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Placental-specific expression from the mouse placental lactogen II gene promoter

(hormone/placenta/transcription/transgenic)

MIHO M. SHIDA*, LAURIE L. JACKSON-GRUSBY*†, SUSAN R. ROSS‡, AND DANIEL I. H. LINZER*§

*Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, IL 60208; and ‡Department of Biochemistry, University of Illinois, Chicago, IL 60612

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ABSTRACT **The gene for mouse placental lactogen II (mPL-II) has been isolated and characterized. This gene contains five exons, with a transcription start site 59 nucleotides upstream of the translation initiation ATG. Introduction of a DNA construct containing 2.7 kilobases of sequence upstream of the mPL-II transcription initiation site directed the synthesis of a linked coding region for the simian virus 40 large and small tumor antigens in placental trophoblast giant cells of transgenic mice. The pattern of simian virus 40 transgene expression in the placenta was indistinguishable from that of the endogenous mPL-II gene. In contrast, the first 569 base pairs upstream of the transcription start site proved insufficient to direct placental expression. Thus, one or more elements required for placental trophoblast giant cell expression have been localized to a region between −2700 and −569 of the mPL-II gene.**

The prolactin (PRL)/growth hormone (GH) family in many mammalian species includes proteins synthesized in the placenta. In rodents, the placenta is the site of synthesis of the PRL-like proteins placental lactogen I (PL-I) and II (PL-II) (1, 2). The two PLs bind to the same receptor as PRL (designated the PRL receptor) and elicit cellular responses identical to those evoked by PRL (3). These placental hormones are essential for maintaining ovarian progesterone production and for developing the mammary gland for postpartum lactation. Additional roles of these hormones may include promoting fetal growth, either directly through fetal PRL receptors or indirectly through maternal effects—for example, by mobilizing glucose from maternal liver glycogen storage for use by the fetus (4). Several species also synthesize PRL-like placental proteins with unknown function. In the mouse, these have been designated proliferin (5) and proliferin-related protein (6). Mouse PL-I (mPL-I) and PL-II (mPL-II), as well as proliferin, are synthesized in trophoblast giant cells (7, 8), while proliferin-related protein synthesis appears to be confined to a distinct set of trophoblast cells (9).

Surges in PRL secretion occur during the first half of gestation in rodents but cease with the onset of PL-I synthesis at midgestation (10). Since PL-I and PL-II achieve much higher serum concentrations than PRL, they effectively replace PRL in pregnant mice from middle through late gestation. A peak level of mPL-I protein in serum is achieved transiently at day 10 of pregnancy; the amount of serum mPL-I then declines rapidly, although low levels of this hormone persist throughout gestation (11). Production of mPL-II is slightly delayed with respect to mPL-I, but this hormone is the major lactogenic hormone in the serum throughout the latter half of gestation (4). Unlike mPL-I levels, which decline precipitously by day 12, mPL-II concentrations remain elevated until parturition.

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Peripheral giant cells synthesize mPL-I predominantly at day 9, both mPL-I and mPL-II at day 10, and mPL-II predominantly at day 11 and thereafter (8); the molecular basis for the switch from mPL-I to mPL-II gene expression is unknown. Later in gestation, mPL-II is also synthesized by giant cells within the labyrinth region of the placenta (12), suggesting that additional changes in the patterns of gene expression or of cell differentiation and migration are occurring. For both mPL-I and mPL-II, changes in these hormone mRNA levels correlate closely with hormone protein levels. Thus, the primary determinant of the concentrations of the circulating PLs appears to be the concentrations of the corresponding mPL-I and mPL-II mRNAs.

The genes for all four of the PRL-like placental hormones and for PRL reside on mouse chromosome 13 (13), so this group of mouse placental hormone genes probably arose by duplication and divergence of the PRL gene. The organization of the PL and PRL genes is therefore likely to be similar, and these genes may share regulatory motifs as well. Relatively little is known about the regulation of expression of the placental members of this hormone family, although elements involved in the growth-related expression of proliferin in established mouse cell lines have been partially characterized (14, 15). Characterizing the genetic elements involved in placental expression of these genes would provide (*i*) insights into the control of endocrine reproductive physiology during pregnancy, (*ii*) molecular handles for dissecting the differentiation pattern and roles of placental trophoblasts, and (*iii*) useful reagents for targeting expression of specific genes to the placenta. We have therefore now isolated and begun to characterize the mPL-II gene, and we report here that a defined region of the mPL-II gene confers placental trophoblast-specific expression in transgenic mice.¶

MATERIALS AND METHODS

Isolation of mPL-II Genomic Clones. A bacteriophage library of BALB/c mouse genomic DNA was screened by hybridization to mPL-II cDNA (16), and two distinct clones were obtained. Plaque-purified bacteriophage DNAs, isolated from bacterial lysates by glycerol step-gradient centrifugation (17), were restriction mapped, and fragments hybridizing to the mPL-II cDNA were sequenced.

