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PLAC1, an Xq26 Gene with Placenta-Specific Expression

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A novel human X-linked gene shows placenta-specific expression and has been named *PLAC1*. The gene maps 65 kb telomeric to *HPRT* at Xq26 and has been completely sequenced at the cDNA and genomic levels. The mouse orthologue *Plac1* maps to the syntenically equivalent region of the mouse X chromosome. *In situ* hybridization studies with the antisense mRNA during mouse embryogenesis detect *Plac1* expression from 7.5 dpc (days postcoitum) to 14.5 dpc in ectoplacental cone, giant cells, and labyrinthine trophoblasts. The putative human and murine PLAC1 proteins are 60% identical and 77% homologous. Both include a signal peptide and a peptide sequence also found in an interaction domain of the ZP3 (zona pellucida 3) protein. These results make *PLAC1* a marker for placental development, with a possible role in the establishment of the mother–fetus interface. © 2000 Academic Press

INTRODUCTION

The placenta forms very early in development as a specialized organ to interface the rapidly growing mammalian embryo with its mother for nourishment and respiration. During mammalian development, the trophoblast is the first cell lineage to differentiate and gives rise to most of the extraembryonic tissues required for implantation and further development of the embryo proper (Copp, 1995; Cross *et al.,* 1994). During implantation in mice, while the embryonic inner cell mass continues to differentiate, trophoblasts attach to the receptive uterine epithelium and the trophectoderm then proliferates to form the ectoplacental cone and, later, the spongiotrophoblast layer of placenta. The outermost trophoblasts of the ectoplacental cone differentiate into secondary trophoblast giant cells, forming the placental interface with the maternal cells in the decidua, where they also exhibit significant endocrine activity (Cross *et al.,* 1994; Soares *et al.,* 1996).

Although speculation has suggested that they might be selectively X-linked, only a few placenta-specific genes have been found on the X chromosome (see Hurst and Randerson, 1999; and Discussion). Among possible candidates, one particular locus in mouse, however, has been reported, near the Hprt locus, that is suggested to be involved in early development. Several mutant mice with large chromosomal deletions spanning 200 to 700 kb around the *Hprt* gene yielded a runty phenotype or caused death at birth (Kushi *et al.,* 1998). Because deletion of the *Hprt* gene itself in the mouse causes no such phenotype, the simplest explanation for these phenotypes is the existence of another gene, near *Hprt,* that functions during early fetal/placental development.

We have sequenced and analyzed the syntenically equivalent human genomic region around HPRT for its gene content. This work has led to the isolation of a novel gene, *PLAC1,* whose expression is localized only in placenta. We have also isolated and mapped the homologue from the corresponding region of the mouse X chromosome and report the comparative transcript sequences and the specific developmental expression of the mouse gene during placenta formation. Its structure and expression in placenta are consistent with a role for the gene in early development.

MATERIALS AND METHODS

Sequencing. EST (expressed sequence tag) DNAs were sequenced on an ABD 377 instrument (Perkin–Elmer, Applied Biosystems). For complete sequencing of cDNA clones, a primer walking strategy was applied. Overlapping sequencing in both directions minimized the sequence error rate to ≤0.1%. Sequencing of bacterial artificial chromosome (BAC) clones in human Xq26 included over 1 Mb of total sequence deposited in GenBank, including, from centromere to telomere, bWXD9 (GenBank Accession No. AC002420, 244 kb); bWXD8 (AF003529, 166 kb); bWXD178 (AC004409, 42 kb); bWXD177 (AC002407, 95.5 kb); bWXD180 (AC002408, 111 kb); three BACs in a core region around HPRT, discussed in the text and including bWXD187 (AC004383, 156,461 bp), bWXD173 (AC004387, 205,643 bp), and bWXD171 (AC004676, 201,175 bp).

Electronic tools and searches. Searches in the EST databases and comparisons of cDNA to genomic DNA sequences were performed by

