological significance of human placental aromatase and the resulting high levels of estrogen production are not known. It has been postulated that estrogens may be required for implantation of the blastocyst; however, pregnancies characterized by placental sulfatase deficiency, wherein the placenta is deprived of C_{19} substrate and, thus, produces very little estrogen, are relatively uncomplicated (21). This also is the case with mutations of the *CYP19* gene in humans, which give rise to complete estrogen deficiency and elevated levels of androgens. Females homozygous for a mutation of the *CYP19* gene are virilized *in utero* and manifest primary amenorrhea with hypergonadotropic hypogonadism at the time of puberty (21). Because the human fetal adrenals produce high levels of dehydroepiandrosterone sulfate, which are converted to testosterone peripherally, it is likely that human placenta evolved the capacity to express elevated levels of aromatase, in part, to metabolize circulating androgens and prevent their virilizing effects. By contrast, the placentae of fetal rats and mice do not express aromatase; however, these species have low circulating levels of C_{19} steroids. It should be noted that in mice homozygous for targeted deletion of the 5α-reductase type I gene, there is increased fetal demise at midgestation (22). It was suggested that decreased 5α-reduction of androgens results in their conversion to estrogens, which cause fetal death at midgestation. Thus, it has been proposed that increased estrogen is deleterious to the fetus and that the human fetal/placental unit also has evolved mechanisms to protect the fetus from toxic effects of estrogen (22).

In this study, transgenic mice were used to delineate the regulatory regions of the human *CYP19* gene involved in placenta-specific expression. Mouse placenta does not express the *CYP19* gene; therefore, it was uncertain as to whether transgenic mice could serve as a suitable model, because it was unknown whether lack of placental expression of aromatase was due to the absence of essential gene regulatory elements or of critical trans-acting factors. Results of the present study indicate that mouse placenta contains the necessary transcription factors to activate the human *CYP19* gene, but that the mouse genome lacks cis-acting elements required for expression of the endogenous *CYP19* gene. It appears, therefore, that the introduction of essential cis-acting elements through mutagenesis or retroviral insertion may be responsible for expression of the *CYP19* gene in the human placenta.

In this respect, the human *CYP19* gene is analogous to that encoding the α-subunit of the glycoprotein hormones, which is expressed in the placentae of only primates and horses (23, 24). Lack of expression of the glycoprotein hormone α-subunit gene in rodent placenta was suggested to be due to a single nucleotide change in a CRE (cyclic AMP-response element) sequence, which prevents it from binding CREB, the transcription factor that mediates responsiveness to cAMP (24). In contrast to the glycoprotein hormone α-subunit gene, which appears to use a single promoter, tissue-specific expression of the *CYP19* gene in humans appears to be mediated by use of tissue-specific promoters, which lie upstream of alternative first exons. Results of studies using RACE (rapid amplification of cDNA ends) cDNA libraries made from cattle, horse, and pig placental tissues have indicated that tissue-specific promoters upstream of placenta-specific exons also regulate placental expression of the *CYP19* gene in these species (25). In those species in which the 5′ flanking regions of the *CYP19* placenta-specific promoters have been cloned, there is low sequence identity among species (25, 26). Furthermore, in the human *CYP19* gene, the placenta-specific promoter lies $\approx$40 kb upstream of the start site of translation, whereas in the bovine *CYP19* gene, the placenta-specific promoter lies $\approx$19 kb upstream of the translation start site (27). In the pig, there is even greater complexity, because there may be as many as three *CYP19* genes encoding tissue-specific isoforms with multiple promoter usage for some of the isoforms (26, 28). Because of this complexity, it is not possible to begin to define the genetic basis for placenta-specific expression of human aromatase by comparison of promoter sequences.

In the present study, we observed that in transgenic founder mice, expression of the *CYP19*(I.1)$_{-2400}$:hGH fusion gene was restricted to the placenta; transgene expression was not detected in the fetal kidney, brain, liver, or lung tissues. In transgenic mice carrying *CYP19*(I.1):hGH fusion genes containing as little as 501 bp of exon I.1 5′ flanking DNA, placenta-specific expression of the reporter gene was observed in three independent mouse lines. Transgene expression was not detected in adult brain, testis, ovary, skin, adipose, adrenal, kidney, spleen, liver, lung, or heart tissues. In two other lines, in addition to high levels of placental expression, relatively low levels of transgene expression were observed in the brain or the ovary. It should be noted that in most vertebrates that have been studied thus far, aromatase is expressed primarily in the gonads and in the brain, although different tissue-specific promoters are utilized. The low levels of *CYP19*(I.1)$_{-501}$:hGH transgene expression in ovary or brain in these two lines may be due to position effects of transgene integration and/or to some degree of overlap of promoter usage among tissues (25, 29).

