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Biochemistry

Nucleotide sequence of a growth-related mRNA encoding a member of the prolactin-growth hormone family

(growth regulation/prolactin-like protein/proliferin)

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ABSTRACT **As part of the proliferative response to serum, mouse 3T3 cells produce a set of growth-related mRNAs identified by hybridization to cloned cDNAs. One of these mRNAs, which is about 1 kilobase long, appears within a few hours after stimulation of resting cells with serum or platelet-derived growth factor and reaches a high level during the transition from the G_1 to the S phase of growth. This mRNA is translated *in vitro* into a protein of approximately 25 kilodaltons. The corresponding cloned cDNA of 791 base pairs has been sequenced; it contains a single open reading frame that encodes a protein of 224 amino acids with extensive sequence homology to mammalian prolactins. The initial 29-amino acid segment of the encoded protein resembles the signal sequences of prehormones. That the growth-related protein is not mouse prolactin is indicated by comparison of its predicted amino acid composition with that of mouse prolactin and by the distinct fragment patterns seen when restricted mouse DNA is probed with the cloned cDNA or rat prolactin cDNA. Therefore, the growth-related protein appears to be a new member of the prolactin-growth hormone family. Because of its relationship to prolactin and growth hormone and its association with cell proliferation, the protein has been called "proliferin."**

Activation of specific genes occurs during the transition of mammalian cells from the resting to the growing state (1). What these genes are and what roles the products of these genes play in the progression of the cell cycle remain, for the most part, to be elucidated. Recently, several groups of workers have reported the molecular cloning of cDNAs corresponding to growth-related mRNAs from cultured mouse cells stimulated with serum or purified growth factors (2–4). One of the growth-related mRNAs that we found in a number of proliferating murine cell lines is a 1-kilobase RNA (referred to as 28H6) that is virtually undetectable in nongrowing cells (2, 5). Following serum stimulation of BALB/c 3T3 fibroblastic cells, 28H6 RNA appears within 3 hr and reaches a maximal level at the time of transition between the G_1 and S phases of growth. Because 28H6 RNA is not always found in proliferating murine cell lines (2, 5), its expression is not essential for growth. However, our interest in this RNA was reinforced when nucleotide sequence analysis of an incomplete cDNA clone suggested that 28H6 RNA encoded a protein related to prolactin (2, 5). We now report the sequence of a cDNA clone that appears to contain the complete coding and 3′-untranslated regions of the mRNA. This cDNA encodes a protein of 224 amino acids with extensive homology to mammalian prolactins. By several criteria the encoded protein is likely to be a new member of the prolactin-growth hormone family. Because of its relationship to prolactin and growth hormone and its association with cell proliferation, the protein will be referred to as "proliferin" or "PLF."

MATERIALS AND METHODS

DNA Sequence Analysis. The cDNA clones were end labeled by filling in 5′ overhangs, left after restriction endonuclease cleavage, with the Klenow fragment of *Escherichia coli* DNA polymerase I and an [α-^{32}P]dNTP. *Pst* I 3′ overhangs were labeled with cordycepin [α-^{32}P]triphosphate and terminal nucleotidyltransferase (6). Fragments labeled at one end were isolated from polyacrylamide gels and sequenced by the method of Maxam and Gilbert (7). The cleavage products were resolved on 8% or 20% polyacrylamide/urea gels (8), and the gels were autoradiographed.

Oligonucleotide Synthesis and Labeling. Defined oligonucleotides were synthesized with an Applied Biosystems 380A DNA synthesizer, and the final products were purified by high performance liquid chromatography. Oligonucleotides were labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP.

Colony Hybridization. The cDNA library (2) was grown on nitrocellulose filters on agar plates containing tetracycline at 6 μg/ml, and replica filters were prepared by the procedure of Hanahan and Meselson (9). For the high-density screen, each 88 × 88 mm filter contained approximately 50,000 colonies. The bacterial colonies on the replica filters were lysed (9), and the filters were baked and incubated prior to hybridization as described (10). Hybridizations were carried out for 12–18 hr at 37°C in 1 M NaCl/50 mM Tris·HCl, pH 7.4/5 mM EDTA/0.5% $NaDodSO_4$/0.2% bovine serum albumin/0.2% Ficoll/0.2% polyvinylpyrrolidone (10, 11) containing denatured *E. coli* DNA at 10 μg/ml and 1×10^6 dpm of kinase-labeled oligonucleotide per ml. The filters were washed with 0.9 M NaCl/0.09 M sodium citrate at 0°C with several changes for a total of 1 hr and then with fresh solution of the same composition at 37°C for two 10-min periods (12). After drying, the filters were exposed to x-ray film. Hybridizing regions were picked and rescreened at low density by the same procedure. Finally, individual colonies were picked into microwells and screened as described (2).

