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Biochemistry

Molecular cloning of a prolactin-related mRNA expressed in bovine placenta

(placental hormone/placental lactogen/bovine prolactin-related cDNA I/nucleic acid hybridization)

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ABSTRACT **Bovine (*Bos taurus*) prolactin-related cDNA I (bPRC-I), distinct from the isolated bovine placental lactogen, was derived from bovine fetal placental mRNA by molecular cloning. The nucleotide sequence is 63% homologous to bovine prolactin cDNA and only 45% to bovine growth hormone. The region of bPRC-I corresponding to the 5′ portion of the signal peptide and 5′ untranslated region of bovine prolactin mRNA is markedly different from prolactin. The predicted protein is 39% homologous to bovine prolactin and about 30% to the related placental hormones in rodents. This identification of a prolactin-related gene in the cow in addition to those reported in rodents suggests that multiple prolactin-related genes expressed in the placenta may be a general phenomenon in nonprimates. The role of these related hormones during gestation remains to be investigated.**

Several of the diverse functions attributed to growth hormone and prolactin are important during pregnancy, including growth of the fetus and placenta, control of maternal metabolism, and development of the maternal mammary gland. Control of these activities may involve the evolutionarily related placental hormones, known as placental lactogens, either to replace or assist the pituitary hormones (1–7). Placental members of the prolactin–growth hormone family have been identified in several species, including ruminants (cow, sheep, and goat) (8–13), rodents (rats, mice, and hamsters) (14–17), and primates (18). In most studies to date, these proteins have been identified by their prolactin or growth hormone activity or receptor binding. In rodents, this methodology has resulted in the isolation of two placental products and one decidual protein (14–16), whereas in ruminants, only a single protein has been identified (8–13). In the human, a single protein closely related to growth hormone has received considerable attention (18). In contrast, protein characterization as well as recent reports of placental mRNAs in rodents (19–23) suggest that these placental hormones in non-primates are more closely related to prolactin.

In the pregnant cow, circulating levels of prolactin-like and growth hormone-like activity, as measured by classical assays, are much lower than those of other species, including other ruminants (6, 7). The isolated bovine placental lactogen is 32 kDa, which is quite large for a member of this gene family (8–10, 24). Specific radioimmunoassays and bioassays have confirmed the low levels of this hormone in the maternal circulation, although fetal levels are comparable to those in other species (25, 26).

Little is known about these placental hormones in nonprimates and nonrodents. To further characterize the structure, regulation, and activity of the placental members of the prolactin–growth hormone gene family, we examined a bovine (*Bos taurus*) genomic library and a cDNA library prepared from bovine fetal placental mRNA for sequences related to prolactin and/or growth hormone.‡ Here we report the isolation and characterization of a cDNA that is more homologous to bovine prolactin (bPRL) than bovine growth hormone (bGH) and is distinct from the reported placental lactogen in this species.

MATERIALS AND METHODS

Bovine Genomic Library. The construction and amplification of the bovine genomic library in λ Charon 28 have been described (27). Plaques were lifted to nitrocellulose (28), and filters were hybridized as described (29), except that temperatures of prehybridization and hybridization were at 32°C (low stringency) or 42°C (high stringency). Nick-translated bPRL or bGH cDNA (about 10^8 cpm/μg) (27, 30, 31) was used as the hybridization probe.

Construction of a cDNA Library. Bovine fetal cotyledons from about 6 months gestation were collected into liquid nitrogen at the time of slaughter. Frozen tissue was homogenized in 100 mM Tris·HCl, pH 8/100 mM NaCl/40 mM vanadyl-ribonucleoside complex (synthesized as described, ref. 32) and 1 mg of heparin per ml. The mixture was repeatedly extracted with phenol/chloroform, 1:1 (vol/vol), followed by ether. RNA was pelleted through a cesium chloride cushion (33) and enriched for poly(A)$^+$ RNA by passing it twice over an oligo(dT)-cellulose affinity column (34). RNA from bovine liver and pituitaries was prepared in a similar manner.

Three cDNA libraries were made by using essentially the same procedures (35). cDNA was synthesized by reverse transcriptase, and the second strand was synthesized using *Escherichia coli* DNA polymerase I large fragment, followed by reverse transcriptase. The double-stranded cDNA was treated with S1 nuclease and cloned into *Pst* I-cut pBR322 using dG-dC homopolymer tailing (36). For the second library, only cDNA between 600 and 1300 base pairs (bp) was cloned. The cDNA-containing plasmids were transformed into *E. coli* HB101 (37), and the libraries were screened with nick-translated genomic DNA or placental cDNA restriction enzyme fragments (38).