We also observed in the present study that expression of the *CYP19*(I.1)$_{-501}$:hGH transgene was detectable in mouse placenta as early as E10.5 and increased further on E15.5 and E17.5. In human pregnancy, by 7 weeks of gestation, >50% of the estrogens (estradiol-17β and estriol) in the maternal

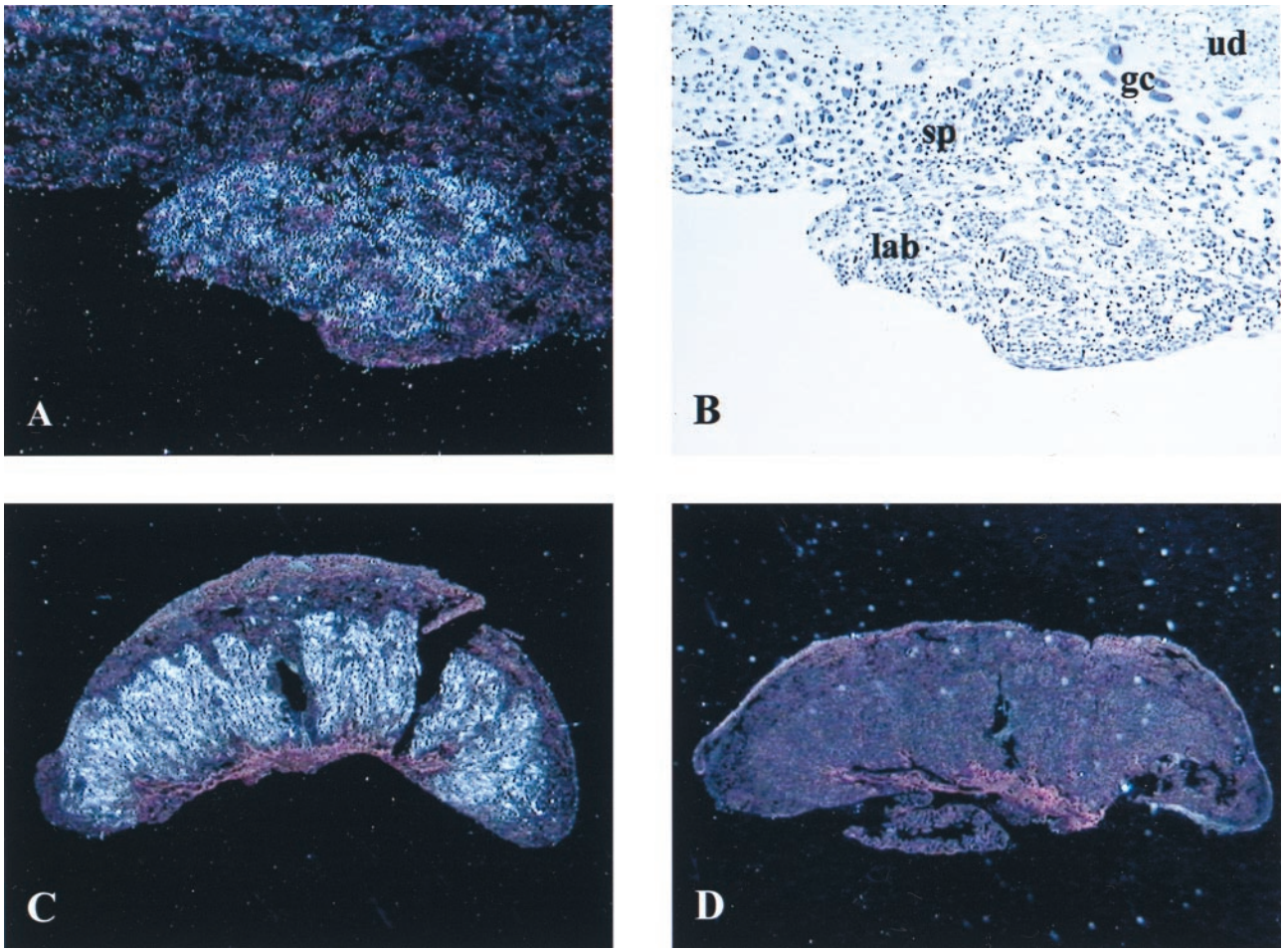

FIG. 5. Expression of a *CYP19*(I.1)$_{-501}$:hGH fusion gene is restricted to labyrinthine trophoblast. Placental tissues obtained from E10.5 or E17.5 fetal mice carrying the *CYP19*(I.1)$_{-501}$:hGH (*A*– *C*) transgene were processed for *in situ* hybridization by using a ^{35}S-labeled antisense hGH cRNA probe and exposed to photographic emulsion for 1–2 weeks. Bright- and dark-field microscopy then were performed. (*A*) Dark-field micrograph of placental tissue section from an E10.5 transgenic mouse carrying the *CYP19*(I.1)$_{-501}$:hGH fusion gene hybridized with radiolabeled hGH antisense cRNA probe. (*B*) Bright-field micrograph of the hematoxylin-stained E10.5 placental tissue section shown in *A*. (*C*) Dark-field micrograph of placental tissue section from an E17.5 transgenic mouse carrying the *CYP19*(I.1)$_{-501}$:hGH fusion gene incubated with radiolabeled hGH antisense cRNA probe. (*D*) Dark-field micrograph of E17.5 placental section from a nontransgenic mouse incubated with radiolabeled hGH antisense cRNA probe. gc, trophoblast giant cell; sp, spongiotrophoblast; lab, labyrinthine trophoblast; ud, uterine decidual tissue.

circulation are produced by the placenta (20). Estrogen production by the placenta increases progressively to very high levels near term. Therefore, it appears that although aromatase is not produced endogenously by mouse placenta, fusion genes containing 501 bp of human *CYP19*(I.1) 5′ flanking sequence are expressed in a developmentally regulated fashion.

Humans and rodents possess a hemochorial placenta wherein the trophoblast is bathed in maternal blood; however, there are differences in their placental structures and in the endocrine functions of the different trophoblast cells (30). In humans, soon after implantation, the trophoectoderm of the blastocyst gives rise to an outer, multinucleated syncytiotrophoblast layer, which is bathed in maternal blood and derived from an underlying layer of mononuclear cytotrophoblast cells. The syncytiotrophoblast layer, which expresses aromatase, serves both as an endocrine tissue and as a transporting epithelium. On the other hand, in mice, the main fetal components of the placenta are the labyrinthine trophoblast, spongiotrophoblast, and trophoblast giant cells. Transport functions are carried out by the labyrinthine trophoblast, whereas the spongiotrophoblast and the trophoblast giant cells, which abut the uterine decidua, possess endocrine