Purification of RNA and DNA. Krebs ascites carcinoma cells (from American Type Culture Collection) were isolated from the peritoneal cavity of BALB/c mice 10 days after injection. The cells were lysed in guanidinium thiocyanate solution (13), and the RNA was pelleted through a CsCl cushion (14). Poly(A)$^+$ RNA was selected by two cycles of binding to oligo(dT)-cellulose (15). Recombinant plasmid DNAs were purified by two rounds of CsCl/ethidium bromide density gradient centrifugation (10). The rat prolactin cDNA clone PRL-2 (16, 17) was the gift of R. Maurer. BALB/c liver DNA was provided by K. Peden.

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Abbreviations: bGH, bovine growth hormone; bPRL, bovine prolactin; mPRL, mouse prolactin; mPLF, mouse proliferin; rPRL, rat prolactin.

DNA Filter Hybridization. Mouse genomic DNA was digested with the restriction endonuclease *Eco*RI, extracted with phenol/chloroform, and ethanol precipitated prior to electrophoresis in 1% agarose gels. The DNA was transferred to nitrocellulose (18), baked, and then treated as described for colony hybridizations, except that salmon sperm DNA was used in place of *E. coli* DNA, and cDNA clones nick-translated (19) to 5×10^8 dpm/μg were used as probes instead of oligonucleotides. Hybridizations with rat prolactin clone PRL-2 were carried out at 60°C for 60 hr; filters were hybridized with the mouse proliferin clone at 67°C for 60 hr. Filters were washed at 60°C or 67°C, respectively, as described (10) and then autoradiographed.

Hybrid-Selected Translation. The proliferin cDNA clone (20 μg) was linearized with *Eco*RI, denatured with 0.1 M NaOH, neutralized with an equal volume of 45 mM NaOAc, pH 4.8/2.5 M NaCl and spotted on nitrocellulose. After baking, the filter was washed, and hybridized to 1 mg of Krebs ascites carcinoma total cellular RNA, essentially as described (20). The hybridization mixture (in 50% formamide/20 mM Pipes, pH 6.4/0.2% $NaDodSO_4$/400 mM NaCl) was heated at 70°C for 10 min, then incubated at 50°C overnight. After washing the filter, bound RNA was eluted, extracted with phenol/chloroform, and ethanol precipitated (20). The RNA was translated in a rabbit reticulocyte lysate (New England Nuclear), and the [^{35}S]methionine-labeled translation products were resolved by $NaDodSO_4$/polyacrylamide gel electrophoresis (21). The gel was treated with EN^{3}HANCE (New England Nuclear) prior to exposure.

Computer Analysis. Nucleotide sequences were compared with sequences in the Los Alamos Data Bank using the algorithm of Wilbur and Lipman (22).

RESULTS

Nucleotide Sequence of Proliferin cDNA. Our initial clone of proliferin cDNA (designated 28H6) was isolated from a plasmid cDNA library prepared from mRNA of serum-stimulated BALB/c 3T3 cells and screened with [^{32}P]cDNA synthesized from resting versus growing cell mRNA (2). 28H6 cDNA hybridizes to a 1-kilobase mRNA that is abundant in several lines of proliferating mouse cells but is virtually absent in resting cells (2, 5). By nucleotide sequence analysis, the 28H6 cDNA insert contains 422 base pairs, representing approximately the 3′ half of the mRNA. To obtain a more complete cDNA, a synthetic oligonucleotide representing a 14-nucleotide sequence from the 5′ end of the 28H6 cDNA was used to probe the original cDNA library. Of 5×10^5 colonies screened, ≈400 hybridized to the probe. Thus, this cDNA is at least 0.08% of the library. Several clones with longer cDNA inserts than clone 28H6 were isolated and partially sequenced, but none of these extended to the expected methionine codon at the translation initiation site. Therefore a second synthetic oligonucleotide, representing 5′ sequences from the longest cDNA insert, was used to probe the cDNA library once more. Only 20 out of 5×10^5 colonies hybridized to this 17-nucleotide probe. One of these (designated clone PLF-1) contains a cDNA insert extending beyond the putative translation start site. A restriction map of this clone is shown in Fig. 1, together with a display of fragments sequenced.

