



Published in final edited form as:

Am J Physiol. 1994 May ; 266(5 Pt 1): G914–G921.

Rat pancreatic lipase and two related proteins: enzymatic properties and mRNA expression during development

R. Mark Payne, Harold F. Sims, Martha L. Jennens, and Mark E. Lowe

Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri 63110

Abstract

We report the cDNA sequences of rat colipase, rat pancreatic lipase (rPL), and a rat pancreatic lipase-related protein (rPLRP). Comparison to the human PLRP cDNA suggests that the isolated clone encodes rPLRP-2. Both cDNA and a third cDNA encoding rPLRP-1 are secreted from Sf9 cells infected with recombinant baculovirus. rPL and rPLRP-2 hydrolyze triolein, 8.0 and 4.4 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \mu\text{g}^{-1}$, respectively. They are inhibited by bile salts, and activity is restored by (pro)colipase. PLRP-1 has barely detectable activity against triolein, even with (pro)colipase present. The pattern of mRNA expression during development in the rat reveals that all mRNA are low in the fetal rat pancreas. Both PLRP mRNA rise just before birth to a maximum 12 h after birth. They fall to low levels in the adult. In contrast, the PL mRNA is low at birth and rises rapidly during the suckling-weanling transition. In conclusion, the rat has at least three genes encoding different lipases, and these related genes have separate regulatory controls.

Keywords

triglycerides; baculovirus; protein expression

Pancreatic Triglyceride Lipase (PL), lipoprotein lipase (LPL), and hepatic lipase have striking amino acid homology (6, 7). This homology led to the proposal that the three lipases form a gene family. The concept of a lipase gene family is supported by structural analysis of the genes (5). Positions of exons and introns are highly conserved among the three genes. Also, an *A/u* sequence is conserved in the homologous intron of PL and LPL (15). These findings suggest that the three genes evolved from a common ancestral gene.

Additional members of the lipase gene family were isolated from a human pancreatic library (2). Of the three related clones, one is identical to a cDNA for human PL (hPL) (12). The other two cDNA have a predicted protein sequence with 65 and 68% amino acid identity to PL. These proteins are named pancreatic lipase-related proteins 1 and 2 (PLRP-1 and PLRP-2). Although the similarities suggest that the three proteins are related, the differences prove that the proteins are encoded by different genes of the growing lipase gene family.

Like the human, the rat may have PLRPs. A cDNA that encodes a protein with homology to PL was cloned from a rat pancreas cDNA library (18). The predicted amino acid sequence of the cDNA is 85% identical to that of the hPLRP-1, only 68% identical to hPL, and 63% identical to hPLRP-2, suggesting that the reported clone encodes rat PLRP-1 (rPLRP-1).

The presence of the PLRPs in the pancreas raises questions about the biological function, gene regulation, and expression pattern of these proteins. We report the isolation of two homologous cDNA clones from a rat pancreas library that encode rPL and rPLRP-2. Infection of an insect cell line with recombinant baculovirus containing the cDNA for these clones and an rPLRP-1 clone produces secreted proteins, enabling us to test the activity of each protein against triolein. Additionally, we determined the expression, during development, of the genes encoding rPL, the two rPLRPs, and colipase. The results suggest that the proteins have different functions and that the genes have different regulatory controls.

METHODS

Terminology

In this report, the terminology for triglyceride lipase and the related proteins will follow that proposed by Giller et al. (2). mPLRP-2 designates the cDNA isolated from an interleukin-4-stimulated, mouse cytotoxic T-lymphocyte cell line (3). The mouse cytotoxic T-lymphocyte lipase clone is most homologous to hPLRP-2 (2).

