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A new member of the prolactin-growth hormone gene family expressed in mouse placenta

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Mouse placenta has been found to contain an mRNA that encodes a previously unidentified member of the prolactin-growth hormone family. This 1.1-kb mRNA (designated PRP mRNA) was detected as a cDNA clone that hydridized to a cDNA clone of mouse proliferin, a recently described growth-associated placental protein related to prolactin. PRP mRNA levels are highest in the fetal part of the placenta and peak at day 12 of gestation, decreasing gradually until term. The 972-bp sequence of PRP mRNA, determined from two cDNA clones, encodes a protein of 244 amino acid residues that has a hydrophobic leader sequence. The protein encoded by PRP mRNA has significant homology to all of the members of the prolactin family, yet is different from each of them; it also differs from mouse placental lactogen. Nucleotide sequence homology is most extensive between PRP and proliferin mRNAs, particularly at their 5′ ends, where they share 92 of the first 97 nucleotides.

Key words: hormone/placenta/prolactin/proliferin/proliferin-related protein

Introduction

The placenta is the source of several peptide hormones that are homologues of hormones synthesized in other endocrine tissues (Krieger, 1982; Boime *et al.*, 1982). These placental hormones are thought to play crucial roles in normal fetal development. During the latter half of gestation in rodents, removal of the pituitary does not block progression of the pregnancy if a functional placenta is retained (Selye *et al.*, 1933; Newton and Beck, 1939; Gardner and Allen, 1942), suggesting that placental hormones can substitute for the pituitary hormones prolactin and growth hormone. One of the major placental hormones is placental lactogen, which is a member of the prolactin-growth hormone family (for review, see Talamantes *et al.*, 1980). In rodents, two forms of placental lactogen have been reported that have activities similar to prolactin and bind tightly to prolactin receptors (Robertson and Friesen, 1981; Robertson *et al.*, 1982; Colosi *et al.*, 1982; Soares *et al.*, 1983).

The known placental lactogens may not account for all of the prolactin-like activity in the placenta. Recently we described another member of the prolactin family called proliferin, whose mRNA was found in certain proliferating mouse cell lines in culture, but was absent in quiescent cells (Linzer and Nathans, 1983, 1984a, 1984b). Subsequently proliferin mRNA and protein were shown to be synthesized in mouse placenta and the protein was shown to differ from placental lactogen (Linzer *et al.*, 1985). During the isolation of proliferin cDNA from a placental cDNA plasmid library we noted that there was a class of recombinant plasmids that hybridized weakly to proliferin cDNA. We report here that the weakly hybridizing cDNA encodes yet another protein related to prolactin. Because of its close relationship to proliferin, we refer to this new member of the prolactin-growth hormone family as proliferin-related protein or PRP.

Results

Isolation of PRP cDNA clones

After identification of mouse placenta as an *in vivo* source of mRNA hybridizing to proliferin cDNA sequences (Linzer *et al.*, 1985), we sought to clone the proliferin-related mRNA present in placenta. Placental poly(A)$^+$ RNA from late gestation BALB/c mice was transcribed into cDNA and cloned into pBR322. Approximately 1500 bacterial colonies transformed with these recombinant plasmids were screened by hybridization to the proliferin cDNA clone PLF-1 (Linzer and Nathans, 1984b). As shown in the autoradiograph of one such filter (Figure 1), hybridizing clones were detected that gave either strong or weak signals. Of the 11 positive colonies, seven showed strong and four showed weak hybridization. The strongly hybridizing clones have been shown to correspond to the placental form of proliferin (Linzer *et al.*, 1985). The weakly hybridizing clones were similar to each other, but differed from proliferin plasmids as judged by restriction enzyme cleavage patterns. We refer to the weakly hybridizing plasmids as PRP (proliferin-related protein) clones.

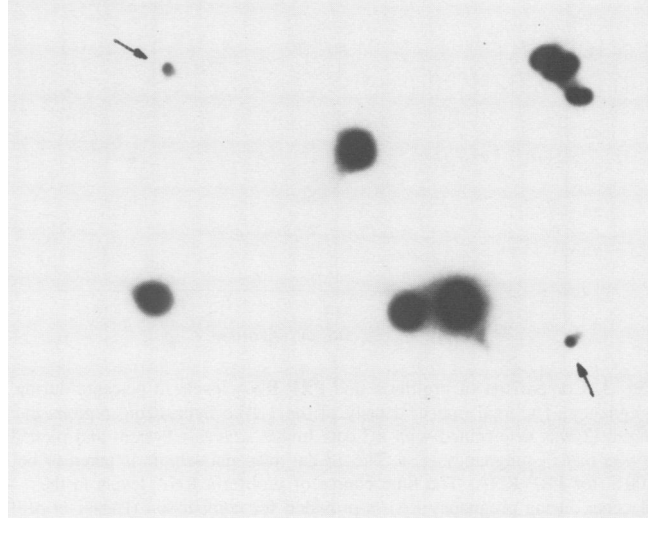

Fig. 1. Detection of proliferin-related placental cDNA clones. Bacterial colonies containing mouse placental mRNA sequences cloned into pBR322 were grown on nitrocellulose filters and hybridized to the proliferin cDNA clone PLF-1. Upon over-exposure of the filters to X-ray film, two sets of hybridizing colonies became evident: strongly- and weakly-hydrizing clones. The arrows point to two weakly-hybridizing clones on a filter that has ~750 colonies.