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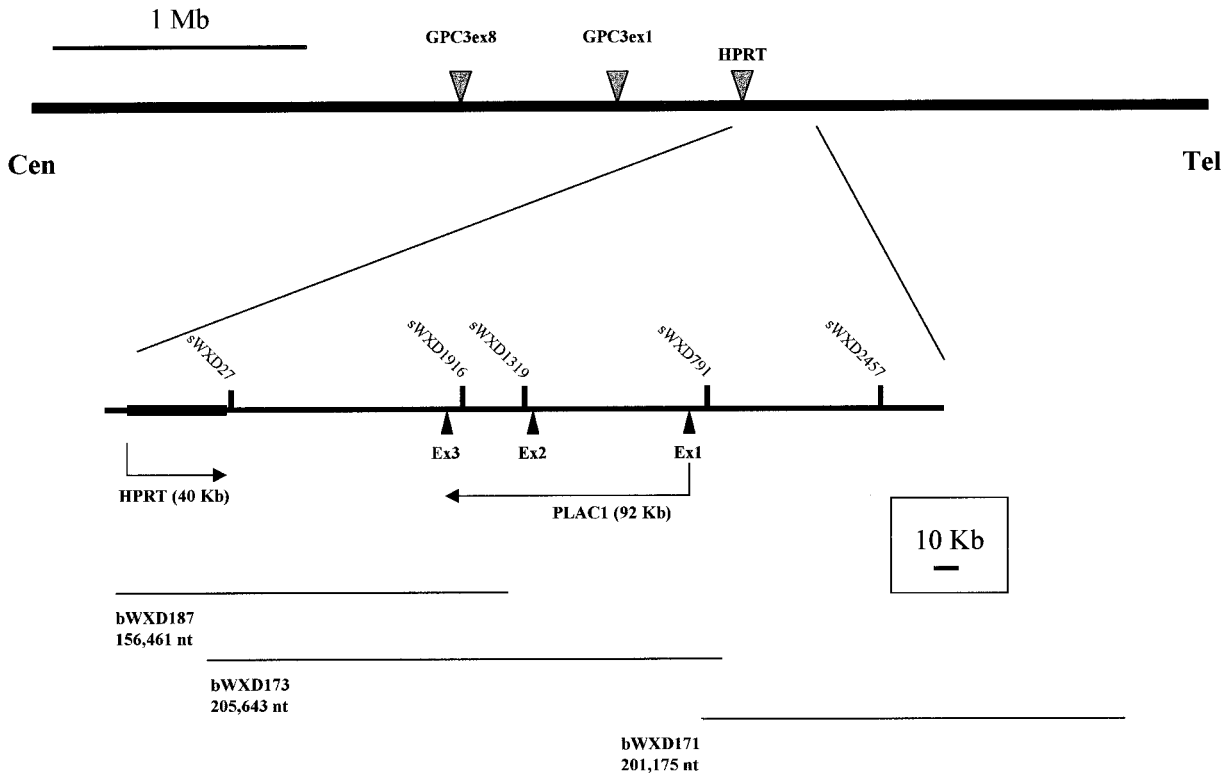

FIG. 1. Genomic location of *PLAC1* and *HPRT* in Xq26. The 3′ ends of the genes are 65 kb apart, compared to a skeletonized representation of the more detailed map in Pilia *et al.* (1996) and the complete sequence of three overlapping BAC clones (bWXD187, 173, and 171; see text). Details of the indicated STSs ("sWXD") and others in the region are available at the Web site for the Washington University Center for Genetics in Medicine at http://www.ibc.wustl.edu/cgm/.

BLAST programs on the server of the National Center for Biotechnology Information. For the analysis of genomic DNA, the CENSOR program (Jurka *et al.*, 1996) was used to mask repetitive sequences. Then the unique sequence portions were examined by GRAIL2 (Uberbacher and Mural, 1991) for the prediction of possible exons and were compared to the PLAC1 cDNA sequence by BLAST (Altschul *et al.*, 1990).

Northern blot hybridization. Standard human Northern MTN, MTII, fetal, Master Blot, and mouse embryo blots (Clontech) were used for the expression studies. The whole human fragment (*Eco*RI–*Not*I digestion) from EST 148524 (Accession No. H12503) was used as a probe for human tissue RNAs. The whole cloned insert from mouse EST C0026B06 (Accession No. AA408322) was used as a probe for mouse RNAs.

In situ hybridization. The mouse *in situ* probe was obtained using primers *Plac1* Forward (CGCGGATCCCGTCCGGGCTAAGGGACC) and *Plac1*Reverse (CGCGGATCCCTCACAGGAGACAAGAAGG) to amplify a fragment of about 200 bp from the 3′ end of the cDNA. These primers have a sequence (underlined) at their 5′ ends of the genomic tracts that includes a *Bam*HI restriction site sequence (linker), facilitating subcloning into the plasmid pSPORT1 (Gibco). The sequence of the insert was verified, and strand-specific RNA probes were transcribed using a digoxigenin–UTP labeling kit (Boehringer Mannheim) according to the manufacturer's instructions.

Mouse embryos and placentas at 6.5, 7.5, 8.5, 9.5, 11.5, 13.5, and 16.5 dpc were studied for *in situ* hybridization according to the protocol of Wilkinson and Nieto (1993), with sections embedded in gelatin.

RESULTS

Genomic and cDNA Sequence of PLAC1

We sequenced about 1 Mb of human Xq26 DNA from the *GPC3* gene to about 200 kb telomeric of *HPRT* (Materials and Methods and Fig. 1). Small gaps remain in some reported sequenced clones, but it is now clear that this entire region of Xq26 apparently encodes only a few genes. At the centromeric end of the zone, the large *GPC3* gene itself extends over more than 600 kb in a single transcription unit (Huber *et al.*, 1997). Moving toward the telomere, the paucity of genes is shown by several criteria. The content of repetitive sequences is very high (see GenBank entries referred to above), and in the entire region, GRAIL detects only two of the CpG islands that are telltales for about half of all genes (Antequera and Bird, 1993). One of those is associated with *GPC3;* the other is associated with *HPRT*. *HPRT* also contains all but one of the human ESTs in GenBank that match genomic sequence in the region by BLAST searches.