Cloning and sequence analysis

A rat pancreas cDNA library (Clontech) was screened with random primer ^{32}P -labeled probes derived from human colipase and PL cDNA (11, 12). Multiple positive clones for each probe were purified, and the λ -DNA was isolated (14). Restriction analysis with *EcoR* I identified several potential full-length clones. These inserts were subcloned into pGEM32, and the nucleotide sequence was determined with universal primers and synthetic oligonucleotide primers by the dideoxynucleotide method with Sequenase. The sequences were previously deposited in Genbank (rat triglyceride lipase, M58369; rat colipase, M58360).

To isolate the cDNA encoding the rPLRP-2, cDNA was synthesized from rat pancreas RNA with oligo(dT) primer and reverse transcriptase. A region of cDNA encoding lipases was amplified by polymerase chain reaction (PCR) using nested primers. The first set was derived from regions that were identical in the cDNA for hPL, mPLRP-2, rPL, and rPLRP-1 (see Fig. 1). The first PCR reaction contained these primers and the cDNA synthesized from 5 μg of rat pancreas RNA. The mixture was denatured at 94°C for 1 min, annealed, and extended at 60°C for 2 min for 25 cycles. The 285-bp product was isolated from a 2% agarose gel with diethylaminoethyl paper (14). This product was expected to contain sequences of PL, PLRP-1, and PLRP-2. A second set of primers was derived from the mPLRP-2 sequence in a region that has little identity to hPL, rPL, or rPLRP-1; this set of primers was used to amplify the putative rPLRP-2 sequence from an aliquot of the first PCR product (Fig. 1) (2,3). This product was the template for a ^{32}P -labeled probe that was used to screen the rat pancreas cDNA library. The probe was generated with Klenow using the PCR oligonucleotides as primers (14).

Six positive clones were isolated and the λ -DNA prepared. Each had similar size inserts as determined by *EcoR* I restriction analysis. Each was subcloned into pGEM3Z and sequenced partially as described above. All were identical over the region sequenced, ~ 600 bp. One clone was fully sequenced on both strands.

The rPLRP-1 cDNA was a gift from Dr. Catherine Wicker-Planquart (Marseille, France) (18).

Tissue collection and RNA isolation

Adult tissue was obtained from breeding male Sprague-Dawley rats. Fetal tissue was obtained from pregnant Sprague-Dawley rats weighing between 250 and 300 g. The rats were maintained on a 12: 12-h light-dark schedule and fed ad libitum with standard rat chow. Gestational stage was based on the appearance of the vaginal mucoid plug after introduction of the male. Protocols for use of live vertebrate animals were approved by the Committee on Humane Care of Laboratory Animals at Washington University. After animals were anesthetized by intraperitoneal injection of pentobarbital, 60 mg/kg body wt, the pancreas was rapidly excised, snap-frozen, and stored in liquid nitrogen.

Total cellular RNA was isolated from pancreas by the RNazol method following the manufacturer's instructions (Cinna/Biotech Laboratories, Friendswood, TX). All of the organs from a single litter were pooled for the prenatal and birth time points. Single or two pooled organs were extracted for the other postnatal time points. RNA integrity was determined by formaldehyde-agarose gel electrophoresis and a ratio of optical density at 260 nm to optical density at 280 nm between 1.9 and 2.1.

Dot-blot analysis of mRNA

Relative quantitative mRNA analysis was performed on supported nitrocellulose. Total RNA was measured by absorbance at 260 nm. RNA (3 µg) was applied to the membrane in a dot-blot manifold after denaturing in 7.5~ SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) and 25% formaldehyde. All samples had 3 µg of yeast tRNA as carrier. Dots containing 3 µg of yeast tRNA and 3 µg of total liver RNA were included on all blots. Each time point was done in duplicate or triplicate. The repeat determinations were done on RNA isolated from separate animals or separate litters. The membrane was baked at 80°C in a vacuum oven for 2 h.