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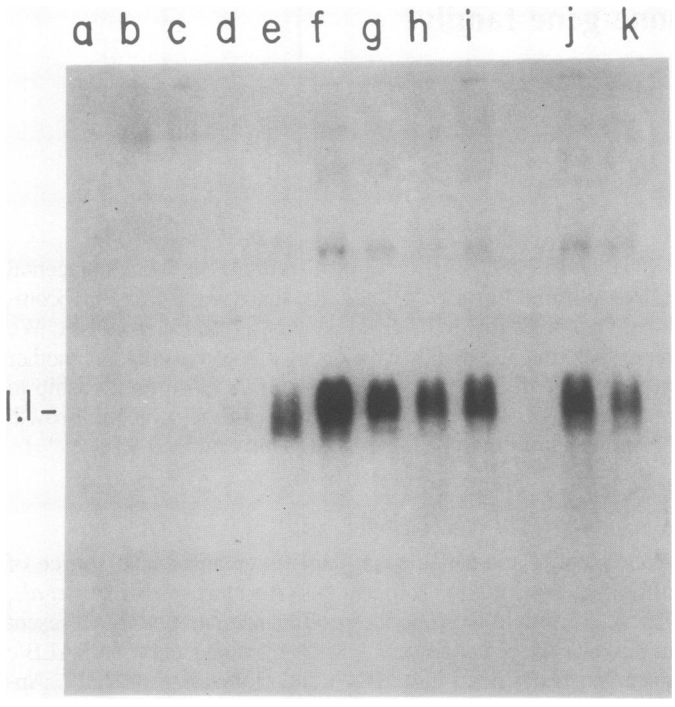

Fig. 2. Levels of PRP RNA in mouse placenta. Total RNA was purified from mouse placenta, brain, and whole fetus and fractionated by formaldehyde-agarose gel electrophoresis. Each lane contains 10 μg of RNA. After transfer to nitrocellulose, the RNA was hybridized to a PRP cDNA clone. The size of the major hybridizing RNA species was determined to be 1.1 kb. **(a)** Adult brain RNA; **(b)** fetal brain; **(c)** whole fetus; **(d)** placenta, day 8 of pregnancy; **(e)** placenta, day 10; **(f)** placenta, day 12; **(g)** placenta, day 14; **(h)** placenta, day 16; **(i)** placenta, day 18; **(j)** fetal-derived portion of the placenta; **(k)** maternal-derived portion of the placenta.

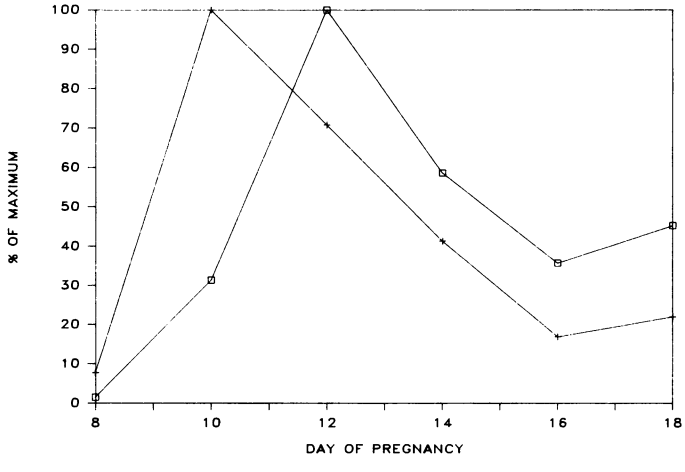

Fig. 3. Comparison of proliferin and PRP RNA levels in placenta during pregnancy. The total optical density of the 1.1-kb hybridizing regions in Figure 2 was determined with a Loats Image Analysis System and plotted *versus* day of pregnancy (□). The 12-day placenta sample is taken to be 100% for PRP RNA. The time course of proliferin RNA levels in the placenta during pregnancy (+) is provided for comparison (Linzer *et al.*, 1985).

If the frequency of proliferin and PRP clones in the total cDNA population accurately reflects the concentration of their mRNAs in placental tissue, then PRP and proliferin mRNAs are quite abundant, each representing 0.2−0.5% of the total poly(A)$^+$ RNA in mouse placenta.