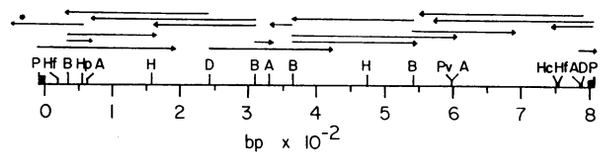

FIG. 1. Restriction and sequence analyses of mouse proliferin (mPLF) cDNA. The restriction endonuclease sites in the mPLF clone PLF-1 are diagrammed above: A, *Alu* I; B, *Bst*NI; D, *Dde* I; H, *Hpa* II; Hc, *Hin*cII; Hf, *Hin*fI; Hp, *Hph* I; P, *Pst* I; Pv, *Pvu* II. The boxed areas at the ends of the clone represent the G-C homopolymer tails added for cloning. The arrows correspond to fragments sequenced by the method of Maxam and Gilbert (7). Each fragment was labeled at the 3′ end, and the direction of the arrow indicates the DNA strand sequenced. Fragments were derived from several cDNA clones, all of which have identical restriction maps. The asterisk marks the region sequenced by primer extension. bp, Base pairs.

As shown in Fig. 2, PLF-1 cDNA has a single open reading frame, coding for a protein with 224 amino acid residues, on the assumption that the ATG beginning at nucleotide 5 is the start codon. [By extension of the 17-nucleotide primer hybridized to proliferin mRNA prepared from growing Krebs ascites carcinoma cells (5), an additional 37 nucleotides were sequenced; the position of the putative start codon was confirmed, and no other in-phase methionine codons were found.] The translation termination codon TGA appears at nucleotides 677–679, and a polyadenylylation sig-

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        Met Leu Pro Ser Leu Ile Gln Pro Cys Ser Trp Ile Leu Leu Leu Leu Val Asn Ser Ser Leu Leu Trp Lys Asn
  1 AGAG ATG CTC CCT TCT TTG ATT CAA CCA TGC TCC TGG ATA CTG CTC CTA CTA CTG GTG AAC AGC TCG TTA TTG TGG AAG AAT   82

        Val Ala Ser Phe Pro Met Cys Ala Met Arg Asn Gly Arg Cys Phe Met Ser Phe Glu Asp Thr Phe Glu Leu Ala Gly Ser
 83  GTT GCC TCA TTT CCC ATG TGT GCA ATG AGG AAT GGT CGT TGC TTT ATG TCC TTT GAA GAC ACA TTT GAA TTA GCC GGC AGT  163

        Leu Ser His Asn Ile Ser Ile Glu Val Ser Glu Leu Phe Thr Glu Phe Glu Lys His Tyr Ser Asn Val Ser Gly Leu Arg
164  TTG TCT CAT AAT ATC AGT ATA GAA GTT TCA GAA CTG TTC ACT GAA TTT GAA AAA CAT TAT TCT AAC GTG TCT GGG CTC AGA  244

        Asp Lys Ser Pro Met Arg Cys Asn Thr Ser Phe Leu Pro Thr Pro Glu Asn Lys Glu Gln Ala Arg Leu Thr His Tyr Ser
245  GAC AAA AGC CCC ATG AGA TGC AAT ACT TCT TTC CTT CCA ACT CCA GAA AAC AAG GAA CAA GCC AGG CTC ACA CAC TAT TCA  325

        Ala Leu Leu Lys Ser Gly Ala Met Ile Leu Asp Ala Trp Glu Ser Pro Leu Asp Asp Leu Val Ser Glu Leu Ser Thr Ile
326  GCT CTT CTG AAA TCA GGA GCC ATG ATT TTG GAT GCC TGG GAA AGC CCT CTG GAC GAT CTA GTG AGT GAA TTA TCT ACC ATA  406

        Lys Asn Val Pro Asp Ile Ile Ile Ser Lys Ala Thr Asp Ile Lys Lys Lys Ile Asn Ala Val Arg Asn Gly Val Asn Ala
407  AAA AAT GTC CCT GAT ATA ATC ATC TCC AAA GCC ACA GAC ATA AAG AAA AAG ATC AAC GCA GTC CGG AAC GGG GTT AAT GCC  487