The only other possible gene in the region suggested by computer-assisted gene prediction programs contains three likely exons and includes the other human EST that matches sequence in the region (Accession No. H12503). Reported here as *PLAC1,* the gene lies 65 kb telomeric to *HPRT*. The relative placement and extent of the transcription units of the two genes are schematized in Fig. 1, based on the complete sequence of three contiguous overlapping human X chromosome BACs covering a 350-kb region (bWXD173, bWXD187, and bWXD171).

The sequence of the human PLAC1 cDNA was derived by fully sequencing the 1131-bp insert of clone

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GGCACGAGGATCAGACCATCAGAAGGATTTGTATAAAGAGTGACTCTCCTATGAAGGTAAAGGCCACCC
CTCTTCAGTTCCGGTGACTGAGATACATTTTTCCAATCCTGGGGGCAAATACAGACACAGCAAGTTCCTTC
TTCCCTTTGGAAATTTGGCAGCTGCCTTCACCAGTGAGCACAAAGCCACATTTCAAAGGAAACTGACAAA
TTATCCCCAGCTGCCAGAAGAAGAAATCCTCACTGGACGGCTTCCTGTTTCCTGTGGTTCATTATCTGATT
GGCTGCAGGG
      ATGAAAGTTTTTAAGTTCATAGGACTGATGATCCTCCTCACCTCTGCGTTTTCAGCCGGT
  292 --------+---------+---------+---------+---------+---------+- 351
      M  K  V  F  K  F  I  G  L  M  I  L  L  T  S  A  F  S  A  G  -

      TCAGGACAAAGTCCAATGACTGTGCTGTGCTCCATAGACTGGTTCATGGTCACAGTGCAC
  352 --------+---------+---------+---------+---------+---------+- 411
      S  G  Q  S  P  M  T  V  L  C  S  I  D  W  F  M  V  T  V  H  -

      CCCTTCATGCTAAACAACGATGTGTGTGTACACTTTCATGAACTACACTTGGGCCTGGGT
  412 --------+---------+---------+---------+---------+---------+- 471
      P  F  M  L  N  N  D  V  C  V  H  F  H  E  L  H  L  G  L  G  -

      TGCCCCCCAAACCATGTTCAGCCACACGCCTACCAGTTCACCTACCGTGTTACTGAATGT
  472 --------+---------+---------+---------+---------+---------+- 531
      C  P  P  N  H  V  Q  P  H  A  Y  Q  F  T  Y  R  V  T  E  C  -

      GGCATCAGGGCCAAAGCTGTCTCTCAGGACATGGTTATCTACAGCACTGAGATACACTAC
  532 --------+---------+---------+---------+---------+---------+- 591
      G  I  R  A  K  A  V  S  Q  D  M  V  I  Y  S  T  E  I  H  Y  -

      TCTTCTAAGGGCACGCCATCTAAGTTTGTGATCCCAGTGTCATGTGCTGCCCCCCAAAAG
  592 --------+---------+---------+---------+---------+---------+- 651
      S  S  K  G  T  P  S  K  F  V  I  P  V  S  C  A  A  P  Q  K  -

      TCCCCATGGCTCACCAAGCCCTGCTCCATGAGAGTAGCCAGCAAGAGCAGGGCCACAGCC
  652 --------+---------+---------+---------+---------+---------+- 711
      S  P  W  L  T  K  P  C  S  M  R  V  A  S  K  S  R  A  T  A  -

      CAGAAGGATGAGAAATGCTACGAGGTGTTCAGCTTGTCACAGTCCAGTCAAAGGCCCAAC
  712 --------+---------+---------+---------+---------+---------+- 771
      Q  K  D  E  K  C  Y  E  V  F  S  L  S  Q  S  S  Q  R  P  N  -

      TGCGATTGTCCACCTTGTGTCTTCAGTGAAGAAGAGCATACCCAGGTCCCTTGTCACCAA
  772 --------+---------+---------+---------+---------+---------+- 831
      C  D  C  P  P  C  V  F  S  E  E  E  H  T  Q  V  P  C  H  Q  -

      GCAGGGGCTCAGGAGGCTCAACCTCTGCAGCCATCTCACTTTCTTGATATTTCTGAGGAT
  832 --------+---------+---------+---------+---------+---------+- 891
      A  G  A  Q  E  A  Q  P  L  Q  P  S  H  F  L  D  I  S  E  D  -