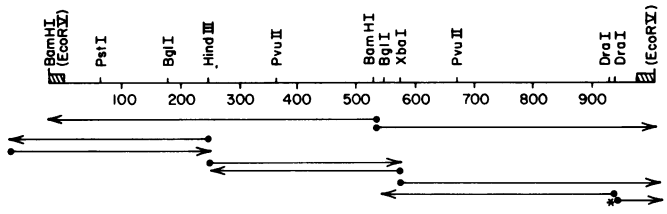

Fig. 4. Restriction map and strategy for sequencing PRP cDNA clones. The sites of cleavage by a variety of restriction enzymes are marked above the linear representation of the cDNA insert in PRP-1. The cDNA region is numbered in bp; hatched regions represent the G-C homopolymer tails used for insertion of the cDNA into the *Eco*RV site of pBR322. Arrows indicate the restriction fragments cloned into the M13 vectors mp18 and mp19 and the direction of sequencing. The asterisk marks the region sequenced by end-labeling and chemical degradation.

Appearance of PRP mRNA during pregnancy

To determine the levels of placental PRP mRNA at different stages of pregnancy, total RNA isolated from the placentas of Swiss-Webster mice at defined stages of pregnancy was fractionated on formaldehyde-agarose gels, transferred to nitrocellulose, and hybridized with the PRP cDNA clone designated PRP-1. As seen in Figure 2, PRP-hybridizing RNA was detected at very low levels on day 8 of pregnancy. The level increased to a peak at day 12 and then decreased gradually through day 18. The size of this mRNA is estimated to be 1.1 kb, slightly larger than the 1.0-kb proliferin mRNA. Low levels of high mol. wt. RNAs that hybridized to the PRP probe were also detected, but the relationship of these RNAs to the 1.1-kb RNA is not clear. Under these hybridization conditions, the proliferin and PRP sequences did not appear to cross-hybridize, as judged by rehybridizing this filter with the PLF-1 probe. In fact, as seen in Figure 3, although the time curve of PRP and proliferin RNAs during pregnancy have similar shapes, PRP RNA was delayed in appearance relative to proliferin RNA. A comparison of the hybridization signals of the PRP and proliferin RNAs indicates that the maximum amounts of these RNAs are approximately equal.

Proliferin RNA has been found predominantly in the fetal portion of the placenta and has not been found in a number of other mouse tissues examined (Linzer *et al.*, 1985). Identical results have been obtained for PRP (Figure 2). The fetal part of the placenta is richer in PRP than is the maternal part, and no PRP RNA is observed in the whole fetus or in brain tissue from either adults or embryos. However, unlike proliferin, PRP RNA has not been detected in growing BALB/c 3T3 cells.

Sequence of PRP cDNA

The inserts from two PRP cDNA clones have been sequenced. Restriction fragments of PRP-1 and PRP-2 were subcloned into M13 vectors and sequenced by the dideoxy chain termination method. A small region near the 3′ terminus of PRP-1 was also sequenced by chemical degradation. The fragments sequenced are shown in Figure 4, and the nucleotide sequence determined for PRP-1 and PRP-2 is given in Figure 5 along with the derived amino acid sequence. Only two differences were found between the two clones: the first T at the beginning of the 5′-untranslated region is present in PRP-2, but not PRP-1; and PRP-1 extends seven nucleotides beyond the end of PRP-2 in the 3′-untranslated region and includes a remanant of the poly(A) tail from PRP mRNA. Translation is assumed to start at the first ATG (nucleotides 69−71 in PRP-2) and terminate after 732 nucleotides at positions 801−803. (Note the in-phase TAG termination codon upstream of the first ATG.) The 3′-untranslated