[³²P]dCTP-labeled antisense cRNA probes for colipase were generated from linearized plasmids with the Riboprobe system following the manufacturer's directions (Promega, Madison, WI). A random primer probe for rPLRP-2 was generated from the rPLRP-2 PCR product with the specific oligonucleotides for the second PCR reaction as primers (Fig. 1). Oligonucleotide probes were labeled with [³²P]dATP with T4 polynucleotide kinase (14). Prehybridization and hybridization were performed at either 42°C (cDNA and oligonucleotide probes) or 65°C (cRNA probes) in 50% formamide, 0.03~ Denhardt's solution (14), 5.2~ SSC, 0.026 M sodium phosphate, pH 7.0, and 0.1% sodium dodecyl sulfate (SDS). Blots were washed at a final stringency of 60°C for oligonucleotide probes or 65°C for cDNA and cRNA probes in 1× SSC and 1% SDS for 45 min and exposed to Kodak XAR film at -70°C with intensifying screens.

The blots were analyzed by radioisotopic scanning of the membrane with an AMBIS radioanalytic imaging system (AMBIS Systems, San Diego, CA). After scanning, all blots were stripped and probed with an oligonucleotide to 18S rRNA. Each signal was normalized to the amount of 18S rRNA to account for loading differences.

Expression of rPL, rPLRP-1, and rPLRP-2 in Sf9 cells

The cDNA for rPL, rPLRP-1, and rPLRP-2 were subcloned into the baculovirus transfer vector pVL1392. Sf9 cells were transfected, and recombinant virus was prepared as described (16). Sf9 cells in spinner culture were infected with the purified recombinant virus, and the media were collected 4–5 days postinfection. The cells and debris were removed by centrifugation. The media were analyzed by immunoblot after SDS polyacrylamide gel electrophoresis (PAGE) (10), and lipase activity was determined. Protein concentrations were determined by immunoblot, using hPL and antibody against hPL as

standards (10). Expression levels ranged from 20 to 50 µg/ml for each protein. Alternatively, the proteins were purified by ion exchange chromatography (M. L. Jennens and M. E. Lowe, unpublished results) before analysis. Lipase activity was determined against [³H] triolein (10). Human procolipase or trypsin-activated human colipase gave identical results in the assay. Most determinations were done with procolipase.

RESULTS AND DISCUSSION

Cloning and sequence analysis of rat cDNA for procolipase, PL, and PLRP-2

A cDNA encoding a protein homologous to hPLRP-1 was previously isolated from a rat pancreas library (18). A cDNA corresponding to rat PLRP-2 has not been described. To determine if the rat pancreas has mRNA for a PLRP-2, a putative, partial rat cDNA for PLRP-2 was isolated from pancreas RNA by PCR. Direct sequence analysis of this cDNA demonstrates 95% identity to the nucleotide sequence of the corresponding region in the mPLRP-2 cDNA. Screening of a rat pancreas library probe derived from this cDNA produced a complete cDNA.

Figure 2 shows the nucleotide sequence and the predicted amino acid sequence of this 1,546-bp clone. A protein sequence of 482 amino acids is predicted, although the actual protein may be 468 amino acids. The sequence Lys-Glu-Val-Cys-Tyr is the predicted NH₂-terminus of the mature protein by analogy to other lipases, but two methionines are candidates for the translation start site, and the length of the signal peptide is not certain. It may be 16 or 30 amino acids long. Neither potential initiation site is homologous to the initiation consensus sequence, so that criterion does not discriminate between the two sites (8). Most pancreatic exocrine proteins have short leader sequences, 16 to 20 amino acids. Analogy to these proteins predicts that the second methionine is the start site (12). There is no poly(A) tail on the clone, indicating that part of the 3'-untranslated region is missing.

The predicted amino acid sequence has 63% identity to hPL, 67% to rPLRP-1, 93% to mPLRP-2, and 75% to hPLRP-2. Alignment of the predicted amino acid sequence with hPL reveals that the catalytic triad of hPL is conserved (10). The nucleophilic serine is present in the Gly-X-Ser-X-Gly sequence found in lipases from many different sources (5). A histidine and an aspartic acid complete the triad and are also conserved. The close homology to the PLRP-2 proteins suggests that this clone is rPLRP-2 and demonstrates that the rat, like the human, also has at least two gene products that are closely related to PL.