Sequence Analysis. Sequence of overlapping pieces of both strands was determined by the dideoxynucleotide chain-termination method (39) after subcloning into M13mp8, -mp18, or -mp19 (40). Sequences were analyzed using the University of Wisconsin Genetics Computer Group programs (41).

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Abbreviations: bPRC-I, bovine prolactin-related cDNA I; bPRL, bovine prolactin; bGH, bovine growth hormone.

‡The nucleotide sequence reported in this paper is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J02944).

RESULTS

To identify placental relatives of the growth hormone gene family, we looked for sequences that were similar but not identical to bPRL or bGH cDNAs. About 10^6 plaques of the bovine genomic library were screened with these cDNAs under conditions of low and high stringency (see *Materials and Methods*). The genomic clone bP04 hybridized to bPRL cDNA under conditions of low stringency but not under conditions of high stringency (Fig. 1). bP04 did not hybridize to bGH cDNA at either level of stringency. One of the regions of bP04 that hybridized to bPRL cDNA was located in a 1.8-kilobase (kb) *Pst* I fragment, bP04-PstI, which was used as a probe in subsequent studies.

To ascertain if these sequences were expressed in the placenta, bP04-PstI was hybridized to poly(A)$^+$ RNA prepared from bovine placenta, pituitary, and liver using RNA transfer blot hybridization under the high stringency conditions (Fig. 2). This genomic fragment hybridized only to placental RNA (lane 1) of about 1100 nucleotides in length. Pituitary prolactin RNA (lane 2) did not hybridize to this genomic restriction enzyme fragment under these conditions. These data indicated that the gene corresponding to bP04 was closely related to a placental member of the prolactin–growth hormone gene family.

The bP04-PstI genomic fragment was used to screen 237 clones from the first cDNA library prepared from mRNA purified from fetal cotyledonary tissue. One clone was identified that contained an insert of 360 bp. A secondary library (1650 recombinants) was screened with this cDNA to obtain a longer clone. Several additional clones were identified, the longest of which was pbPL11 (820 bp). Sequence analysis indicated that this cDNA was lacking sequences coding for the amino terminus. A third library was screened with the 5′ *Pst* I fragment of pbPL11 (denoted by the bar in Fig. 3), resulting in the identification of several clones including pbPL582.

Nucleotide sequences of pbPL11 and pbPL582 were determined using the sequencing strategy shown in Fig. 3. The sequences of overlapping regions of these clones were identical. The combined sequence of this cDNA, bovine prolactin-related cDNA I (bPRC-I), is shown in Fig. 4.

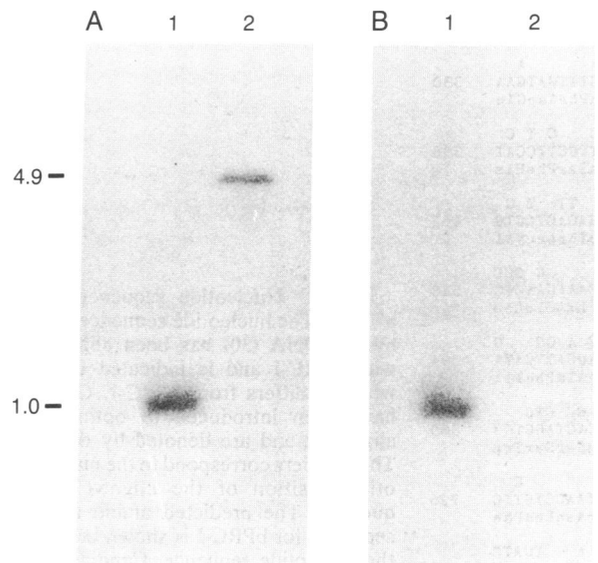

FIG. 1. Hybridization of bPRL cDNA to bovine genomic clone bP04. Lanes 1, 1 μg of bPRL cDNA recombinant plasmid cut with *Pst* I; lanes 2, 5 μg of genomic clone bP04 cut with *Bam*HI. Fragments were separated on a 0.8% agarose gel, transferred to GeneScreen (42), and hybridized to nick-translated bPRL cDNA under conditions of low stringency (*A*) and high stringency (*B*). Under conditions of low, but not high stringency, bPRL cDNA hybridized to a 4.9-kb *Bam*HI fragment of bP04.