      TGGTCTCTTCACACAGATGATATGATTGGGTCCATGTGA
  892 --------+---------+---------+-------- 930
      W  S  L  H  T  D  D  M  I  G  S  M  -

TCCTCAGGTTTGGGGTCTCCTGAAGATGCTATTTCTAGAATTAGTATATAGTGTACAAATGTCTGACAAAT
AAGTGCTCTTGTGACCCTCATGTGAGCACTTTTGAGAAAGAGAAACCTATAGCAACTTCATGAATTAAGC
CTTTTTCTATATTTTTATATTCATGTGTAAACAAAAAATAAAATAAAATTCTGATCGCATAAAAAAAAAA
AAAAAAAAAAATTCCT
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FIG. 2. Sequence of *PLAC1* cDNA and the deduced encoded protein. The initiating ATG codon and the 24 amino acids in the putative leader peptide are in boldface type.

H12503 (Fig. 2; the sequence is deposited in GenBank under Accession No. AF234654). Attempts to extend the 5′ end of the available cDNA sequence on mRNA preparations from placenta have thus far been unsuccessful, but the cDNA sequence and poly(A) tail would account for all or nearly all of the 1.7-kb RNA species detected in Northern analyses. This is consistent with a likely promoter start site just upstream of the cDNA sequence (see below).

The 5′ untranslated sequence contains stop codons in all three possible translational frames, and the entire cDNA includes 291 bp of 5′ untranslated sequence followed by a 636-bp open reading frame (ORF) and 204 bp of 3′ untranslated sequence before the poly(A) addition site. By comparison to the genomic sequence, the transcription of *PLAC1* is in the telomeric to centromeric direction, opposite to the orientation of *HPRT*, and three exons are inferred. All have standard exon–intron junctions and are present within clone bWXD173 (Fig. 1). The first two exons are relatively small, 152 and 72 bp. Using the nucleotide numbering in the GenBank entry for bWXD173 (centromeric to telomeric), they extend from the genomic position 187,955 to 187,804 and from 131,589 to 131,518 within the yWXD173 sequence, respectively. The third, longer exon (885 bp) contains the entire open reading frame. It extends from position 96,175 to 95,296. Thus, the primary transcript of *PLAC1* would span about 92,700 bp, only about 2% of which is present in the mRNA.

Using the genomic sequence, computer-assisted analysis was extended to look for possible promoter motifs. TFSEARCH (www.genome.ad.jp/SIT/TFSEARCH.html),

```
      1                                                          60
PLAC1 MKVFKFIGLMILLTSAFSAGSGQSPMTVLCSIDWFMVTVHPFMLNNDVCVHFHELHLGLG
Plac1 MNLRKFLGGTVLVAFMLFSYSEQNQVNVLCSTDWFMVTVHPFLLNNDVYVHFYEVHLGLG

     61                                                         120
PLAC1 CPPNHVQPHAYQFTYRVTECGIRAKAVSQDMVIYSTEIHYSSKGTPSKFVIPVSCAAPQK
Plac1 CPPNHVHPHFYQFHYRVTECGIRIKAVSPDVVIYSSEIHYASKGSSTKYVIPVSCAAPRR

    121                                                         180
PLAC1 SPWLTKPCSMRVASKSRATAQKDEKCYEVFSLSQSSQRPNCDCPPCVFSEEEHTQVPCHQ
Plac1 SPWLTKPYSAKAPSNNMGATPKNDTSYHVFTLPEPSEQPNCSCPPYVYNQKSM-------
    181                                 212
PLAC1 AGAQEAQPLQPSHFLDISEDWSLHTDDMIGSM
Plac1 --------------------------------
```

FIG. 3. Alignment of the *PLAC1* and *Plac1* orthologous proteins. Identical residues are shaded.

a program that looks for putative transcription factor-binding sites, detected a TATAA sequence 19 bp upstream of the 5′ end of the cDNA, and a TATA-binding protein site was predicted at −25 bp (a score of 86.7, where 100 is maximum certainty). Two binding sites for standard human CCAAT/enhancer-binding proteins are also detected (at −60 to −54 residues from the 5′ end of the cDNA, with scores of 91.2 and 88.0). Of more specific possible relevance for a gene expressed early in development, TFSEARCH also detected three putative binding sites for chicken CdxA (scores of 100.0, 96.2, and 92.3) around nucleotides −27 and −20. CdxA is a homeobox protein that is first expressed in early epiblast and primitive streak and later continues to affect transcription in epithelial cells of embryonic gut and yolk sac as well as adult intestine, heart, kidney, liver, and lung (Frumkin *et al.*, 1991; Doll and Niessing, 1993; Margalit *et al.*, 1993).