Another homologous cDNA was isolated from a rat pancreas library with a probe derived from the hPL cDNA. The clone is 1,492 nucleotides encoding an open reading frame of 465 amino acids (Fig. 3A). A 16-amino acid signal peptide is predicted. The sequence has 78% identity to the hPL sequence, 66% identity to hPLRP-1, 62% identity to hPLRP-2, and 63% identity to mPLRP-2. These comparisons suggest that the cDNA encodes rPL. The predicted amino acid sequence of this clone is 66% identical to rPLRP-1 and 65% identical to rPLRP-2.

Because of the intimate relationship between PL and another pancreatic exocrine protein, colipase, a cDNA for procolipase was isolated to allow comparisons of procolipase expression with the expression of PL and the PLRPs. The rat procolipase clone is 485 bp plus a poly(dA) tail (Fig. 3B). An open reading frame encodes 112 amino acids. The predicted amino acid sequence is 72% identical to that of human procolipase. Like the human protein, the rat protein has a 17-amino acid signal peptide and a 5-amino acid propeptide. The sequence is identical to another sequence for rat procolipase except that the first amino acid of the propeptide is alanine rather than valine (14). The NH₂-terminus of the mature protein is predicted to be Gly-Leu-Phe-Ile-Asn-Leu.

Activity of rPL, rPLRP-1, and rPLRP-2 expressed in Sf9 cells

The cDNA for rPL, rPLRP-1, and rPLRP-2 were subcloned into baculovirus transfer vectors, and recombinant virus was produced. Sf9 cells were infected with the virus, and the media were tested for the presence of lipase. After separation by SDS-PAGE, the proteins were transferred to a membrane and probed with an antibody against hPL. A single protein band is detected in both the rPL and rPLRP-2 media, but a doublet is present for rPLRP-1 (Fig. 4). The two PLRPs migrated at a higher molecular weight than rPL. The larger size of PLRP-1 is due in part to an additional six amino acids at the carboxy terminus. If the signal peptide of rPLRP-2 is 16 amino acids instead of 30, then that protein would have an additional 14 amino acids at the amino terminus, and rPLRP-2 would appear larger than rPL on SDS-PAGE. The presence of oligosaccharides on the PLRPs may also contribute to the larger size. rPLRP-1 binds to concavalin A Sepharose beads and elutes with α -methylmannoside, whereas rPL and rPLRP-2 do not bind to the resin (data not shown). The cross-reactivity with a polyclonal antibody to hPL suggests the presence of common antigenic epitopes and provides further evidence that the proteins have similar structures.

To test for lipase activity in the media, aliquots were incubated with [3 H]triolein emulsified with deoxycholic acid (DOC) or taurodeoxycholic acid. Lipolytic activity is present in the media from Sf9 cells for all proteins when (pro)colipase is present (Table 1). No activity is present in the medium of Sf9 cells infected with the wild-type virus. The activity of rPLRP-1 is 15- to 40-fold less than the activity of rPL and rPLRP-2. In some preparations of rPLRP-1 no activity can be detected. The rPLRP-1 activity shows a pH optimum of 8.0 and no NaCl dependence from 0 to 200 mM (data not shown). rPLRP-1 activity is not inhibited by bile salts, nor is activity stimulated by procolipase or colipase. In contrast, the activity for both rPL and rPLRP-2 is inhibited by bile salts and is reactivated by (pro)colipase (Table 1 and Fig. 5A).