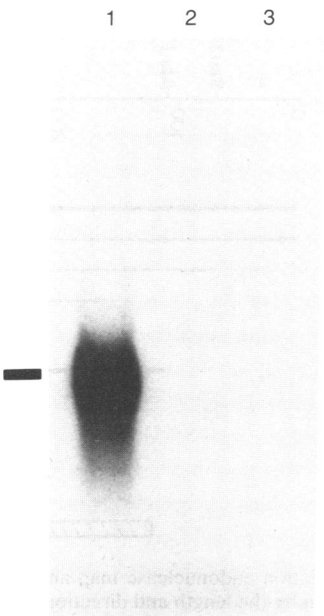

FIG. 2. Transfer blot analysis of mRNA from placenta (lane 1), liver (lane 2), and pituitary (lane 3). Five micrograms of poly(A)$^+$ RNA from each tissue was fractionated on formaldehyde gels (43), transferred to GeneScreen, and hybridized to nick-translated bP04-PstI. The bar marks the position of the placental RNA (1100 nucleotides) hybridizing to the genomic fragment.

bPRC-I contains an open reading frame of 267 amino acids beginning with the first nucleotide and ending with the termination codon TAA at nucleotide positions 808–810. Three AUGs are present in the correct reading frame in the 5′ region of this cDNA (marked with asterisks in Fig. 4). Because the protein predicted by this cDNA has not been identified, the initiating methionine cannot be designated with certainty (see *Discussion*). A region coding for strongly hydrophobic amino acids is present from nucleotides 151 to 186, which is 80% homologous to the carboxyl terminus of the signal peptide of bPRL. Consensus sites for N-glycosylation, Asn-Xaa-Ser/Thr (44), are predicted at three sites in the protein (denoted by dashed lines in Fig. 4). Following the termination codon is a 3′ untranslated region of ≈150 bp, with a poly(A) signal at nucleotides 931–936 (45).

The placental mRNA described by bPRC-I is related to the bovine prolactin cDNA throughout most of its length (Fig. 4). bPRC-I is 63% homologous overall to bPRL at the level of nucleotide sequence compared to only 45% to bGH. The region of bPRC-I from nucleotide 141 through the 3′ untranslated region is quite similar to bPRL cDNA. However, 5′ to nucleotide 141 of bPRC-I, they are quite different, sharing only 5 of the next 24 nucleotides. Although a short stretch of slightly greater homology (14/25) is present, this 5′ region is only about 30% (31/102) homologous overall to bPRL.

Consistently, the protein predicted by bPRC-I is much more similar to bPRL than bGH (Fig. 5). Overall homology to bPRL at the level of amino acid sequence is 39% compared to 17% to bGH. It shares about 30% amino acid sequence with the rodent placental proteins. bPRC-I exhibits a pattern of codon usage similar to prolactins, demonstrating a lesser preference for G/C in the third codon position than do growth hormones and using TAA as the termination codon (24).

DISCUSSION

The members of the prolactin–growth hormone gene family are believed to have evolved by gene duplication events (24,