Plac1, the Mouse Orthologue

Homology searches in the public EST database (Wheeler *et al.*, 2000) revealed strong conservation with only two mouse 5′ ESTs [clones C0008F04 and C0026B06 (GenBank Accession No. AA409600 and AA408322)] that our previous studies had recovered from transcripts in the ectoplacental cone of 7.5-dpc (days postcoitum) embryos (Ko *et al.*, 1998). The source of the homologous ESTs is consistent with the Northern analysis of human tissues, given that the ectoplacental cone gives rise to the murine placenta (see below). Mouse cDNA C0008F04 was completely sequenced; its 1074-bp insert encodes the full ORF, providing the predicted amino acid sequence in Fig. 3. The mouse and human genes are 75% identical at the DNA level (nucleotide sequences are deposited in GenBank under Accession No. AF3234653 for mouse and Accession No. AF234654 for human) and 60% identical at the amino acid level (Fig. 3).

cDNA clone C0008F04 was previously mapped as an anonymous cDNA on The Jackson Laboratory BSS backcross mouse DNA panel (http://www.jax.org/resources/documents/cmdata/bkmap/BSS.html) and designated *DXWsu72e* (Ko *et al.*, 1998). In the composite genetic map compiled by The Jackson Laboratory (http://www.informatics.jax.org/), *Plac1* (*DXWsu72e*) is placed 16.0 cM from the centromere, and *Hprt* is localized 17.0 cM from the centromere. This is in accord with the syntenic equivalence of the mouse and human regions containing these genes.

To confirm the physical proximity of *Hprt* and *Plac1* in mouse DNA, we studied three yeast artificial chromosomes (YACs), I114e9 (about 420 kb) and I27F11 (about 300 kb) from the UK Mouse Genome Center YAC library and M1C10 (about 280 kb) from the MIT mouse YAC collection. All had been demonstrated to contain the *Hprt* gene (by Dr. C. Huxley, who kindly provided the clones). A sequence-tagged site (STS) primer pair from the 3′ end of *Plac1* was positive on all three YACs. These results are consistent with a distance between *Plac1* and *Hprt* sequences, which is comparable to the 65 kb between the human orthologues.

The Putative PLAC1/Plac1 Protein

The human ORF encodes a putative protein of 212 amino acids, whereas the mouse encodes a highly homologous but shorter product of 173 amino acids (Figs. 2 and 3). This is apparently an intrinsic property rather than an artifact, because two independently recovered mouse cDNA clones were identical in their coding regions and 3′UTRs (untranslated regions), with a stop codon in the same location, and the longer putative human protein was inferred from both cDNA and genomic sequence.

Despite their putative different lengths, the mouse and human orthologues share a number of features. In their very similar amino acid sequences, the PSORT (psort.ims.u-tokyo.ac.jp) program, which looks for protein sorting signals and localization sites, suggests that PLAC1 and *Plac1* are both extracellular (with a 56 and 66.7% likelihood for human and mouse, respectively), and each contains a cleavable signal peptide of 23 residues, with a cleavage site between residues 23 and 24. It may be significant that both the human and the mouse proteins end as well as begin with methionine.

In addition, significant homology was detected for both mouse and human genes with the ZP3 (zona pellucida 3) protein of several species of eutherian mammals, including mouse. ZP3 is the specific sperm-binding glycoprotein in the zona pellucida. This binding is thought to confer species-specificity during fertiliza-

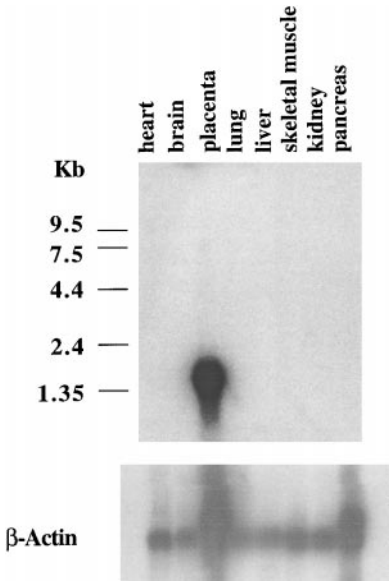

FIG. 4. Northern blot analysis of expression of *PLAC1*, tested against placenta and seven adult tissues in a Clontech MTN blot (see text). β-actin control hybridization is shown in the lower panel.

tion. Computer-aided prediction using the MOTIF program (www.motif.genome.ad.jp), searching against the PRINTS database of Attwood and Beck (1994) shows that both human and murine putative proteins contain the "zona pellucida sperm-binding protein signature," between amino acids 89 and 106 in each case (QDmVIYSTEIHYSSkgtP; see Discussion). Significant homology with the ZP3 protein family was inferred using the EMBL BLAST2 algorithm (dove.embl-heidelberg.de/Blast2). The predicted human protein shows 28% identity and 45% similarity ($P = 0.026$) to the ZP3 protein of a marsupial (AF079524) and is 30% identical and 45% similar ($P = 0.0018$) to the mouse equivalent. Significant homology to ZP3 was also inferred by the Blitz program (www2.ebi.ac.uk/bic_sw) of the European Bioinformatics Institute, which compares protein sequences to the nonredundant SWISS-PROT + TREMBL + SWISSNEW + TREMBLNEW databases.