        Leu Met Ser Thr Met Leu Gln Asn Gly Asp Glu Glu Lys Lys Asn Pro Ala Trp Phe Leu Gln Ser Asp Asn Glu Asp Ala
488  CTC ATG AGC ACC ATG CTT CAG AAT GGA GAT GAA GAA AAG AAG AAC CCT GCC TGG TTC TTG CAA TCT GAC AAT GAA GAT GCT  568

        Arg Ile His Ser Leu Tyr Gly Met Ile Ser Cys Leu Asp Asn Asp Phe Lys Lys Val Asp Ile Tyr Leu Asn Val Leu Lys
569  CGC ATT CAT TCT TTA TAT GGC ATG ATC AGC TGC CTA GAC AAT GAC TTT AAG AAG GTT GAT ATT TAT CTC AAC GTC CTG AAG  649

        Cys Tyr Met Leu Lys Ile Asp Asn Cys STOP
650  TGT TAC ATG TTA AAA ATA GAT AAC TGC TGA  TATTTCTTTCATGTGCTCTGCTTCTGAAATATCATGTAATATCCTTTCAATTTGTATCTTTTGAAT  745

746  TTGTTGTTGACTCATTTAAAAATAAAAAGTAGCTCTCAGAAATATA    791
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FIG. 2. Nucleotide sequence of mPLF. The sequence of the 791-bp cDNA insert in clone PLF-1 is presented, as is the amino acid sequence translated from the open reading frame. The original cDNA clone (28H6) corresponds to nucleotides 360–781, inclusive, and the oligonucleotide synthesized from this region is the complement of nucleotides 360–373. A longer cDNA clone (28H6-20), isolated by hybridization to this oligonucleotide, contains residues 31–784, inclusive; the oligonucleotide synthesized from this region is complementary to nucleotides 50–66. A single difference detected in the primer-extended cDNA synthesized from this second oligonucleotide with Krebs ascites carcinoma RNA as template is a change of T-A to C-G at position 18.

nal A-A-T-A-A-A (23) is present around nucleotide 770. The sequence of all the other proliferin cDNA clones analyzed agree with that of PLF-1, except for variability in the position of the poly(A) track. In a separate cDNA clone, the poly(A) stretch follows nucleotide 788. This variability does not represent rearrangement during cloning (24), as determined by sequencing of the corresponding region of genomic DNA (unpublished observations).

Relationship of Proliferin to Prolactin. The first indication that proliferin is related to prolactin came from comparison of the nucleotide sequence of 28H6 cDNA with the sequences stored in the Los Alamos DNA data bank (2, 5). We also noted that proliferin mRNA from mouse 3T3 cells or Krebs ascites carcinoma cells is about 1 kilobase long, about the same size as prolactin mRNA (25). Furthermore, purification of Krebs cell proliferin mRNA by hybridization to proliferin cDNA and translation *in vitro* results in a polypeptide with an estimated molecular weight of 25,000 (Fig. 3), about the same as that of preprolactin (26).

The nucleotide sequence homology between the coding regions of PLF-1 and bovine prolactin cDNA (27) is 55% and is reflected in the amino acid sequence comparisons of the two proteins. In Fig. 4 the predicted amino acid sequence of mPLF is compared with the sequences of bovine prolactin (bPRL) and bovine growth hormone (bGH), which is related to prolactin (26). The results are summarized in Table 1 together with related sequence comparisons. Analysis of the proliferin amino acid sequence reveals that (*i*) mPLF and bPRL are more closely related than bPRL and bGH; (*ii*) mPLF has significantly less homology to bPRL than do other mammalian prolactins—e.g., rat prolactin (rPRL); (*iii*) the sequence of the first 29 amino acids of mPLF closely resembles that of the signal peptide of bPRL; (*iv*) this mPLF leader sequence satisfies the empirical rules for cleavage of a signal peptide after the serine residue at position 29 (28); (*v*) the sizes of the presumed precursor and mature mPLF polypeptides are similar to those of bPRL; (*vi*) the locations of the six cysteine and two tryptophan residues in mature bPRL and mPLF, diagrammed in Fig. 5, correspond almost precisely [these residues are highly conserved in the mammalian prolactins (26)]; (*vii*) mPLF contains the sequences Lys-Lys-Lys (positions 149–151 in the full-length polypeptide) and Lys-Lys (174-175 and 205-206), which are often proteolytic cleavage sites in peptide hormones (29, 30), as well as three