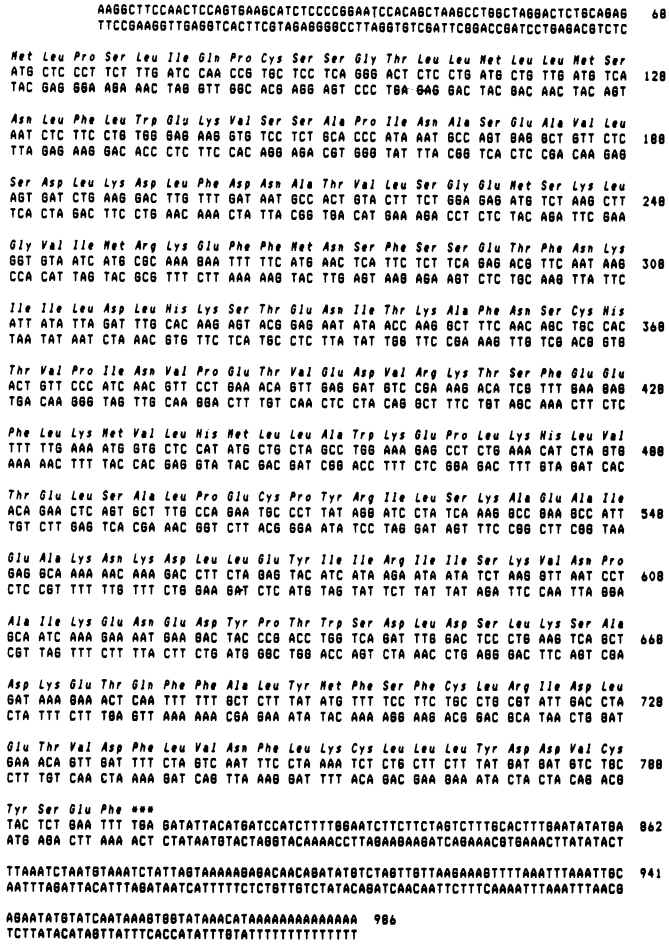

Fig. 5. Nucleotide and amino acid sequence of PRP. The nucleotide sequence of the cDNA inserts from clones PRP-1 and PRP-2 were determined as detailed in the legend to Figure 4. The combined sequence is presented above: the PRP-1 cDNA sequence begins at nucleotide 2, while the PRP-2 sequence terminates at position 965. Translation is assumed to initiate at the first ATG (positions 69–71) and continue until the TGA codon at nucleotides 801–803. The poly(A) addition signal is found at nucleotides 954–959, 14 nucleotides upstream from the remanant of the poly(A) tract.

region extends for 169 nucleotides after the termination codon to the poly(A) addition site, and includes the poly(A) addition signal AATAAA (Proudfoot and Brownlee, 1974) at positions 954–959.

The encoded PRP has 244 amino acids with an aggregate mol. wt. of 27 956. A protein with the predicted size was detected by translation of placental RNA hybrid-selected by the PRP plasmid (data not shown). The amino-terminal region of PRP is quite hydrophobic and may function as a signal peptide for secretion. Three potential N-linked glycosylation sites (Bahl and Shah, 1977) occur within the amino-terminal half of the PRP protein: Asn-Ala-Ser at amino acids 34–36, Asn-Ala-Thr at residues 49–51, and Asn-Ile-Thr at positions 91–93. Also in this half of the protein are two Arg-Lys dipeptides (65–66 and 114–115) that may be locations of proteolytic cleavage often utilized in the processing of peptide hormones (Docherty and Steiner, 1982).

Comparison of PRP with the prolactin family

The hybridization of PRP cDNA clones to proliferin sequences suggests that PRP is a member of the prolactin family of peptide hormones. A comparison of the predicted PRP sequence with

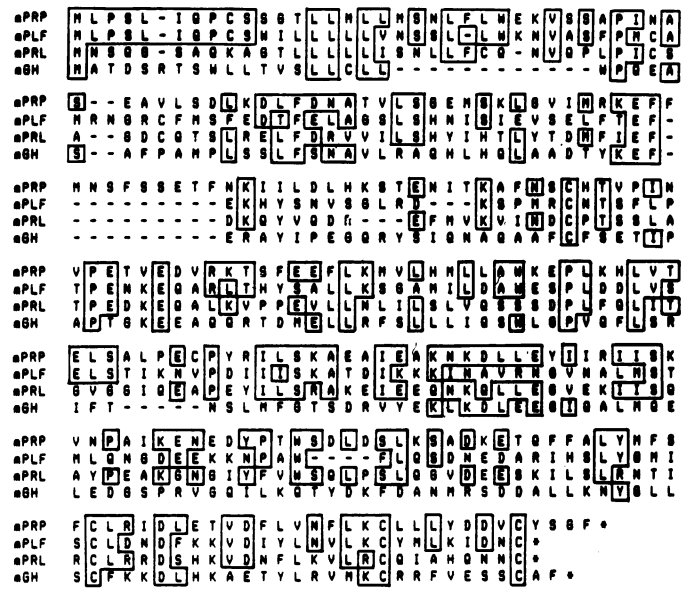

Fig. 6. Comparison of the amino acid sequences of mouse prolactin-family members. The predicted amino acid sequence of PRP is compared with the sequence of mouse proliferin (derived from the cDNA clone PLF-1), mouse prolactin and mouse growth hormone. The proliferin sequence is from Linzer and Nathans (1984b) and the prolactin and growth hormone sequences are from Linzer and Talamantes (1985). Regions of identity to PRP are boxed. Gaps have been added to align the sequences and do not represent missing amino acids.