The behavior of the lipases may reflect important functional differences that may provide clues to the mechanism of bile salt inactivation and (pro)colipase reactivation of lipase. Although rPLRP-1 has a primary structure closely related to rPL and rPLRP-2, some of the differences must determine reactivity toward triglycerides. The low activity of rPLRP-1 against triolein argues that the protein acts on other substrates, possibly phospholipids, cholesterol esters, or vitamin esters, or that another protein cofactor, analogous to colipase, may be required for full rPLRP-1 activity.

The inactivation by bile salts and (pro)colipase dependence of rPLRP-2 are in contrast to the properties reported for hPLRP-2 and for mPLRP-2 (6, 10). The human protein has significant activity in the presence of bile salts, and that activity is minimally stimulated by colipase (6). The available data on the mPLRP-2 indicate significant activity without colipase present and only a three- to fourfold stimulation by colipase (10). rPLRP-2 has little activity in the presence of bile salts and is stimulated 20- to 40-fold by colipase, as is rPL.

To confirm that the rPLRP-2 is inhibited by bile salts and reactivated by (pro)colipase, the activity of rPLRP-2 was tested over a range of DOC concentrations with and without procolipase present (Fig. 5B). rPLRP-2 is inhibited by increasing concentrations of DOC, and procolipase clearly reactivated rPLRP-2 over a broad DOC concentration range. The results suggest that rPLRP-2 is more sensitive to bile salt inhibition than hPLRP-2 or mPLRP-2 and that rPLRP-2 is more responsive to (pro)colipase reactivation than PLRP-2 from the other species. There may be differences in the properties of rPLRP-2, such as optimum pH, inhibition by substrate or product, substrate specificity, or sensitivity to bile salts, that explain the different properties of the PLRP-2s from various species. Alternatively, despite the homology to hPLRP-2 and mPLRP-2, the rat protein may represent a fourth form of lipase with distinctly different catalytic properties.

Expression during rat development of procolipase, PL, PLRP-1, and PLRP-2

The intimate relationship of PL function and the presence of (pro)colipase in the duodenum argues that the genes for these proteins are coordinately regulated. The developmental patterns of procolipase, PL, and the PLRPs were determined by RNA blot analysis. Procolipase mRNA was detected with a Riboprobe for procolipase. Because a PL Riboprobe would detect PL and PLRP mRNA and confound the interpretation of the results, probes complementary to regions of low homology among the lipase mRNA were hybridized to the RNA blots (Fig. 6). When tested against cRNA for rPL and the rPLRPs, each probe specifically detects the mRNA from which the probe is derived (Fig. 7A). Furthermore, each probe recognizes a single mRNA species in pancreas RNA and does not hybridize to RNA from rat liver (Fig. 7B).

A difference in expression during development is suggested by these blots. The PLRP mRNA is present at 12 h after birth, but the PL mRNA is not detected. All three species are present in 28-day and adult rat pancreas RNA. The levels of the PLRP mRNA decrease with age, whereas the levels of PL mRNA increase with age. To extend this observation, the presence of mRNA for each lipase and for procolipase was determined by dot-blot analysis of pancreas RNA harvested at various prenatal and postnatal ages. The blots were hybridized to probes specific for each mRNA.

The profiles of all four mRNA are given in Fig. 8. rPL mRNA is low during gestation and in the first weeks after birth. By 21 days after birth, the rPL mRNA rises rapidly to levels that are 40-fold higher than at birth. The highest levels of rPL mRNA are present in the adult, 100-fold higher than are present at birth. The profile of the mRNA for rPL follows the profile of rPL activity previously reported for the developing rat (1, 13). The activity is low at birth and rises through the suckling-weanling period to reach adult levels by the time of weaning.

rPLRP-1 and rPLRP-2 mRNA have markedly different patterns compared with rPL mRNA (Fig. 8). Both rPLRP mRNA are abundant before birth, and both peak by 12 h. rPLRP-1 falls tenfold to adult levels by 21–28 days. rPLRP-2 falls 15-fold from 12 h to 7 days. The pattern of mRNA expression suggests that regulation of the rPLRPs is coordinate as opposed to the discoordinate regulation of the rPLRP and rPL mRNA. Although differences in mRNA stability cannot be excluded, these results suggest that the genes for the rPLRPs are under different regulatory controls during development than is The rPL gene.