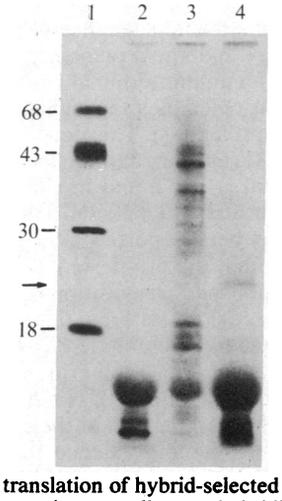

FIG. 3. *In vitro* translation of hybrid-selected RNA. Total RNA from Krebs ascites carcinoma cells was hybridized to the mPLF clone 28H6-20, which was immobilized on nitrocellulose. The RNA was eluted and translated in a reticulocyte lysate system. The [^{35}S]methionine-labeled translation products were resolved by Na-DodSO$_4$/polyacrylamide gel electrophoresis, and the gel was fluorographed prior to exposure. Lanes: 1, molecular weight standards ($\times 10^{-3}$); 2, translation reaction with no added RNA; 3, translation of unselected Krebs RNA; 4, translation of proliferin-selected RNA. The arrow points to the selected translation product of approximately 25 kilodaltons. This protein is not observed when pBR322-selected RNA is used as mRNA.

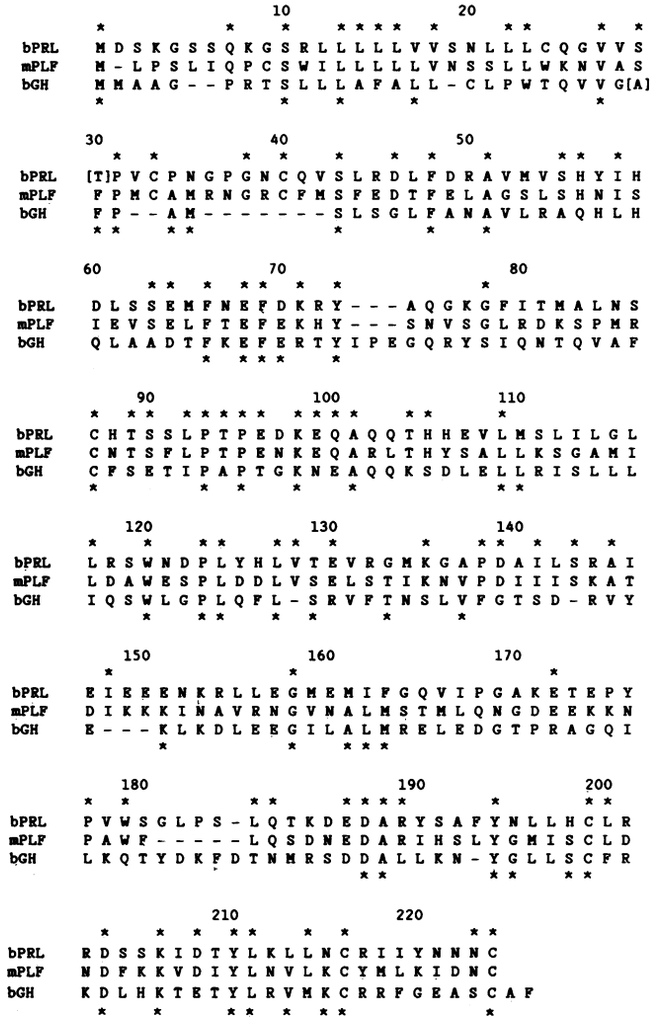

FIG. 4. Comparison of the amino acid sequences of prolactin, growth hormone, and proliferin. The amino acid sequence from the major open reading frame of mPLF is aligned with the sequences of bPRL and bGH. The initial residues in mature bPRL and bGH are bracketed, and asterisks mark identical amino acids between mPLF and the two hormones. Gaps have been added to align the sequences; these gaps do not represent missing amino acids. Data for bPRL and bGH are from Miller and Eberhardt (26). The single-letter amino acid code is used: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.