activities. Various hormones (placental lactogen family, prolactin family, growth hormone-releasing hormone) and steroid hydroxylases (cytochrome P450 side-chain cleavage enzyme, cytochrome P450 17α hydroxylase) are synthesized in the trophoblast giant cells and in the spongiotrophoblast cells of mouse placenta (31, 32). Surprisingly, in the present study, we observed that 501 bp of DNA flanking the 5′ end of placenta-specific exon I.1 directed transgene expression specifically to the mouse labyrinthine trophoblast. This layer, which is highly vascularized, is trilaminar; its outer cellular layer, which covers two layers of syncytium, is bathed by maternal blood in the placental sinuses (30). Recently, Jacquemin *et al*. (33) reported that expression of the transcriptional enhancer factor 5 (TEF-5) occurs specifically in the labyrinthine region of the murine placenta during later stages of development. Interestingly, TEF-5 also is expressed specifically in the syncytiotrophoblast of the human placenta. Thus, the labyrinthine layer of the mouse placenta contains syncytial cells that appear to be analogous to the human syncytiotrophoblast, which expresses high levels of aromatase and also is bathed in maternal blood.

Our previous studies using primary cultures of human placental cells transfected with *CYP19*(I.1):hGH fusion genes suggest that the region between 501 and 42 bp upstream of exon I.1 contains response elements required for trophoblast-specific expression of the *CYP19* gene, whereas the region between –501 and −246 bp of exon I.1 may bind transcriptional repressors that prevent *CYP19* gene expression in nonplacental

cells (10). Results of the present transgenic studies provide definitive evidence that placenta-specific transcription factors, conserved between humans and rodents, bind to cis-acting elements present within the 501 bp of *CYP19* exon I.1 5′ flanking sequence to mediate placenta-specific expression of aromatase. Transgenic studies are in progress to map the genetic elements that mediate placenta-specific expression of human aromatase. Identification of critical cis-acting elements and characterization of transcription factors involved in placenta-specific expression of the human *CYP19* gene will contribute to a better understanding of the mechanisms involved in syncytiotrophoblast differentiation and in placenta-specific gene expression.

We thank Mr. John Shelton for carrying out the *in situ* hybridization analysis of the transgenic fetal mouse placental tissues and for helpful discussions, John Ritter and Christine Stricker for generation of transgenic mice, and Vickey Chau for technical assistance. This research was supported by National Institutes of Health Grant 5 RO1 DK33775.

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