The relative abundance of the PLRP mRNA around birth and in the first weeks of life raises the possibility that they may be important in the digestion of rat colostrum, or rat milk, which is high in fat relative to rat chow (9). The large, rapid increase of the rPLRP mRNA during the first 12 h of life may be a response to the onset of feeding. Thus the composition of the diet may regulate the expression of these genes directly or through hormones (4). Alternatively, hormonal changes accompanying birth, such as surges in corticosteroids or thyroid hormone, may trigger the rise in rPLRP mRNA independent of diet or the onset of suckling.

The mRNA profile of procolipase also differs from the lipase profiles. Procolipase mRNA is readily detectable during gestation and reaches adult levels by 17–20 days gestation. Procolipase mRNA rises markedly at 7 days of age but falls to adult levels by 14 days and remains at that level throughout the suckling-weanling period. The different patterns of mRNA argue against the coordinate regulation of rPL and colipase expression. The requirement of rPLRP-2 for (pro)colipase may explain the differences in expression of colipase and rPL mRNA. The early procolipase expression may reflect its role in rPLRP-2 activity immediately after birth.

In this paper, we describe the cDNA for a novel rat protein that resembles hPLRP-2. The expressed protein has lipase activity and displays a developmental mRNA profile similar to the previously described rPLRP-1 but different from rPL. The data raise the possibility that these closely related members of the lipase gene family have evolved different regulatory elements. The close homology of the three proteins requires that specific probes be used in studies of lipase mRNA changes with diet, hormones, or development to unravel the relationship of PL and the two PLRPs and to avoid erroneous conclusions based on changes in a homologous protein.

During the preparation of this manuscript, the cDNA of a pancreatic acinar cell zymogen granule protein, GP-3, was published (19). The cDNA is identical to the rPLRP-2 cDNA reported in this paper. The role of an active, colipase-requiring lipase tightly associated with zymogen granule membranes is not known. It is possible that rPLRP-2 has activity against other substrates that may explain its presence on zymogen granule membranes.

Acknowledgments

We thank Drs. David Perlmutter, Sherrie Hauff, and Arnold Strauss for their critical reading of the manuscript.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-33487 and was done during the tenure of an Established Investigatorship (M. E. Lowe) from the American Heart Association.

REFERENCES

1. Deschodt-Lanckman M, Robberecht P, Camus J, Baya C, Christophe J. Hormonal and dietary adaptation of rat pancreatic hydrolases before and after weaning. *Am. J. Physiol.* 1974; 226:39–44. [PubMed: 4358852]
2. Giller T, Buchwald P, Blum-Kaelin D, Hunziker W. Two novel human pancreatic lipase related proteins, hPLRP1 and hPLRP2. *J. Biol. Chem.* 1992; 267:16509–16516. [PubMed: 1379598]
3. Grusby MJ, Nabavi N, Wong H, Dick RF, Bluestone JA, Schotz MC, Glimcher LH. Cloning of an interleukin-4 inducible gene from cytotoxic T lymphocytes and its identification as a lipase. *Cell.* 1990; 60:451–459. [PubMed: 2302735]
4. Kern HF, Rausch U, Scheele GA. Regulation of gene expression in pancreatic adaptation to nutritional substrates or hormones. *Gut.* 1987; 28:89–94. [PubMed: 3319815]
5. Kirchgessner TG, Chuat JC, Heinzmann C, Etienne J, Guilhot S, Svenson K, Ameis D, Pilon C, D'Auriol L, Andalibi A, Schotz MC, Galibert F, Lusic AJ. Organization of the human lipoprotein lipase gene and evolution of the lipase gene family. *Proc. Natl. Acad. Sci. USA.* 1989; 86:9647–9651. [PubMed: 2602366]
6. Kirchgessner TG, Svenson KL, Lusic AJ, Schotz MC. The sequence of cDNA encoding lipoprotein lipase. *J. Biol. Chem.* 1987; 262:8463–8466. [PubMed: 3597382]
7. Komaromy MC, Schotz MC. *Proc. Natl. Acad. Sci. USA.* 1987; 84:1526–1530. [PubMed: 3470738]
8. Kozak M. Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell.* 1986; 44:283–292. [PubMed: 3943125]
9. Lee PC, Kim OK, Lebenthal E. Effect of early weaning and prolonged nursing on development of the rat pancreas. *Pediatr. Res.* 1982; 16:470–473. [PubMed: 6179036]
10. Lowe ME. The catalytic site residues and interfacial binding of human pancreatic lipase. *J. Biol. Chem.* 1992; 267:17069–17073. [PubMed: 1512245]
11. Lowe ME, Rosenblum JL, McEwen P, Strauss AW. Cloning and characterization of the human colipase cDNA. *Biochemistry.* 1990; 29:823–828. [PubMed: 2337598]
12. Lowe ME, Rosenblum JL, Strauss AW. Cloning and characterization of human pancreatic lipase cDNA. *J. Biol. Chem.* 1989; 264:20042–20048. [PubMed: 2479644]