Table I. Amino acid sequence comparison

Proteins	Identical	Related	Total
mPRP x mPLF	77 (32%)	13 (5%)	90 (37%)
mPRP x mPRL	73 (30%)	21 (9%)	94 (39%)
mPRP x mGH	40 (16%)	22 (9%)	62 (25%)

those determined for mouse prolactin and growth hormone (Linzer and Talamantes, 1985) and for proliferin (Linzer and Nathans, 1984b) is presented in Figure 6 and a summary is given in Table I. These data indicate that the four mouse proteins are members of a single family. Overall, the amino acid homology between PRP and proliferin (37%) is similar to the homology between PRP and prolactin (39%). The homology of PRP to growth hormone is less extensive (25%) even though PRP has structural features that are close to those of growth hormone (see Discussion). PRP is also distinct from mouse placental lactogen, since the amino-terminal sequence of placental lactogen (Linzer *et al.*, 1985) is not found in PRP.

While the amino acid homology between PRP and proliferin reveals that they are closely related proteins, the observation that the cDNAs cross-hybridize indicates that they must share a region with extensive nucleotide homology. Figure 7 displays the nucleotide sequences of proliferin and PRP mRNAs aligned to maximize homology. Of the first 97 nucleotides in proliferin mRNA, 92 are identical to the PRP sequence. This region includes the complete 5′-untranslated region and the first 31 translated nucleotides of each mRNA. This degree of homology (95%) over nearly 100 nucleotides not only explains the ability of these sequences to cross-hybridize, but also suggests that these two sequences have an unusual evolutionary relationship. Additionally, the two mRNAs share considerable nucleotide sequence homology throughout the remainder of their sequences (54%), with a total homology of 59%.

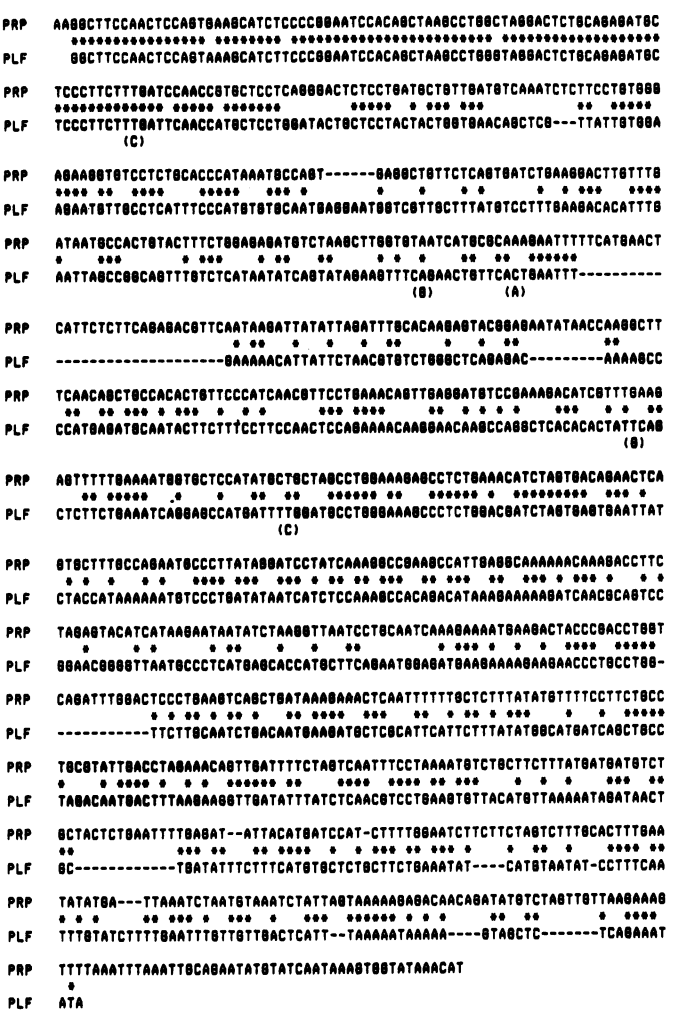

Fig. 7. Nucleotide homology of PRP and proliferin mRNAs. The nucleotide sequences determined for PRP and proliferin cDNA clones are compared. Where the proliferin clones PLF-1 and PLF-2 differ (Linzer *et al.*, 1985), the sequence of PLF-2 is provided in parentheses. Asterisks mark the locations of matching nucleotides between PRP and proliferin. Gaps have been added to optimize homology.