Table 1. Relationship of mPLF, bPRL, and bGH

Proteins	Amino acid sequence comparison		
	Identical	Related*	Total
mPLF vs. bPRL	82 (37%)	20 (9%)	102 (46%)
mPLF vs. bGH	50 (22%)	23 (10%)	73 (32%)
mPLF vs. bPRL or bGH	99 (44%)	20 (9%)	119 (53%)
bPRL vs. bGH	60 (26%)	24 (10%)	84 (37%)
bPRL vs. rPRL	135 (59%)	19 (8%)	154 (68%)

Sequences of bPRL and bGH are from Miller and Eberhardt (26).
*Related amino acids are lysine and arginine; aspartic and glutamic acids; asparagine and glutamine; serine and threonine; and valine, leucine, and isoleucine.

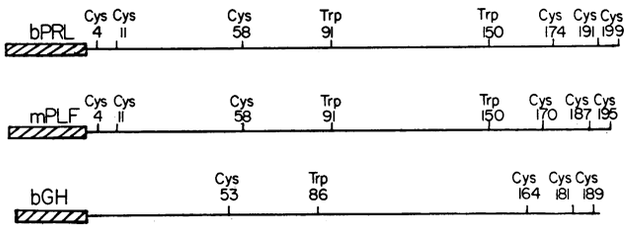

FIG. 5. Locations of cysteine and tryptophan residues. The locations of each of the cysteine and tryptophan residues in bPRL (26), mPLF, and bGH (26) are indicated. Numbers refer to the location in the mature protein, and the hatched regions mark the signal peptides. Proliferin is assumed to have a signal peptide of 29 amino acids, and the processed protein is assumed to start with a phenylalanine residue.

Asn-X-Ser regions (58–60, 75–77, and 88–90), the consensus signal for glycosylation (31).

Evidence that Proliferin Is Not Mouse Prolactin. The degree of amino acid homology between mPLF and bPRL suggests that mPLF is not mouse prolactin, since the sequenced mammalian prolactins show considerably greater interspecies homology (Table 1). Although the amino acid sequence of mouse prolactin (mPRL) is not available for direct comparison, two less-direct lines of evidence indicate that mPLF and mPRL are different proteins. First, the secreted form of mPRL shows striking differences in the content of several amino acids (32) when compared with the predicted composition of mPLF (Table 2). For example, mPLF contains nine methionine residues, while mPRL has only two. (It is notable that mPRL is reported to have only one tryptophan, unlike all other mammalian prolactins analyzed thus far.) Second, the genes for prolactin and proliferin are distinguishable by restriction analysis of mouse DNA. As shown in Fig. 6, different sets of restriction fragments of mouse DNA hybridize with mPLF cDNA and rPRL cDNA. Thus, mouse DNA has one or more genes that are more homologous to *rPRL* than is the *mPLF* gene.

DISCUSSION

The identification of genes that are activated during the transition of mammalian cells from the resting to the growing state is an essential step in understanding the mechanism of cell cycle control. As cells pass through the G_1 phase of growth, new species of mRNA appear at defined times (2–4, 33, 34), some of which are likely to encode proteins involved in cell growth. We have undertaken to identify these growth-related mRNAs and their encoded proteins by cloning and analyzing corresponding cDNAs. In this communication, we present evidence that in a mouse fibroblastic cell line one of these mRNAs codes for a protein closely related to mammalian prolactins. This protein, which we refer to as "proliferin," appears to be a new member of the prolactin-growth hormone family.

Table 2. Amino acid composition of mPRL and mPLF

Amino acid	mPRL	mPLF	Amino acid	mPRL	mPLF
Ala	9	11	Lys	12	15
Arg	9	7	Met	2	9
Asp + Asn	20	29	Phe	7	9
Cys	6	6	Pro	9 or 10	6
Glu + Gln	30	16	Ser	15	19
Gly	8	7	Thr	7	9
His	3	4	Trp	1	2
Ile	15	13	Tyr	6	5
Leu	26	20	Val	14	8

Data for the secreted form of mouse prolactin are from Colosi and Talamantes (32). The mature proliferin protein is assumed to begin with the phenylalanine residue at position 30 in Fig. 2.