Primer Extension and S1 Nuclease Analysis. A ^{32}P-end-labeled antisense oligonucleotide from the mPL-II 5′ untranslated region was hybridized to 20 μg of total RNA from mouse

Abbreviations: CRE, cAMP-responsive element; GH, growth hormone; mPL-I, mouse placental lactogen I; mPL-II, mouse placental lactogen II; PRL, prolactin; SV40, simian virus 40; T antigen, large tumor antigen; t antigen, small tumor antigen.

†Present address: Department of Genetics, Harvard Medical School, Boston, MA 02115.
§To whom reprint requests should be addressed.

¶The sequence reported in this paper has been deposited in the GenBank data base (accession nos. M85062–M85067).

placenta or control tissues and extended with reverse transcriptase. For S1 nuclease analysis, a plasmid subclone of mPL-II genomic DNA was cut and end-labeled within the putative first exon, hybridized to 10 μg of total placental RNA, and treated with S1 nuclease. For both reactions, products were analyzed on 8% polyacrylamide sequencing gels.

Transgenic Analysis. A construct containing 2.7 kilobases (kb) upstream of the mPL-II transcription start site was attached to the simian virus 40 (SV40) early region encoding large (T) and small (t) tumor antigens. The mPL-II fragment extended from an upstream *Eco*RI site to the *Ava* I site in the 5′ untranslated region; the SV40 sequences were from an *Avr* II site 5′ of the coding region to the unique *Bam*HI site. DNA was injected into the male pronuclei of fertilized CD-1 mouse eggs, which were then implanted into pseudopregnant hosts. Tail DNAs from 3- to 4-week-old offspring were hybridized to SV40 DNA to identify transgenic animals. Total RNA was isolated from maternal and fetal mouse tissues by homogenization in guanidinium thiocyanate (18) and centrifugation through CsCl cushions (19). RNAs were fractionated by electrophoresis on formaldehyde/agarose gels (20), transferred to nitrocellulose (21), and hybridized to SV40 or mPL-II sequences.

***In Situ* Hybridization.** Ten-micrometer cryostat sections were prepared from rapidly frozen placentas. Sections were mounted on gelatin-treated microscope slides and hybridized to ^{35}S-labeled antisense or sense mPL-II and T-antigen RNAs generated by *in vitro* transcription of full-length mPL-II cDNA (16) or SV40 early region DNA. Hybridization, washing, and emulsion autoradiography were as described (22).

RESULTS

Cloning and Structural Organization of the mPL-II Gene. Screening of a BALB/c mouse genomic library by hybridization to mPL-II cDNA (16) yielded two positive clones (1b3 and a1g) from 600,000 plaques. Restriction maps of the genomic inserts were generated, and locations of mPL-II exons were determined by hybridization of these restriction fragments to mPL-II-specific oligonucleotides and to mPL-II cDNA (Fig. 1). Genomic fragments containing exon and flanking intron sequences were subcloned into plasmid vectors and sequenced to obtain the precise location of the 5′ and 3′ exon–intron boundaries (Fig. 2).

The mPL-II gene has five exons, like all other characterized members of the PRL/GH gene family (23–25). Clone a1g includes exons 1 and 2 plus 9 kb upstream of the first exon; clone 1b3 contains exons 3, 4, and 5 plus 4 kb of downstream sequences. These five exons and four introns encompass at least 8 kb. (The size of the second intron is not known, since the a1g and 1b3 genomic fragments both end within this intron, but these ends do not overlap.) All of the splice junctions have the expected GT and AG dinucleotides at the 5′ and 3′ ends of each intron, and all four introns begin with the same pentanucleotide sequence GTAAG.