Expression of PLAC1 and Plac1

The reported human and mouse cDNA clones were derived from placenta and protoplacental tissue, respectively, and Northern blot analyses with the cDNAs as probes have firmly supported the selective expression of the gene in developing placenta. For example, Fig. 4 shows a single human mRNA species of about 1.7 kb in term placenta, with no detectable signal in 7 other organs (Clontech blot MTN, bearing RNAs from heart, brain, lung, liver, skeletal muscle, kidney, and pancreas). In additional Northern analyses (data not shown), no signal was observed from spleen, thymus, testis, prostate, ovary, small intestine, colon, or peripheral blood leukocytes (Clontech blot MTII) or in fetal brain, liver, kidney, or heart ("fetal" blot). In further confirmation of specific expression, a dot blot (Clontech "Master Blot") containing 45 adult tissues and fetal spleen, thymus, and lung gave a strong, unique signal from placenta. The gene was thus named *PLAC1* (placenta 1), a name that has been approved by the HUGO International Nomenclature Committee.

The expression of the mouse homologue was analyzed with greater resolution in placentas at different stages of development. In agreement with the data for human *PLAC1*, preliminary Northern blot experiments with a Clontech "mouse embryo blot" detected an RNA species of about 1.6–1.7 kb, with very strong expression at 7 dpc that gradually declined during development to term. The analysis was confirmed and extended with more discriminating *in situ* analyses, as follows.

The expression of placenta-specific genes, such as *Mash-2* (Guillemot *et al.*, 1994) and the structural gene *4311* (Lescisin *et al.*, 1988; the gene was also known as *Tpbp*), was used as a control to orient and define the spatial limits of *Plac1* expression. *Mash-2* is expressed strongly in trophoblast cells in the labyrinth and more weakly in spongiotrophoblasts, in the labyrinth, and in the chorion; *4311*, is expressed in the spongiotrophoblast cells and their precursors in the ectoplacental cone.

Plac1 was expressed in the ectoplacental cone and trophoblastic giant cells at 7–7.5 dpc (Figs. 5A–5C). The giant cells (gc in Figs. 5A and 5B and specifically enlarged in Fig. 5C) are the specialized polyploid cells derived from the conceptus and are directly in contact with maternal tissues at implantation sites. At this stage, expression of a control trophoblast-specific marker *Mash-2* (Fig. 5B) is restricted to the ectoplacental cone/plate. At 8.5 dpc (Fig. 5E), *Mash-2* expression is restricted to the ectoplacental (or chorionic) plate, as previously reported (Guillemot *et al.*, 1994). In contrast (Fig. 5D), *Plac1* signal is seen in both giant cells and ectoplacental plate. In three independent experiments, two of them exemplified in Fig. 5, the level of expression of *Plac1* is consistently lower than that of *Mash2*. Probing at 9.5 and 10.5 dpc showed the same pattern, though with lower intensity of hybridization at 10.5 dpc (data not shown).

Three days later, by 11.5 dpc (and at 12.5 dpc, in additional probings not shown), the embryonic portion of the placenta is divisible into three well-defined layers: giant cells, spongiotrophoblasts, and labyrinth. In controls, as expected (Lescisin *et al.*, 1988), *4311* showed hybridization limited to the spongiotrophoblast layer (not shown), and *Mash-2* (Fig. 5G; cf. Guillemot *et al.*, 1994) hybridized to cells in the labyrinth and some of the spongiotrophoblast cells. Like *Mash-2*, *Plac1* (Fig. 5F and the enlargement in Fig. 5I) is expressed in patches of cells localized throughout the trophoblast layers, including the placental labyrinth and some spongiotrophoblasts (sp). Notably, faint expression of *Plac1* was observed in the trophoblast giant cells (Figs. 5H and