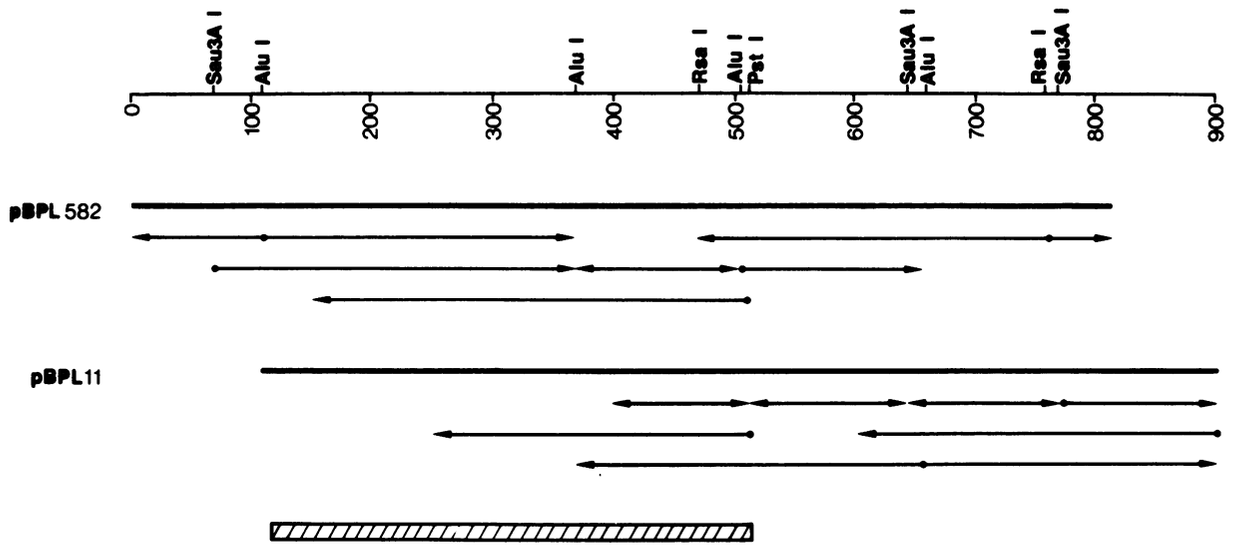

FIG. 3. Restriction endonuclease map and sequencing strategy for pbPL582 and pbPL11. The sequence obtained from a particular M13 subclone is shown by the length and direction of the arrow. The hatched bar under the 5′ portion of pbPL11 is the fragment used to screen the third cDNA library from which pBPL582 was isolated.

46, 47). We have described a cDNA (bPRC-I) corresponding to a mRNA in the bovine fetal placenta that is more closely related to bPRL than bGH, like the placental hormones in rodents, and in contrast to those in primates.

The 5′ region of bPRC-I that is of low homology to bPRL corresponds to the first exon of prolactin, which contains a portion of the signal peptide and 5′ untranslated region (30). If the aligned sequences of bPRC-I function similarly for this placental gene, these differences would not contribute to the mature protein but may have significant implications for regulation of expression of this gene.

Future studies must confirm that the mRNA corresponding

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bPRL    ........................................TG TTGGCTG     C A AGGA
bPRCI 1 GAAATGAACATCAGTATGTCCCATCCCATCAGGCTGACTCCCCACGGTCAACAGGAGTCCTCACCC   66
        GluMetAsnIleSerMetSerHisProIleArgLeuThrProHisGlyGlnGlnGluSerSerPro
           *          *
        GA AGCTTCCTGGTG AGTGTG TT  TG AA  ATCA CA  A      G  AA  .....TTCG
     67 TGGGATCATTCCACAATCCCCATGGCTCCAGCTCCCAGCTTCCGTGGACACCAGTGGACTTACAAC  132
        TrpAspHisSerThrIleProMetAlaProAlaProSerPheArgGlyHisGlnTrpThrTyrAsn
                           **
        T GCAGAA       C       C          G GG    A     A   T       G  TGTG
    133 CCTGTCCGAGGGTCCTGCCTGCTGCTGCTGCTCATGTCTAATCTGCTCCTGTGCCAAGGCAAA  198
        ProValArgGlySerCysLeuLeuLeuLeuLeuMetSerAsnLeuLeuLeuCysGlnGlyLys

        GTC C A CC  GT T    CA T G CC  G AA  GC A GT    C  CG    CT    GAC
    199 TCATGCCCGTCCTGCGGTCCTGACGTGTTTGTCTCCTTACGGAAATCCTTTACAGACAGGTTTATG  264
        SerCysProSerCysGlyProAspValPheValSerLeuArgLysSerPheThrAspArgPheMet

        CGG  A T  TGG G     CT  A  C  G    C   T GGA         C  A      A
    265 AATGCCGCCAGCCTCTCCCATGACTTCTATAACCTTTCCACAATAATGTTCAATGAGTTTGATGAA  330
        AsnAlaAlaSerLeuSerHisAspPheTyrAsnLeuSerThrIleMetPheAsnGluPheAspGlu
                                  - - - - -
        CGG               GGG T AT  C  TG C CT   C        T   TCC   C T C
    331 AAATATGCCCAGGGCAAACTCTACTATATCAATGTCACCAAGAGCCTGCCACACCAATTCCTTCCAT  396
        LysTyrAlaGlnGlyLysLeuTyrTyrIleAsnValThrLysSerCysHisThrAsnSerPheHis
                                  - - - - -
        A C  G     T A   ACA  C   A     CC TCA     T     TG GC T  TT  T G
    397 GCTCCCGAAGAAAGAGATATAGTCCAGCAGACGAACATTGAAGACCTTAGTAAGTGGACACTCGTG  462
        AlaProGluGluArgAspIleValGlnGlnThrAsnIleGluAspLeuSerLysTrpThrLeuVal