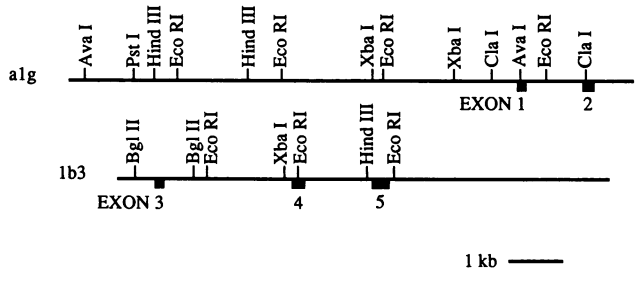

FIG. 1. Structure of mPL-II genomic clones. Restriction maps of the genomic DNA inserts in clones a1g and 1b3 are shown, along with the locations of the five mPL-II exons.

Identification of the mPL-II Transcription Start Site. Exon 1 includes the 5′ untranslated region and part of the secretion signal sequence coding region (Fig. 2). To confirm that no additional exons lie further upstream in the mPL-II gene, the 5′ terminus of the mPL-II mRNA was identified by primer-extension analysis, using as a primer an antisense oligonucleotide specific for mPL-II mRNA. One major extension product was detected from placental (but not brain) RNA, corresponding to a 5′ end of the mRNA 59 nucleotides upstream of the translation initiation AUG (Fig. 3*A*). These 59 nucleotides in the genomic DNA sequence exactly match the 5′ untranslated region in the mPL-II cDNA (16).

The 5′ end of the mPL-II mRNA was also mapped by S1 nuclease analysis (Fig. 3*B*), revealing a major mRNA start site identical to that mapped by primer extension. Both the primer-extension and S1 nuclease analyses indicate that minor mPL-II transcripts are also present in placental RNA that initiate within a few nucleotides of the major start site. That the 5′ end of the mPL-II mRNA is actually a transcription start site and not a processed site is supported by the location of a TATA box 30 base pairs (bp) upstream of this site (Fig. 2). Inspection of the mPL-II promoter sequence reveals a consensus cAMP-responsive element (CRE) at positions −255 to −248 (CTGCGTCA), suggesting that expression of this gene may be sensitive to the action of factors that regulate intracellular cAMP levels.

Placental-Specific Expression in Transgenic Mice. To examine the ability of the mPL-II gene promoter to drive placental-specific expression, a construct was generated linking 2.7 kb of mPL-II sequences from an upstream *Eco*RI site to the *Ava* I site within the 5′ untranslated region (see Fig. 1) to the SV40 early region, encoding the T and t antigens. Expression of this construct, PL-II(2.7)TAg, was assayed in transgenic mice. Three transgenic strains were produced, and each displayed placental expression of SV40 mRNA (Fig. 4*A*). No other tissues in males (Fig. 4*B*) or females were positive for T-antigen expression with the exception of the thymus. Although thymic expression of T antigen was detected in some adults in two of the strains, no mPL-II mRNA was observed in the thymus of any of these animals (data not shown). The production of T antigen in the thymus gave rise to epithelial cell hyperplasia in two strains (Table 1). No aberrant growth of the thymus was observed in the third transgenic mouse strain, which has a comparatively low transgene copy number (<5 copies per cell genome). In contrast to the effect of SV40 tumor antigens on the thymus, no obvious effects were detected on placental structure, embryonic development, or progression of gestation in these three transgenic mouse strains.

A second construct containing only the first 569 bp upstream of the mPL-II gene transcriptional start site, PL-II(0.5)TAg, was also tested in transgenic mice; this region of the promoter was unable to drive expression of linked SV40 sequences in the placenta in two transgenic strains (data not shown). Thus, at least one essential element for placental expression is located between 569 bp and 2.7 kb upstream of exon I in this gene. Interestingly, both male and female transgenic mice bearing the PL-II(0.5)TAg DNA did display abnormal behavior, most notably continuous, rapid circling motions and premature death; this circling motion was also evident in PL-II(2.7)TAg mice. This behavior was found in multiple transgenic strains and seemed to be more pronounced in animals with higher transgene copy numbers, suggesting that some SV40 tumor antigen expression may occur in the brain from the minimal mPL-II gene promoter, although a thorough analysis of the brains from these mice has not been made.