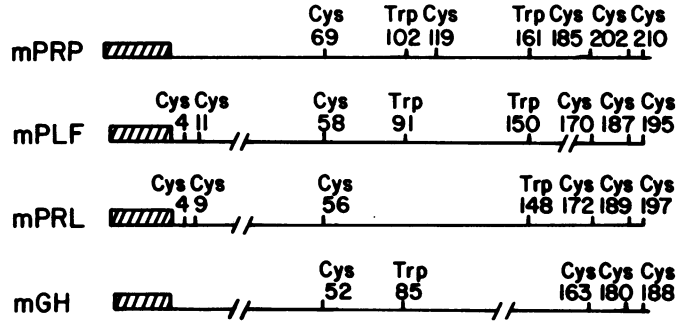

Fig. 8. Conserved structure of mouse prolactin-family members. The locations of the cysteine and tryptophan residues in the secreted forms of mouse PRP, proliferin, prolactin and growth hormone are compared. Numbers refer to amino acid positions in the mature proteins, and the hatched regions represent the signal sequences. The signal peptide in PRP is assumed to be 30 amino acids long, while that for proliferin is predicted to be 29 amino acids.

Discussion

This paper reports the identification of a new member of the prolactin-growth hormone family, proliferin-related protein or PRP, defined in terms of an inferred amino acid sequence encoded in mRNA rather than its physiological activity or protein isolation. As in the case of proliferin, PRP mRNA has been found in mouse placenta; unlike proliferin, PRP mRNA was not detected in proliferating BALB/c 3T3 cells in culture. Based on the frequency of PRP cDNA clones in the placental library (~0.25%) PRP mRNA appears to be moderately abundant. It is especially abundant around the 12th day of pregnancy, after which it gradually decreases as a function of total RNA. Although the precise location of its synthesis in the placenta is unknown, PRP mRNA is present predominantly, if not exclusively, in the fetal portion of mouse placenta.

The nucleotide sequence of PRP mRNA has a single long open reading frame encoding a protein of 244 amino acids. A protein of the expected size is detected upon translation of placental mRNA hybrid-selected with PRP sequences. No charged residues occur in the first 25 amino acids of the protein sequence. This hydrophobic region is predicted to function as a signal sequence for secretion, consistent with PRP being a peptide hormone. The protein may be secreted in a glycosylated form, as is proliferin (Linzer *et al.*, 1985), since the sequence after the hydrophobic leader region contains three consensus glycosylation sites. PRP protein may be further modified by proteolytic processing of the two Arg-Lys dipeptides.

Structurally, PRP is closely related to proliferin and prolactin, but also has features resembling growth hormone. Some of the structural similarities are summarized in Figure 8, which shows common features of mouse prolactin and growth hormone in relation to PRP and proliferin. Proliferin and all of the mammalian prolactins sequenced to date have six cysteine residues positioned approximately as shown for mouse proliferin, and all but mouse prolactin have two tryptophan residues in the mature protein. Mammalian growth hormones have four cysteines and one tryptophan, as shown. As seen in Figure 8, encoded PRP begins with a hydrophobic N terminus and has five cysteines outside the hyrophobic leader, four of which are spaced like those of growth hormone, and two tryptophan residues positioned like those of most prolactins and proliferin. Again like growth hormone, the polypeptide chain of PRP extends beyond the last cysteine. Unique features of PRP include an additional segment of 10 amino acids and a cysteine residue at position 119 already referred to.

The relationship of PRP to proliferin is especially striking at the nucleotide sequence level. These two mRNAs have 95% homology in the 5′-untranslated region and the first 31 nucleotides of the coding region. Significant but much less extensive homology is found throughout the remainder of these two mRNA sequences. The mouse PRP and proliferin genes presumably arose from a duplication event with subsequent divergence, but it seems unlikely that evolutionary pressure would conserve the 5′-untranslated and signal peptide encoding regions more than the regions encoding the mature proteins. We consider it probable that a recent correction process has taken place, realigning the sequences of these two genes near their 5′ ends. Since the point of transition from the region of 95% homology to that of weaker homology is marked in the proliferin gene by an exon/intron boundary (D.Linzer, unpublished observations), this correction would be expected to terminate somewhere within this downstream intron. This strong homology region probably continues upstream of the transcribed sequences, and may include control elements for tissue-specific expression.

As noted earlier, the placenta is the source of other prolactin or growth hormone-related proteins, the placental lactogens. Human placental lactogen is structurally very closely related to

growth hormone (Miller and Eberhardt, 1983), and the *decidua basalis* of human placenta is known to produce prolactin (Golander *et al.*, 1978; Riddick *et al.*, 1978). Two different rodent placental lactogens have been described, both with prolactin-like activities, including tight-binding to prolactin receptors (Robertson and Friesen, 1981; Robertson *et al.*, 1982; Colosi *et al.*, 1982; Soares *et al.*, 1983). The predominant mouse placental lactogen has a molecular size and biological activity consistent with a prolactin-like protein (Colosi *et al.*, 1982). However, partial amino acid sequence data clearly distinguish mouse placental lactogen from other members of the prolactin-growth hormone family, including proliferin and PRP (Linzer *et al.*, 1985). Thus mouse placenta produces at least three prolactin-related proteins. How they differ in activity and what roles they may play in fetal development are interesting questions for the future.