              CG          G C      T    C         C   G A G GG         G GCC
    463 TTGCTGTACTCCTGGAATAATCCTCTGCATCATCTAGTCACGGAGCTGCAGCATATGAAAGAACTG  528
        LeuLeuTyrSerTrpAsnAsnProLeuHisHisLeuValThrGluLeuGlnHisMetLysGluLeu

        C  G T  TA        G  G     T GA A      G AGAAAACA ACG      TG A GG   G
    529 TCAAACGCCTTCCTATCAAGCGCCACAAGGTTTGAGAACATGTCAGAGAAACTTCAAGCATTCATA  594
        SerAsnAlaPheLeuSerSerAlaThrArgPheGluAsnMetSerGluLysLeuGlnAlaPheIle
                                     - - - - -
           ATGAT   TG  C  G      CC GG  C ......  AAGA  CTG  C CTA CC GTG
    595 GAGCGTCAATTCAGCAAGATTATTGTTCCAGTCTTGAATACGATGATCCAGGCTCGCAGTAGCTGG  660
        GluArgGlnPheSerLysIleIleValProValLeuAsnThrMetIleGlnAlaArgSerSerTrp

        T          G      GCAAA T AG A        GCA    T     T CT            C
    661 ACAGGACTCCCATCCCTAATGTCCAGCGCTGAAGATAGGCGTCATTCGGAATTTTATAACCTGTTC  726
        ThrGlyLeuProSerLeuMetSerSerAlaGluAspArgArgHisSerGluPheTyrAsnLeuPhe

        C                      A C  GA       CT   C T   C       AT   A   TCATC
    727 TACTGCCTGCGCAGGGATTCACGTAAAGTTGACATGTACATCAAGATCCTGACGTGCCGAA.....  792
        TyrCysLeuArgArgAspSerArgLysValAspMetTyrIleLysIleLeuThrCysArgThr...

        TA A     C AC       GC      T       CT      TT          G T  TT   G  CC
    793 .CCCACAAAACGTGCTAAAATCCACA.TCCATCTCATCCAGCTCTGAGATAG.TCAAAATCATGTAT  858
           HisLysThrCys

        T  CTG   AAA   T TCTG GC   T T     T G              GGC C          T  A
    859 CCCACAGCGGGCTACCTGAAATTTCACAGCTCTTTAATGCATGCTT...TGTAATGGGTCTCCTCT  924

        ....          A C     T  G    C   G C
    925 TAAAAAAATAAACATAGACGCTTTAGAGATATGAAAATC  963
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FIG. 4. Nucleotide sequence of bPRC-I. The nucleotide sequence for bPRL cDNA (30) has been aligned with bPRC-I and is indicated only where it differs from bPRC-I. Gaps have been introduced to optimize alignment and are denoted by dots. The numbers correspond to the nucleotide position of the aligned sequences. The predicted amino acid sequence for bPRC-I is shown below the nucleotide sequence. Candidates for the initiation methionine are labeled with asterisks; the preferred residue is marked with two asterisks. Consensus sequences for glycosylation are underlined with a dashed line. The termination codon is designated with a solid horizontal line above and below. The poly(A) signal AATAAA is underlined with a solid line.

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bPRCI   MAPAPSFRGH QWTYNPVRGS CLLLLLLMSN LLLCQGK.SC PSCGPDVFVS  50
bPRL    .........m dskgssqkGS rLLLLLvvSN LLLCQGvvSt PvCpngpg.n
mPL     .........mk lslsqPcsfS gaLLLLavSN LLvwekvtSl Pn.......
mPLF    .........ml pssiqPcs.. wiLLLLLvns sLLwknvaSf PmCamrngr.
mPRP    .........ml psliqPcs.S gtLLmLLMSN LfLwekvsSa Pinas...ea
rPLII   .........vq lsltqPcf.S gtLLmLavSt LLLweqvtSa P........n
rPLPA   .........mH lslshqws.S wtvLLLLvSN LLLwentaSa mrakllnvh.
bGH     .........mm aagprts... .LLLafall. cLpwtqvvga fpa.......