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-569                                                                atcgatgtg
-560   agaatcttca gcttttgtaa agtacatatt atattgctat tgaggtaaat ttctttttca caaaatatat
-490   actgtttaat aatatttgct gattacagac agaaattcat cttttaatta aatgccaaaa actaatggaa
-420   cactgctgtg atgcgccagt caaagctaaa atagaactta tggagggctt tcgttaccac ctgaaacaca
-350   taggggaatt aacagatgac aggaacagca gagagaatat agacttttga tctagagaaa aggtttaaag
-280   aaacatattt tcttacttac ctggtctgcg tcactatgga accaagatga cttttgtgat ttctgaacta
-210   atttgaaccc tgcactgttg caaactgcaa aacaattcat acacgaaaca ggactgtgac aagagtaagg
-140   aatattttta tacttcctac agggatataa tctaagaacc tttaccttga caaaacacat acttccctct
- 70   gcctcgtgac ctggatgacc ttggagagaa tctgttagta tatatgggag gcatagagct gagggaaggc
┌►
AGCACTCGGGGAACAGCAGCCTTCTGGTGTTGTCAGCACTTCAAGAGAACTCCTCAGAG ATG AAG CTG TCT TTG AGT
                                                             Met Lys Leu Ser Leu Ser

CAA CCA TGC TCC TTC T gtaag... INTRON 1 ...tatag CT GGG GCA CTC CTG TTG CTG GCA GTG
Gln Pro Cys Ser Phe S                          er Gly Ala Leu Leu Leu Leu Ala Val

TCA AAC CTA CTT GTC TGG GAG AAG GTG ACT TCC TTA CCA AAT TAT CGA TTA CCC ACT GAA AGC
Ser Asn Leu Leu Val Trp Glu Lys Val Thr Ser Leu Pro Asn Tyr Arg Leu Pro Thr Glu Ser

CTA TAC CAA CGT GTG ATT GTG GTG TCA CAC AAC GCC CAT GAT CTT GCT TCA AAA GCC TTC ATG
Lue Tyr Gln Arg Val Ile Val Val Ser His Asn Ala His Asp Leu Ala Ser Lys Ala Phe Met

GAA TTT  gtaag... INTRON 2 ...tgcag GAA ATG AAG TTT GGT AGG ACA GCT TGG ACC TAT GGC
Glu Phe                            Glu Met Lys Phe Gly Arg Thr Ala Trp Thr Tyr Gly

CTG ATG TTA AGC CCC TGC CAC ACT GCT GCA ATC CTT ACT CCA GAA AAC AGC GAG CAA GTC CAC
Leu Met Leu Ser Pro Cys His Thr Ala Ala Ile Leu Thr Pro Glu Asn Ser Glu Gln Val His

CAG ACA ACA gtaag... INTRON 3 ...tttag  TCG GAA GAC CTT CTG AAA GTG TCC ATC ACT ATT
Gln Thr Thr                            Ser Glu Asp Leu Leu Lys Val Ser Ile Thr Ile

TTA CAA GCC TGG GAA GAG CCT CTG AAA CAC ATG GTG GCA GCA GTG GCT GCT CTT CCA CAT GTA
Leu Gln Ala Trp Glu Glu Pro Leu Lys His Met Val Ala Ala Val Ala Ala Leu Pro His Val

CCT GAT ACT CTG CTG TCA AGA ACA AAG GAG TTG GAG GAA AGA ATT CAA GGG CTT CTG GAA GGA
Pro Asp Thr Leu Leu Ser Arg Thr Lys Glu Leu Glu Glu Arg Ile Gln Gly Leu Leu Glu Gly

CTG AAG ATC ATA TTC AAT AGG  gtaag... INTRON 4 ...tttag GTT TAC CCA GGA GCT GTT GCA
Leu Lys Ile Ile Phe Asn Arg                            Val Tyr Pro Gly Ala Val Ala

AGT GAC TAT ACT TTC TGG TCT GCA TGG TCA GAT TTG CAG TCA TCT GAT GAA TCC ACT AAG AAC
Ser Asp Tyr Thr Phe Trp Ser Ala Trp Ser Asp Leu Gln Ser Ser Asp Glu Ser Thr Lys Asn

AGT GCT CTT AGA ACC TTA TGG CGG TGC GTG CGC AGG GAT ACA CAT AAA GTT GAC AAT TAC CTC
Ser Ala Leu Arg Thr Leu Trp Arg Cys Val Arg Arg Asp Thr His Lys Val Asp Asn Tyr Leu

AAG GTC CTG AAG TGT CGT GAT GTT CAT AAC AAC AAC TGC TGA ACATCTATCCCTCTTCTCTGTCTCTTA
Lys Val Leu Lys Cys Arg Asp Val His Asn Asn Asn Cys END

GAAGGTCCCTCGTGATCTAGACCTTCAAAGCACCAATGAATTTCCCCTTCTTTGGTGCCTTTCTAGATTGAGTTGTCATCTTA
CCCCCAAATAAACTGATTCTTTGCAAATGCTAAATTGAAAA
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FIG. 2. Nucleotide sequence of the mPL-II gene. The sequence of the mPL-II exons is given in capital letters; the upstream nontranscribed region and the pentanucleotide sequences at the beginning and end of each intron are in lowercase letters. The TATA box and a consensus CRE are underlined, and the transcription start site is designated by the bent arrow.