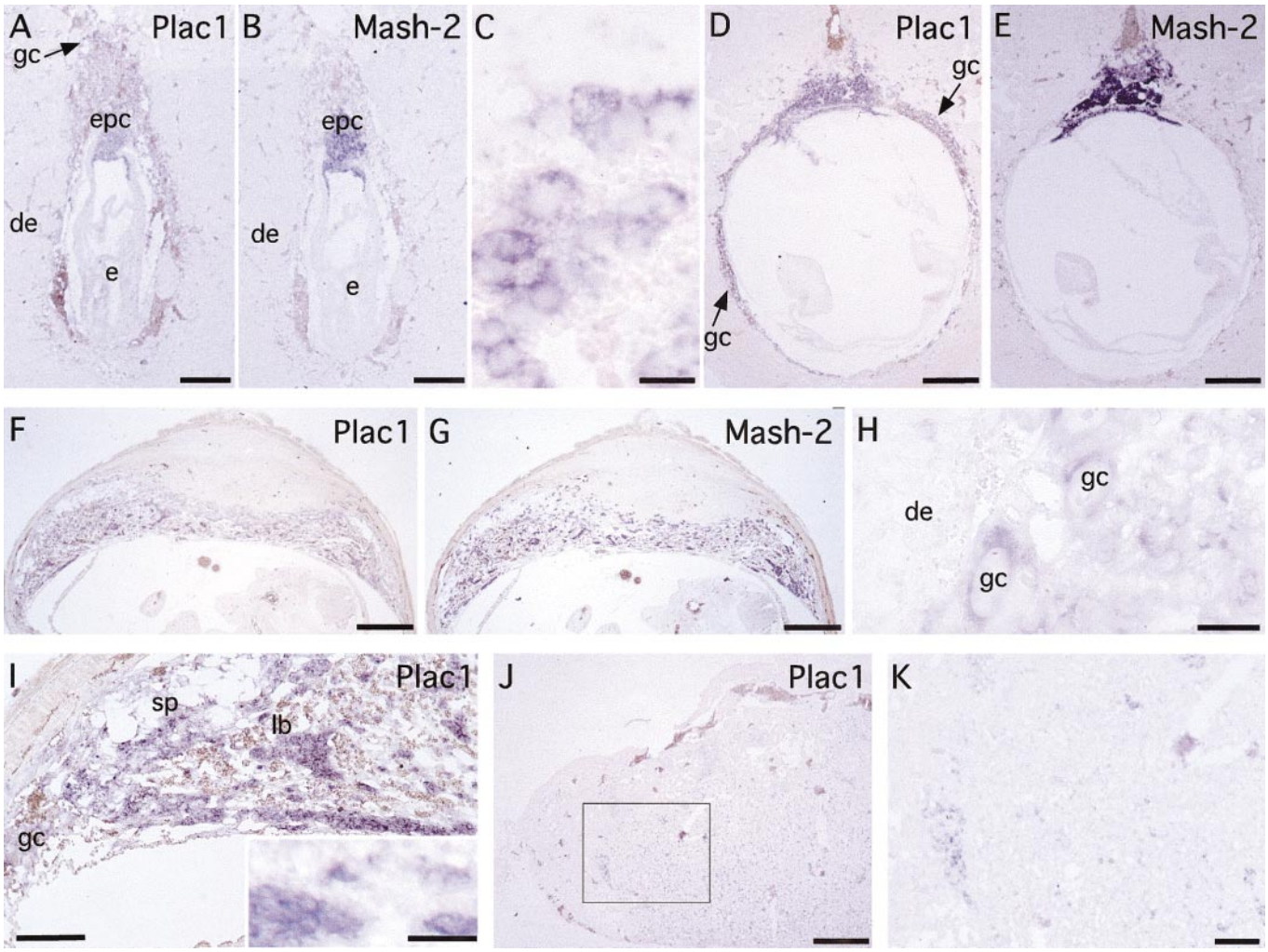

FIG. 5. Expression of *Plac1* in developing mouse placenta. Positive hybridization is purple; in all cases, control hybridization with Plac1 sense strand as a probe gave no signal. (**A–C**) 7.5 dpc. (**A**) *Plac1* hybridizes to the ectoplacental cone (epc) and the giant cells (gc) surrounding the embryo. Also indicated are the decidua (de) and embryo proper (e). (**B**) Control hybridization of a *Mash-2* probe, which is expressed only in epc. (**C**) Enlargement of a gc region of the embryo of **A,** with *Plac1* as a probe. (**D** and **E**) 8.5 dpc. Hybridization of probes of *Plac1* (**D**) to chorion/epc and gc, and of *Mash-2* (**E**) to chorion/epc. (**F–I**) 11.5 dpc. Comparative hybridization to placenta of probes for *Plac1* (**F**) and *Mash-2* (**G**). (**H**) Enlargement of a portion of gc at 11.5 dpc, at the border with the decidua (de), with *Plac1* as probe. (**I**) Enlargement of a portion of **F,** showing the hybridization of *Plac1* throughout the trophoblast layers: spongiotrophoblasts (sp), labyrinth (lb), and gc. The inset shows a fourfold further enlargement of one section, showing the stained trophoblastic cells. (**J** and **K**) 14.5 dpc. (**J**) *Plac1* shows weak hybridization to lb and sp. (**K**) Enlargement of the framed portion of **J,** showing remaining *Plac1* hybridization. Scale bars represent 200 μm in **A, B** and **I;** 20 μm in **C;** 400 μm in **D, E,** and **J;** 900 μm in **F** and **G;** 50 μm in **H** and the inset of **I;** and 100 μm in **K.**

5I)), where no *Mash-2* signal was reported (Guillemot *et al.*, 1994). These data are thus consistent with *Plac1* expression in all trophoblast-derived cells but no other detected cell type.