bPRCI   LRKSFTDRFM NAASLSHDFY NLSTIMFNEF DEKY...... ....AQGKLY 100
bPRL    cqvSlrDlFd rAvmvSHyih dLSseMFNEF DkrY...... ....AQGKgf
mPL     yRlpteslyq rvivvSHnah dLaskaFmEF emKf...... ....grtawt
mPLF    cfmSFeDtFe lAgSLSHnis ievselFNEF ekhY...... ....snvsgl
mPRP    vlsdlkDlFd NAtvLSgems kLgvIMrkEF fmnsfssetf nkiildlhks
rPLII   yRmStgslyq rvveLSHyth dLaskvFiEF DmKf...... ....grtvwt
rPLPA   nytSygDtwn qAikiSqDmn qyisdlsthv kifY...... ....AQGrgf
bGH     .msls.glFa NAvlraqhlh qLaadtFkEF ertY...... .ipegQrys.

bPRCI   Y...INVTKS CHTNSFHAPE ERDIVQQTNI EDLSKWTLVL LYSWNNPLHH 150
bPRL    ...itmalnS CHTsSlptPE dkeqaQQThh EvLmsliLgL LrSWNdPLyH
mPL     Yg...lmlsp CHTaailtPE nseqVhQTts EDLlKvsiti LqaWeePLkH
mPLF    rd...kspmr CnTsflptPE nkeqarlThy aaLlKsgami sdaWesPLdd
mPRP    tenitkafnS CHTvpinvPE tvedVrkTsf EeflKmvLhm LlaWkePLkH
rPLII   hn...lmlsp CHTaaiptPE nseqVhQaks EDLlKvsiti LqaWqePLkH
rPLPA   e....rrTtr CHTsSlssPE nkeqaQQfql EvLlglshsL LqaWlNPLHH
bGH     ..iqntqvaf CfsetipAPt gkneaQQksd leLlrisLlL iqSWlgPLqf

bPRCI   LVTELQHMKE LSNAFLSSAT RFENMSEKLQ AFIERQFSKI IVPVLNTMIQ 200
bPRL    LVTEvrgMKg apdAiLSrAi eiEeenkrLl egmEmiFgqv IpgakeTep.
mPL     mVaavaalph vpdtlLSrtk elEeriqgLl eglkiiFnrv ypgavasd..
mPLF    LVsELstiKn vpdiiiSkAT dikkkinavr ngvnalmStm lqngdeekk.
mPRP    LVTELsalpE cpyriLSkAe aiEaknkdLl eyIiRiiSKv npaikened.
rPLII   iVaavatlpd gSdtlLSrtk elEeriqgLl eglEtilSrv qpgavgsd..
rPLPA   LwaEmcdrlg stpptLykAl mlkesniKLl daIkniakKg nfeinqkan.
bGH     Lsrvftnslv fgtsd..rvy ek...lkdLe egIlalmrel edgtpragkQ

bPRCI   ARSSWTGLPS L..MSSAEDR RHSEFYNLFY CLRRDSRKVD MYIKILTCRT 250
bPRL    .ypvWsGLPS L..qtkdEDa RySaFYNLlh CLRRDSsKiD tYlKlLnCRi
mPL     .ytfWsaws. .dlqSSdEst knSalrtLwr CvRRDthKVD nYlKvLkCRd
mPLF    .npaW..... .flqSdnEDa RihslYgmis CLdnDfkKVD iYlnvLkCym
mPRP    .yptWsdL.. dslkSadket qffalYmfsf CLRiDletVD flvnfLkCll
rPLII   .ytfWsew.. sdlqSSdkst kngvlsvLyr CmRRDthKVD nflKvLkCRd
rPLPA   .ytaWseL.. gflqSpnrDt RyfaFYNLFh CLkkDSnnVe MYlKlLkCRl
bGH     .ilkqTydkf dtnMrSd.Da llkn.YgLls CfRkDlhKte tYlrvmkCRr