Giant Cell-Specific Expression of the SV40 Transgene. To determine whether the 2.7-kb mPL-II promoter was active specifically in mPL-II-expressing trophoblast giant cells, the distribution of mPL-II and SV40 mRNAs in the transgenic placenta was analyzed by *in situ* hybridization. Placental tissue from transgenic females late in pregnancy was quickly frozen and then sectioned. These sections were hybridized to sense and antisense single-stranded mPL-II or SV40 RNAs.

The patterns of T antigen and mPL-II-expressing cells in the late gestation placenta were indistinguishable (Fig. 5). Cells positive for both mRNAs were found scattered throughout the placental basal zone and labyrinth. Thus, the 2.7-kb upstream sequences of the mPL-II gene appear to drive transcription in only those placental trophoblasts expressing the endogenous mPL-II gene. The giant cells are terminally differentiated cells; these cells may be unable to regain a proliferative potential even with T antigen present, as attempts to culture these transgenic placental cells have thus far failed to give rise to immortalized T-antigen-positive cell lines.

DISCUSSION

We have used transgenic mice to test the ability of fragments of the mPL-II gene promoter to function in the placenta. A transgenic approach was initiated, in part, because of a lack of a suitable tissue culture system for analyzing cell type-specific PL expression. Initially, transient transfections into primary placental cell cultures were attempted, but this approach gave extremely low levels of expression (data not shown). During the course of these investigations, a rat choriocarcinoma cell line (Rcho) that expresses the endogenous PL-I and PL-II genes was reported (26), but transient transfection of constructs containing the 2.7-kb PL-II promoter region into these cells also has not resulted in significant transcription (data not shown).

In contrast, the 2.7-kb region upstream of the mPL-II transcription start site is sufficient to direct gene expression specifically in placental trophoblast giant cells in transgenic mice. At least one critical element for placental-specific expression lies between upstream positions −569 and −2700, since the gene proximal 569-bp upstream region was insufficient to drive transcription in the placenta of transgenic mice. Furthermore, since the transgene is expressed at levels comparable to the endogenous mPL-II gene, and since the distribution of placental cells containing transgene and endogenous mPL-II mRNA is indistinguishable, it seems most likely that all of the essential tissue-specific transcriptional elements lie within the first 2.7 kb upstream of the mPL-II gene.

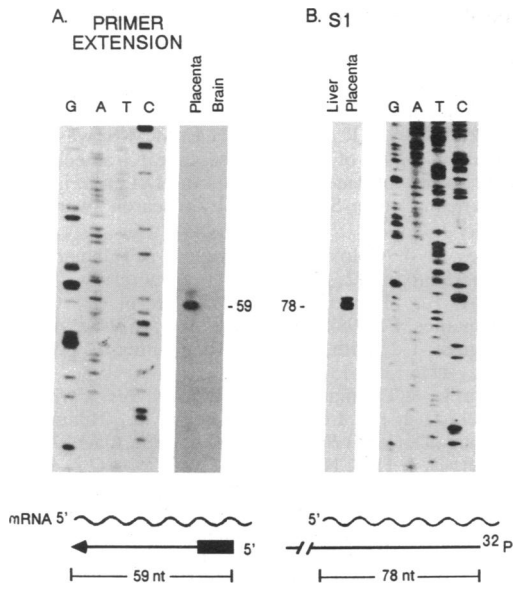

FIG. 3. Mapping of the mPL-II transcription start site. (*A*) An antisense oligonucleotide (solid box), labeled at its 5′ end with ^{32}P, was hybridized to placental or brain RNA and extended with reverse transcriptase. The extension products were fractionated alongside a set of DNA sequencing reactions as length markers. (*B*) A DNA fragment from clone a1g was labeled at the 5′ end of the unique *Hin*cII site within exon I, hybridized to placental or liver RNA, and digested with S1 nuclease. Products were fractionated alongside a set of DNA sequencing reactions. The transcription start site predicted by both methods is identical [the 5′ ends of the oligonucleotide and the *Hin*cII site lie 19 nucleotides (nt) apart].