13. Robberecht P, Deschodt-Lanckman M, Camus J, Bruylants J, Christophe J. Rat pancreatic hydrolases from birth to weaning and dietary adaptation after weaning. *Am. J. Physiol.* 1971; 221:376–381. [PubMed: 5555811]
14. Sambrook, J.; Fritsch, EF.; Maniatis, T. *Molecular Cloning, A Laboratory Manual*. 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; 1989.
15. Sims HF, Jennens M, Lowe ME. The human pancreatic lipase gene: structure and conservation of an *Alu* sequence in the lipase gene family. *Gene*. 1993; 131:281–285. [PubMed: 8406023]
16. Summers, MD.; Smith, GE. *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Bulletin No. 1555. College Station, Texas: Dept. of Entomology, Texas Agricultural Experiment Station and Texas A&M University; 1987.
17. Wicker C, Puigserver A. Rat pancreatic colipase mRNA: nucleotide sequence of a cDNA clone and nutritional regulation by lipidic diet. *Biochem. Biophys. Res. Commun.* 1990; 167:130–136. [PubMed: 2129524]
18. Wicker-Planquart C, Puigserver A. Primary structure of rat pancreatic lipase mRNA. *FEBS Lett.* 1992; 1:61–66. [PubMed: 1730292]
19. Wishart MJ, Andrews PC, Nichols R, Blevins GT Jr, Logsdon CD, Williams JA. Identification and cloning of GP-3 from rat pancreatic acinar zymogen granules as a glycosylated membrane-associated lipase. *J. Biol. Chem.* 1993; 268:10303–10311. [PubMed: 8486693]


```

=====
147 GGGAAAGAGGTTTGCTATGGACACCTTGGATGCTTTTCCAATGACAAGCCCTGGGCAGGG
   : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
121 GGAAAAGAGGTTTGCTATGATAACCTTGGATGCTTTTCTGACGCTGAGCCCTGGGCAGGG
   : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
75 GGAAAAGAAGTTTGCTTTGACAAACTCGGCTGCTTCAGTGATGATGCCCGTGGTCAGGA

207 ATGATTCAGCGGCCTTCGAAGATATTCCCTTGGTCCCCAGAGGACATTGATACCCGCTTT
   : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
181 ACAGCTATCAGGCCCTCAAACCTTCTCCCATGGAGCCCTGAGAAGATCAACACTCGCTTC
   : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
135 ACCATAGACAGACCCCTGAAAGCACTGCCCTGGAGCCCAGCACAGATCAACACCCGCTTT

267 CTTCTGTACACAAACGAGAATCCAACAAC TACCAAATAATCTCTGCCACCGACCCAGCC
   : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
241 CTGCTGTACACCAATGACAACCCAAC TGCTTTTCAGACTCTCCAGCTTTCTGACCCATTG
   : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
195 CTCTCTACACCAACGAGAACCAAGACAAC TACCAGAAAAT---TACTTCAGATGCATCG

327 ACCATCAATGCTTCCAAC TCCAAC TGGACCGCAAGACACGTTTCATTATCCATGGATTC
   : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
301 ACCATTGGGGCCTCAAATTTTCAAGTTGCCACCAACACTCGGTTTATCATCCATGGCTTC
   : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
252 AGTATCAGAAACTCCAATTTCAAACGAACAGAAAACCCGCATCATTATCCACGGCTTC

=====
387 ATAGACAAGGGAGAAGAAGGCTGGTTGCTGGACATGTGCAAGAAAATGTT mPLRP2
   : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
361 ATAGACAAAGGAGAGGAAAAC TGGGTGGTTGACATGTGCAAGAACATGTT rPLRP1
   : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
312 ATTGACAAGGGAGAGGAGAACTGGCTATCTGACATGTGCAAGAACATGTT rPL