Materials and methods

RNA purification

The isolation of placental RNA from Swiss-Webster mice at defined times of pregnancy and from BALB/c mice during late gestation has been described (Linzer *et al.*, 1985). Briefly, mouse tissue was rapidly frozen in liquid nitrogen or in a dry ice-ethanol bath. Guanidinium thiocyanate solution (Chirgwin *et al.*, 1979) was added to the frozen tissue, which was then homogenized with a Brinkman polytron. The lysate was centrifuged through a CsCl cushion (Glisin *et al.*, 1974), the RNA pellet was recovered, and the RNA was further purified by extraction with phenol/chloroform. Poly(A)$^+$ RNA was selected by two cycles of oligo(dT)-cellulose chromatography (Aviv and Leder, 1972).

cDNA cloning and colony hybridization

Th construction of the placental cDNA clones has been described (Linzer *et al.*, 1985). Poly(A)$^+$ RNA was converted into cDNA (Gubler and Hoffman, 1983), modified with homopolymer dG tails (Roychoudhury *et al.*, 1976), and inserted into dC-tailed pBR322 at the unique *Eco*RV site (Gubler and Hoffman, 1983) Recombinant plasmid DNA was used to transform *Escherichia coli* MM294 cells (Meselson and Yuan, 1968) to ampicillin resistance by the method of Hanahan (1983). Transformation mixtures were plated directly onto nitrocellulose filters on L plates containing 200 μg/ml ampicillin and incubated at 37°C overnight. Replica filters were prepared and DNA from the re-established colonies was immobilized on the filters by denaturation and baking (Hanahan and Meselson, 1980). Filters were incubated in 6 x SSC with 0.2% each of bovine serum albumin, ficoll, and polyvinylpyrrolidone at 65°C for 3 h, and then hybridized to the proliferin cDNA clone PLF-1 (Linzer and Nathans, 1984b) that had been nick-translated (Rigby *et al.*, 1977) to 5 x 10^8 d.p.m./μg. Hybridization in a solution (Peden *et al.*, 1982) of 50 mM Tris-HCl, pH 7.4, 1 M NaCl, 0.5% SDS, 5 mM EDTA, 0.2% each bovine serum albumin, ficoll, and polyvinylpyrrolidone, 10^6 d.p.m./ml of probe, and 5 μg/ml salmon sperm DNA was carried out at 65°C for 22 h. The filters were washed (Peden *et al.*, 1982) and exposed to Kodak XAR film.

Preparation of DNA

Recombinant plasmid DNA was purified from lysates of chloramphenicol-treated cultures by banding twice in CsCl-ethidium bromide gradients (Peden *et al.*, 1982). Ethidium bromide was removed by extraction with water-saturated isobutanol, and the DNA was precipitated with ethanol. After solubilization, the DNA was extracted with phenol-chloroform, precipitated, and stored in a solution of 10 mM Tris-HCl, pH 7.4, 1 mM EDTA. Appropriate restriction fragments were subcloned into the M13 vectors mp18 and mp19 (Yanisch-Perron *et al.*, 1985) and propagated in *E. coli* JM105 cells. Phage particles were precipitated from infected cultures with PEG and then extracted with phenol-chloroform to purify recombinant phage DNA.

DNA sequence analysis

PRP subclones in mp18 and mp19 were sequenced by dideoxy chain termination (Sanger *et al.*, 1977). The sequence at the 3′ end of the cDNA insert was verified by chemical degradation (Maxam and Gilbert, 1980). Reaction products were resolved on acrylamide-urea gels (Sanger and Coulson, 1978) that were then fixed in 10% acetic acid and dried prior to exposure to Kodak XAR film. Both strands of each of two PRP cDNA clones were entirely sequenced between nucleotides 1 and 928; the final 44 nucleotides [not including the remnant of the poly(A) tail] were sequenced from one strand by both dideoxy and chemical methods.

Northern analysis of RNA

Total RNA extracted from tissues was electrophoresed on formaldehyde-agarose gels (Lehrach *et al.*, 1977; Goldberg, 1980) and transferred to nitrocellulose (Thomas, 1980). Filters were baked, incubated in 50% formamide, 5 x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, and 0.08% each bovine serum albumin, ficoll and polyvinylpyrrolidone (Fellous *et al.*, 1982) at 42°C for 3 h, and hybridized in fresh solution of the same composition supplemented with nick-translated PRP-1 DNA and 5 μg/ml salmon sperm DNA. Filters were hybridized at 42°C for 12 – 24 h, washed in 0.1 x SSC, 0.1% SDS for 2 h at 50°C (Thomas, 1980), and exposed to Kodak XAR film. Total intensities of hybridizing regions were determined with a Loats Image Analysis System made available by Michael Kuhar.