Other gene control regions have recently been demonstrated to elicit placental expression in transgenic mice. Expression of a human glycoprotein hormone α-subunit transgene in placental trophoblasts of transgenic mice has been reported by some (27), but not all (28), investigators. Unlike the mPL-II gene, the endogenous mouse α-subunit gene is expressed specifically in the pituitary, and the human transgene is also expressed in the pituitary (27, 28). Furthermore, the placental cell type in which the transgene is expressed has not been identified. Transcriptional activity of

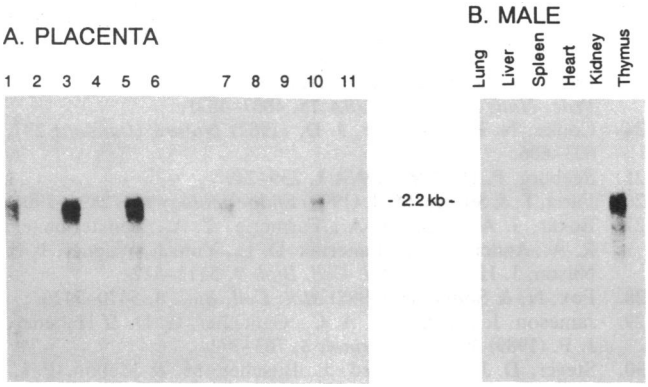

FIG. 4. Placental-specific expression of the mPL-II T-antigen transgene. (*A*) Total RNA (10 μg per lane) from 11 individual placentas from a single transgenic female at day 16 of pregnancy that had been mated to a transgenic male of the same strain was fractionated by electrophoresis and hybridized to SV40 early-region DNA. Strong hybridization (lanes 1, 3, and 5) suggests transmission of the transgene from both parents, while weaker hybridization signals probably indicate transmission from only one parent. (*B*) Total RNA (10 μg per lane) was isolated from tissues of a transgenic male mouse displaying severe thymic hyperplasia, fractionated by electrophoresis, and hybridized to the SV40 DNA probe.

Table 1. Incidence of thymic tumors in PL-II(2.7)TAg transgenic mice

Strain	Sex	No. of mice	Age, days	No. of mice with tumors
1	M	6	116–161	6
1	F	4	73–165	1*
2B	M	8	24–260	2
2B	F	7	164–264	4

*Two of the mice without tumors had enlarged thymuses.

the human α-subunit gene in placental cells has been found to depend on several elements that lie within 180 bp of the transcriptional start site. These include a duplicated CRE (27, 29) and a GATA-like element present in horse and human, but not mouse, genes (30). In addition, two elements—a "junctional" element (31) and an upstream regulatory element (32)—that bind placental-specific factors are located in this region of the gene.

A CRE sequence is present in the mPL-II gene at positions −255 to −248, and a purified CRE binding protein has been found to bind to this site in a DNase I footprint assay (data not shown). A match of 8/9 nucleotides is also observed between the mPL-II promoter sequences at −166 to −158 (GAAACAGGA) and the 3′ portion of the α-subunit gene upstream response element at −162 to −154 (GAAACAAGA). The presence of a CRE and the nearly identical locations of the α-subunit upstream response element and the related mPL-II sequence with respect to the corresponding transcription start sites are consistent with a role for these elements in mPL-II expression in the placenta. However, both of these elements lie within the proximal 569-bp fragment that is unable to confer placental expression, and therefore these elements are insufficient to direct transcrip-