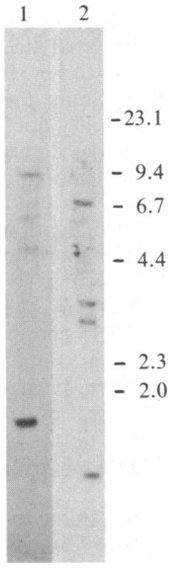

FIG. 6. Restriction patterns of *mPLF* and *mPRL* genes. BALB/c mouse liver DNA samples (10 μg each) were digested with *Eco*RI, electrophoresed, and transferred to nitrocellulose. The filters were hybridized to either the mPLF cDNA clone 28H6-20 (lane 1) or the rPRL cDNA clone PRL-2 (lane 2). The washed filters were then autoradiographed. Size markers (in kb) are from a *Hin*dIII digest of phage λ DNA.

From the nucleotide sequence of proliferin cDNA one can infer that translation of the mRNA would yield a protein of about 25 kilodaltons containing 224 amino acid residues. A 25-kilodalton protein is the major product of hybrid-selected proliferin mRNA translated *in vitro*. The amino acid sequence of proliferin shows a striking resemblance to that of bPRL and other mammalian prolactins. If closely related amino acids are included, mPLF and bPRL have 46% of their amino acid sequence in common, compared with 37% common sequences shared by bGH and bPRL. Especially noteworthy are the nearly identical locations of the six cysteines [which form three disulfide bridges in prolactin (35)] and the two tryptophans in the body of proliferin and prolactin. These residues must have important functional significance since they are found in all the mammalian prolactins sequenced so far.

The predicted amino acid sequence of proliferin suggests that the protein may undergo a number of post-translational changes. There is an amino-terminal hydrophobic region of 29 amino acids that resembles the signal peptide of prehormones, including prolactins. The consensus glycosylation signal Asn-X-Ser (31) appears at three locations in proliferin. Although this signal is not found in rPRL or bPRL, ovine prolactin does have such a signal, and a glycosylated form of ovine prolactin has been reported (36). Recently, we have obtained evidence that the proliferin protein is glycosylated and secreted (unpublished observations). In addition, proliferin contains three regions with contiguous lysine residues, which could be sites for proteolytic cleavage (29, 30).

What is the evidence that proliferin is not mPRL? Since the amino acid sequence of mPRL is not known, we are unable to make a direct comparison of the two proteins. Nonetheless, there is substantial evidence that the proteins are different. First, the degree of amino acid sequence homology between proliferin and mammalian prolactins is considerably less than that between the different mammalian prolactins whose amino acid sequences are known; second, the amino acid composition reported for biologically defined prolactin secreted by cultured mouse pituitary cells (32) differs from that of proliferin; and third, proliferin and rPRL cDNAs hybridize to different restriction fragments derived from mouse DNA. Recently, Talamantes and his colleagues (37) have described a 23-kilodalton murine placental lactogen with potent prolactin-like activity that does not react with antisera

against murine prolactin or growth hormone. The biological properties of this murine placental lactogen suggest that it is similar to prolactin and may be quite different from other placental lactogens (37). While proliferin has only limited homology to human placental lactogen (26), it remains possible that proliferin is closely related to the prolactin-like murine placental lactogen.*

The close structural relationship of proliferin to prolactin suggests a close functional relationship as well. Prolactin is a multifunctional hormone with pronounced physiological effects on growth, reproduction, and osmoregulation (38, 39). It has a marked proliferative effect on the crop sac of pigeons (40) and in mammals is involved in the development and function of various glandular tissues (38, 39). A variety of mammalian cells have receptors for prolactin, including those of the mammary gland, liver, kidney, brain, prostate, testis, and ovary (41). Although prolactin is a pituitary hormone, proteins that react with anti-prolactin sera appear to be synthesized in nonpituitary tissues as well (42, 43). Arguing by analogy to prolactin, we hypothesize that proliferin is secreted by certain types of proliferating cells, binds to cell surface receptors of like or different cells, and stimulates their growth or differentiation. Thus, proliferin may be an augmenting or secondary growth factor akin to transforming growth factors released by tumor cells (44) or to the somatomedins (45). A test of this hypothesis awaits the isolation of purified proliferin.

Note Added in Proof. Since submission of this manuscript, the amino acid sequence of mouse prolactin has been reported (46). Proliferin and mouse prolactin share 32% of their amino acid residues.

*In fact, in collaboration with L. Ogren and F. Talamantes, we have recently detected PLF-related mRNA in mouse placenta (unpublished observations).

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