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References

Aviv,H. and Leder,P. (1972), *Proc. Natl. Acad. Sci. USA*, **69**, 1408-1412.
Bahl,O.P. and Shah,R.H. (1977) in Horowitz,M.I. and Pigman,W. (eds.), *The Glycoconjugates*, Vol. **I**, Academic Press, NY, pp. 385-422.
Boime,I., Boothby,M., Hoshina,M., Daniels-McQueen,S. and Darnell,R. (1982) *Biol. Reprod.*, **26**, 73-91.
Chirgwin,J.M., Przybyla,A.E., MacDonald,R.J. and Rutter,W.J. (1979) *Biochemistry (Wash.)*, **18**, 5294-5299.
Colosi,P., Marr,G., Lopez,J., Haro,L., Ogren,L. and Talamantes,F. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 771-775.
Docherty,K. and Steiner,D.F. (1982) *Annu. Rev. Physiol.*, **44**, 625-638.
Fellous,M., Nir,U., Wallach,D., Merlin,G., Rubinstein,M. and Revel,M. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 3082-3086.
Gardner,W.U. and Allen,E. (1942) *Anat. Rec.*, **83**, 75-91.
Glisin,V., Crkvenjakov,R. and Byus,G. (1974) *Biochemistry (Wash.)*, **13**, 2633-2637.
Golander,A., Hurley,T., Barrett,J., Hizi,A. and Handwerger,S. (1978) *Science (Wash.)*, **202**, 311-313.
Goldberg,D.A. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 5794-5798.
Gubler,U. and Hoffman,B.J. (1983) *Gene*, **25**, 263-269.
Hanahan,D. (1983) *J. Mol. Biol.*, **166**, 557-580.
Hanahan,D. and Meselson,M. (1980) *Gene*, **10**, 63-67.
Krieger,D.T. (1982) *Biol. Reprod.*, **26**, 55-71.
Lehrach,H., Diamond,D., Wozney,J.M. and Boedtker,H. (1977) *Biochemistry (Wash.)*, **16**, 4743-4751.
Linzer,D.I.H. and Nathans,D. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 4271-4275.
Linzer,D.I.H. and Nathans,D. (1984a) in Levine,A., Vande Woude,G., Topp, W. and Watson,J.D. (eds.), *Cancer Cells: The Transformed Phenotype*, Vol. **I**, Cold Spring Harbor Laboratory Press, NY, pp. 111-115.
Linzer,D.I.H. and Nathans,D. (1984b) *Proc. Natl. Acad. Sci. USA*, **81**, 4255-4259.
Linzer,D.I.H. and Talamantes,F. (1985) *J. Biol. Chem.*, in press.
Linzer,D.I.H., Lee,S.-J., Ogren,L., Talamantes,F. and Nathans,D. (1985) *Proc. Natl. Acad. Sci. USA*, in press.
Maxam,A.M. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 499-560.
Meselson,M. and Yuan,R. (1968) *Nature*, **217**, 1110-1114.
Miller,W.L. and Eberhardt,N.L. (1983) *Endocrine Rev.*, **4**, 97-130.
Newton,W.H. and Beck,N. (1939) *J. Endocrinol.*, **1**, 65-75.
Peden,K., Mounts,P. and Hayward,G. (1982) *Cell*, **31**, 71-80.
Proudfoot,N.J. and Brownlee,G.G. (1974) *Nature*, **252**, 359-362.
Riddick,D.H., Luciano,A.A., Kusmik,W.F. and Maslar,I.A. (1978) *Life Sci.*, **23**, 1913-1922.
Rigby,P.W.J., Dieckmann,M., Rhodes,C. and Berg,P. (1977), *J. Mol. Biol.*, **113**, 237-251.
Robertson,M.C. and Friesen,H.G. (1981) *Endocrinology*, **108**, 2388-2390.
Robertson,M.C., Gillespie,B. and Friesen,H.G. (1982) *Endocrinology*, **111**, 1862-1866.
Roychoudhury,R., Jay,E. and Wu,R. (1976) *Nucleic Acids Res.*, **3**, 101-116.
Sanger,F. and Coulson,A.R. (1978) *FEBS Lett.*, **87**, 107-110.
Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463-5467.
Seyle,H., Collio,J.B. and Thomson,D.L. (1933) *Proc. Soc. Exp. Biol. Med.*, **31**, 82-83.
Soares,M.J., Colosi,P., Ogren,L. and Talamantes,F. (1983) *Endocrinology*, **112**, 1313-1317.
Talamantes,F., Ogren,L., Markoff,E., Woodard,S. and Madrid,J. (1980) *Fed. Proc.*, **39**, 2582-2587.
Thomas,P.S. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 5201-5205.
Yanisch-Perron,C., Vieira,J. and Messing,J. (1985) *Gene*, **33**, 103-119.

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