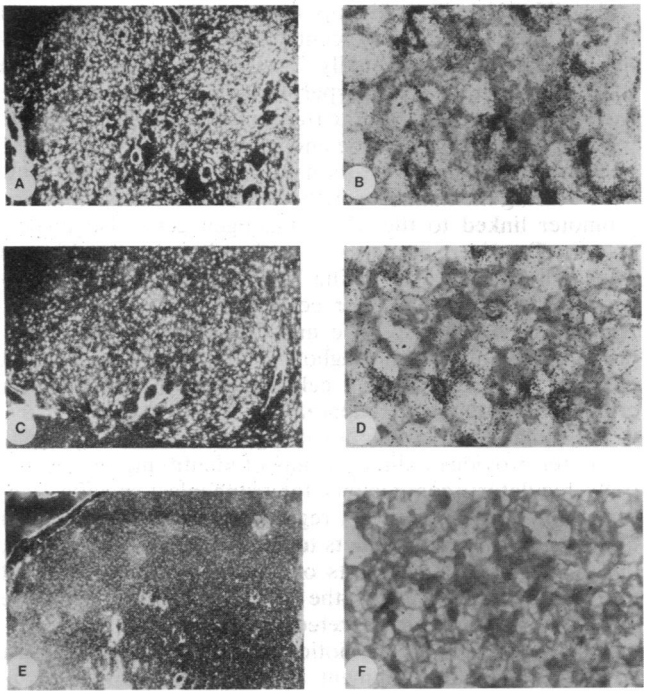

FIG. 5. Localization of transgene expression within the placenta. Frozen placental sections from day 16 of gestation were analyzed by *in situ* hybridization to single-stranded antisense probes for mPL-II (*A* and *B*) or SV40 early (*C* and *D*) mRNAs. (*E* and *F*) Hybridization to a SV40 early-region mRNA sense probe. (*Left*) Low-magnification dark-field views showing extensive hybridization to both anti-sense probes (light regions) throughout the placental labyrinth. (*Right*) High-magnification bright-field view of regions near the base of the labyrinth; hybridization is detected as dark silver grains.

tion in the mouse placenta. The sequence TGGTAAT, which forms the core of the α-subunit gene junctional element (31), is also found in the mPL-II gene in at least two copies at positions further upstream than −569 (data not shown); the relevance of these sites, as well as other regions of the mPL-II gene, for placental trophoblast cell expression remains to be examined.

Placental cell-specific expression has also been reported for constructs containing regions from the human PL gene (33). In this case, transient transfections into a human choriocarcinoma cell line were used to define a cell type-specific enhancer located downstream from this gene. Unlike rodent PL genes, though, human PL is derived from a duplication of the GH gene. Furthermore, the human and rodent placenta differ significantly in structure and development, so that common regulatory motifs and factors governing human and rodent PL expression may not exist.

The transgenic mouse strains used in this study were also generated in an attempt to immortalize mPL-II-expressing giant cells for long-term growth in cell culture. Expression of SV40 T antigen in transgenic mice has been used successfully to isolate a variety of immortalized cell lines, but, even though some placental cell lines were established from the transgenic placental tissue, none of these cell lines expressed T antigen or mPL-II (or any of the other placental members of the PRL family), nor did they display a trophoblast morphology (data not shown). Sustained proliferation of these T-antigen-expressing trophoblasts may not be possible with this approach: mPL-II gene activation occurs in terminally differentiated giant cells that may be unable to be rescued for growth by T antigen. Furthermore, the ≈10 days of mPL-II expression during pregnancy in the mouse represents only a short time for an effect of T antigen on cell proliferation to develop before the tissue is placed in culture.

Not only did SV40 T antigen fail to immortalize giant cells, it also did not appear to disrupt the normal *in vivo* patterns of cellular differentiation, placental development, and gestational progress. Unexpectedly, T-antigen expression did lead to massive epithelial cell hyperplasia in the thymus of male and female mice in two of the transgenic strains; no evidence for thymic expression of the endogenous mPL-II gene could be seen. Other investigators have reported that transgenic mice bearing the human growth hormone-releasing hormone promoter linked to the SV40 T-antigen gene also display ectopic T-antigen expression in the thymus, resulting in thymic hyperplasia (34). The thymus may be a uniquely permissive environment for ectopic transgene expression. Alternatively, the transgene may be activated with a low probability in all cells throughout the transgenic animal, but the rare T-antigen-positive cell may not be restricted for growth in the thymus compared to other tissues.

The isolation of a functional region of the mPL-II gene promoter provides a direct means of identifying the cis and trans regulatory components for trophoblast-specific transcription. This 2.7-kb DNA region should also be useful in targeting other gene products to the conceptus. In this way, the effects of gene products on the placental functions of protecting and nourishing the developing embryo can be examined. In addition, secreted proteins that may not normally be present in the amniotic fluid may be tested for their effects on fetal development by transgenic expression in placental trophoblasts. Utilization of the mPL-II and other placental-specific promoters to alter or eliminate the expressing placental cell may also provide a means of determining the role of distinct trophoblast cell populations during gestation as well as following the differentiation programs of the trophoblast lineage.