bPRCI   ..HKTC
bPRL    iynnnC
mPL     vhnnnC
mPLF    lkidnC
mPRP    lyddvCysgf
rPLII   iynnnC
rPLPA   i.rskC
bGH     fgeasCaf
```

FIG. 5. Comparison of the amino acid sequence predicted by bPRC-I to that of bPRL (30), mouse placental lactogen (mPL; ref. 23), mouse proliferin-related protein (mPRP; ref. 20), mouse proliferin (mPLF; ref. 19), rat placental lactogen II (rPLII; ref. 21), rat prolactin-like protein A (rPLPA; ref. 22), and bGH (27). The AUG at nucleotides 88–90 of bPRC-I is assumed to be the initiating methionine. Gaps, marked by dots, have been introduced to maximize homologies. Numbers correspond to amino acid position of the aligned sequences. Residues identical to the protein predicted by bPRC-I are in uppercase and those differing are in lowercase.

to bPRC-I is translated and address the nature of the bovine gene product. The AUG marked with two asterisks in Fig. 4 appears to be the most likely initiation codon. Consistent with this position, *in vitro* translation of the hybrid-selected mRNA (data not shown) resulted in a protein of about 27.5 kDa. In addition, beginning with the glycine at amino acid position 19 (Fig. 5), the predicted protein is very similar to the signal peptide of bPRL and may function as such for the protein predicted by bPRC-1. However, the position of the hydrophobic region suggests that the signal peptide would be at least 36 amino acids, which is quite long for a eukaryotic signal peptide, even for this gene family (48, 49). Initiation of translation at the favored methionine would yield a prehormone of 238 amino acids. Assuming that the hydrophobic region homologous to bPRL is the signal peptide, the mature protein would be about 200 amino acids. This is similar in size to the other members of this gene family (24).

Depending on the site of cleavage of the signal peptide of bPRC-I, four to six cysteine residues may be present in the mature protein (amino acid positions 40, 43, 111, 231, 248, and 256 in Fig. 5). In most prolactins and mouse proliferin, the six cysteines are believed to form three disulfide bonds, compared with two bonds in growth hormones. In bPRC-I, five of the six cysteines are in positions similar to those in the prolactins, but the remaining cysteine is separated from the first by only two amino acids and may be too close to form a loop (50). Mammalian prolactins contain two tryptophans, and growth hormones contain a single tryptophan. Rat placental lactogen II and rat prolactin-like protein A contain additional tryptophans, and three are predicted for bPRC-I. Two of these residues (at amino acid positions 144 and 205) are in positions similar to bPRL. The third is in a position different from the additional tryptophans present in the rodent hormones.

Bovine prolactin and rat and mouse placental lactogen II do not contain any potential glycosylation sites. However, the consensus sites for N-glycosylation present in the predicted product of bPRC-I indicate that this protein may be modified with carbohydrates *in vivo*. Mouse proliferin and proliferin-related protein as well as rat prolactin-like protein A contain potential glycosylation sites, suggesting that this might not be unusual for these placental proteins.

Bovine placental lactogen described by Bremel and his colleagues is somewhat heterogeneous in size and charge (51), but protein sequence analysis indicates two sequences identical except for the residues on the amino terminus (K. Shimomura and R. D. Bremel, personal communication). The protein predicted by the bPRC-I sequence is distinct from their protein in amino acid composition (11) and in the sequence of its N terminus, sharing only 5 of the 26 N-terminal residues. Thus, it seems that like rodents, the bovine genome also contains multiple members of this gene family, and more than one may be expressed in the placenta. Ongoing work in our laboratory has confirmed the presence of multiple placental prolactin-like mRNAs in this species.§

As was the case with the rodent placental cDNAs that were identified using recombinant DNA technology, the existence of bPRC-I has not been suggested by the extensive studies examining prolactin and growth hormone-like activity in the placenta (6, 7). However, the product of at least one of the rodent genes, mouse proliferin, has been identified *in vivo* using an antibody prepared to a fusion gene product (19). Perhaps these additional placental proteins do not interact with the receptor preparations commonly used to assay lactogenic or somatogenic hormones, either because of species differences in receptors (heterologous systems have commonly been used to study these products) or because these placental proteins may not bind to previously identified lactogenic or somatotropic receptors.

The present report, combined with recent reports of the molecular cloning of placental members of the prolactin-growth hormone family in rodent species (19–23), suggests that placental expression of multiple prolactin-related genes may be a common feature among many mammals, whereas the growth hormone-like placental hormones found in primates may be relatively unique. Failure to detect the product of bPRC-I by classical receptor assays and its marked sequence divergence from the pituitary hormones raises the possibility of specific receptors for this protein. Isolation of the *in vivo* gene product or synthesis of the protein by recombinant DNA methodology may allow us to test this hypothesis.

§Schuler, L. A. & Hurley, W. L., the 68th Annual Meeting of the Endocrine Society, Anaheim, CA, 1986, June 25–27, 1986, p. 139 (abstr.).

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