At 14.5 dpc, when the mouse placenta is structurally complete, only weak expression of *Plac1* is observed in the trophoblast giant cells (Fig. 5J and the enlargement of Fig. 5K). More delimited expression of the spongiotrophoblast-specific marker *4311* was evident in the sp layer of an alternate section of the same embryo (data not shown).

No hybridization signal was detected at later stages, so that *Plac1* is inferred to function only during the development of the placenta and to be dispensable thereafter (see Discussion).

DISCUSSION

Several genes are thus far reported to be predominantly expressed in the murine chorioallantoic placenta. They include placental lactogen (Yamaguchi *et al.*, 1994), 4311 (Lescisin *et al.*, 1988), adrenomedullin (Yotsumoto *et al.*, 1998), and perhaps because of a bias in searches, a number of transcription factors (Morasso *et al.*, 1999) that may be implicated in the complex promoter-restricted expression of proteins like placental lactogen (Liang *et al.*, 1999). The factors specified thus far include the zinc finger factor Rex-1 (Rogers *et al.*, 1991); GATA-3 (Ng *et al.*, 1994); members of the helix-loop-helix family of transcription factors [such as Mash-2 (Guillemot *et al.*, 1994) and Hxt (Cross *et al.*,

1995)]; a homeobox gene, *Dlx-3* (Morasso *et al.*, 1999); and *Gcm* 1 (Basyuk *et al.*, 1999). Similarly, two placenta-specific homeobox genes have been mapped to the X chromosome, *Esx1* (Li *et al.*, 1997; Li and Behringer, 1998) and *Psx* (Chun *et al.*, 1999). In contrast, the gene reported here appears to be a structural protein.

Plac1 expression is distinctly restricted in its time of expression and in its topographical localization, confined to the period of about 7 to 14.5 dpc in mouse trophoblast cells. The timing and location of its expression may be correlated with progressive extensive vascularization, which may provoke the down-regulation of *Plac1* expression in surrounding tissues. Studies of the function of intact and truncated promoters of *Plac1* in trophoblast cultures may clarify the basis for the spatial and temporal selectivity of expression.

PLAC1 falls in a chromosomal region that has been widely used in studies of mutation, because it is possible to select for forward lesions in *HPRT* (and for reversions as well, in a two-way selection scheme). Sizable deletions of hundreds of kilobases in cell culture systems (Fuscoe *et al.*, 1994) and human peripheral blood cells (Nelson *et al.*, 1995) have in general shown no effects other than HPRT deficiency; and in the *in vivo* experiments of Kushi *et al.* (1998), comparable deletions have produced poor fetal development (runtiness and low viability) as the only, though very interesting, phenotype.

Corresponding to the lack of effect of regional deletions in cell cultures, direct sequence analysis of this region of human DNA reveals a plethora of repetitive sequence elements, with *PLAC1* as the only gene detected in a large zone around *HPRT*. The gene-poor region extends all the way to *GPC3* in one direction and at least 200 kb, without the detection as yet of the next telomeric gene, in the other direction. This circumstantial evidence points strongly to loss of *Plac1* as underlying the runty phenotype in the mouse. The critical test for this candidacy will be the direct disruption of the *Plac1* gene to see whether that mimics the effects of loss of function by deletion.

Other independent evidence has indicated that a locus (*Ihpd*, for interspecific hybrid placental dysplasia) that contributes to abnormal placental development in mouse hybrids is linked to the *DXMit8* marker in the same region [Zechner *et al.* (1996), though later analyses have suggested that there may be several X-linked loci involved (Hemberger *et al.*, 1999)]. In this case, opposite phenotypes of placental hypotrophy versus hypertrophy were observed in reciprocal crosses compared to backcrosses. The placental state was associated with growth impairment or increased fetal growth, respectively. Speculatively, Plac1 could contribute to Ihpd, but there are as yet no studies of differences in the structure or interactions of the gene in different mouse species that could test such a notion.

In addition to the circumstantial evidence of its map location and pattern of expression, an independent hint that the gene may be related to early developmental events comes from computer-aided homology searches. In particular, *Plac1* contains the ZP3 motif (see Results) that has been recognized in a variety of extracellularly exposed receptor-like glycoproteins, including TGF-β receptor type III, uromodulin, and glycoprotein GP2 as well as the sperm receptors ZP2 and ZP3 (Bork and Sander, 1992). The PLAC proteins show additional weak but significant overall similarities to ZP-family proteins throughout their extracellular domain.

Based on these first indications, one can speculate that *Plac1* may participate in forming or stabilizing the fetus–mother interface, perhaps as a receptor. It could, however, conceivably function only during the defined period of its expression, from its initial appearance in the ectoplacental cone to its near extinction by 14.5 dpc.

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