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1. Soares, M. J., Colosi, P. C., Ogren, L. & Talamantes, F. (1983) *Endocrinology* **112**, 1313–1317.
2. Colosi, P., Marr, G., Lopez, J., Haro, L., Ogren, L. & Talamantes, F. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 771–775.
3. Harigaya, T., Smith, W. C. & Talamantes, F. (1988) *Endocrinology* **122**, 1366–1372.
4. Ogren, L. & Talamantes, F. (1987) *Int. Rev. Cytol.* **112**, 1–65.
5. Linzer, D. I. H., Lee, S. J., Ogren, L., Talamantes, F. & Nathans, D. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4356–4359.
6. Linzer, D. I. H. & Nathans, D. (1985) *EMBO J.* **4**, 1419–1423.
7. Lee, S. J., Talamantes, F., Wilder, E., Linzer, D. I. H. & Nathans, D. (1988) *Endocrinology* **122**, 1761–1768.
8. Faria, T. N., Ogren, L., Talamantes, F., Linzer, D. I. H. & Soares, M. J. (1991) *Biol. Reprod.* **44**, 327–331.
9. Colosi, P., Swiergiel, J. J., Wilder, E. L., Oviedo, A. & Linzer, D. I. H. (1988) *Mol. Endocrinol.* **2**, 579–586.
10. Barkley, M. S., Radford, G. E. & Geschwind, I. I. (1978) *Biol. Reprod.* **19**, 291–296.
11. Ogren, L., Southard, J. N., Colosi, P., Linzer, D. I. H. & Talamantes, F. (1989) *Endocrinology* **125**, 2253–2257.
12. Hall, J. & Talamantes, F. (1984) *J. Histochem. Cytochem.* **32**, 379–382.
13. Jackson-Grusby, L. L., Pravtcheva, D., Ruddle, F. H. & Linzer, D. I. H. (1988) *Endocrinology* **122**, 2462–2466.
14. Linzer, D. I. H. & Mordacq, J. C. (1987) *EMBO J.* **6**, 2281–2288.
15. Mordacq, J. C. & Linzer, D. I. H. (1989) *Genes Dev.* **3**, 760–769.
16. Jackson, L. L., Colosi, P., Talamantes, F. & Linzer, D. I. H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8496–8500.
17. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
18. Chirgwin, J. M., Przybyla, A. E., Macdonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
19. Glisin, V., Crkvenjakov, R. & Byus, C. (1974) *Biochemistry* **13**, 2633–2637.
20. Lehrach, H., Diamond, D., Wozney, J. M. & Boedtker, H. (1977) *Biochemistry* **16**, 4743–4751.
21. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
22. Woodruff, T. K., Meunier, H., Jones, P. B. C., Hsueh, A. J. W. & Mayo, K. E. (1987) *Mol. Endocrinol.* **1**, 561–568.
23. Barta, A., Richards, R. I., Baxter, J. D. & Shine, J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4867–4871.
24. Cooke, N. E. & Baxter, J. D. (1982) *Nature (London)* **297**, 603–606.
25. Seeburg, P. H. (1982) *DNA* **1**, 239–249.
26. Faria, T. & Soares, M. J. (1991) *Endocrinology* **129**, 2895–2906.
27. Bokar, J. A., Keri, R. A., Farmerie, T. A., Fenstermaker, R. A., Andersen, B., Hamernik, D. L., Yun, J., Wagner, T. & Nilson, J. H. (1989) *Mol. Cell. Biol.* **9**, 5113–5122.
28. Fox, N. & Solter, D. (1988) *Mol. Cell. Biol.* **8**, 5470–5476.
29. Jameson, J. L., Powers, A. C., Gallagher, G. D. & Habener, J. F. (1989) *Mol. Endocrinol.* **3**, 763–772.
30. Steger, D. J., Altschmied, J., Buscher, M. & Mellon, P. L. (1991) *Mol. Endocrinol.* **5**, 243–255.
31. Anderson, B., Kennedy, G. C. & Nilson, J. H. (1990) *J. Biol. Chem.* **265**, 21874–21880.
32. Delegeane, A. M., Ferland, L. H. & Mellon, P. L. (1987) *Mol. Cell. Biol.* **7**, 3994–4002.
33. Walker, W. H., Fitzpatrick, S. L. & Saunders, G. F. (1990) *J. Biol. Chem.* **265**, 12940–12948.
34. Botteri, F. M., van der Putten, H., Wong, D. F., Sauvage, C. A. & Evans, R. M. (1987) *Mol. Cell. Biol.* **7**, 3178–3184.