```

Fig. 1.

The region amplified by polymerase chain reaction (PCR): nucleotide sequence for the region of rat pancreatic lipase (rPL), rat pancreatic lipase-related protein-1 (rPLRP-1) (18), and mouse PLRP-2 (mPLRP-2) (2, 3) amplified by PCR. Top sequence is for mPLRP2, a cDNA originally isolated from interleukin-4-stimulated, mouse cytotoxic T lymphocytes (3). Middle sequence is for rPLRP-1; bottom sequence is for rPL. Numbers refer to the base-pair position in the full-length sequence. Sequence of the oligonucleotide primers for the first PCR is underlined. Dashed double line marks nested primers for the second PCR. The 3'-primers were complementary to the given sequence.

```

10      20      30      40      50      60      70      80      90      100      110      120
agttgttcacatcccgcagtgactctagaattaactgctctctttgagctctggcctctgctggcctcatcctatcctatgacctaggggtgfcagtggaatctgtttccatctgtgcag
M P R G V S G N L F P S V Q
-31
130      140      150      160      170      180      190      200      210      220      230      240
ATGTTGCTCTGCTGGATCGTCAGTCTTCGTGGCCACTGTCGGAGGGAAAGAGGTTTGGCTATGGACACCTTGGCTGCCTTTTCCAATGACAAGCCCTGGGCAGGGATGCTTCAGCGGCT
M L L C W I V S L L L A T V G G K E V C Y G H L G C F S N D K P W A G M L Q R P
-16
250      260      270      280      290      300      310      320      330      340      350      360
TTGAAGATATCCCTTGGTCCCCAGAGGACATCGATACACGCTTCTTCTGTACAAAATGAGAAATCCAAAATACTACCAAAAAATCTCTGCCACCGAACACAGACACCATCAAGTTTCC
L K I F P W S P E D I D T R F L L Y T N E N P N N Y Q K I S A T E P D T I K F S
25
370      380      390      400      410      420      430      440      450      460      470      480
AACTTCAAACGGACCGCAAGACGCGTTTCATCGTCCATGGATTCATAGACAAGGGAGAGGATGGCTGGTGTCTGGACATGTGCAAGAAAATGTTCAAGTGGAGAAAAGTGAAGTGTATT
N F Q L D R K T R F I V H G F I D K G E D G W L L D M C K K M F Q V E K V N C I
65
490      500      510      520      530      540      550      560      570      580      590      600
TGCCTCGATTGGAGCGGAGGATCCAGGACTGAATACACCCAAGCCCTCTACAACACTCGGGTCTGGGGCGCTGAGATTGCCCTTCCCTAGTGCAGAGTTTATCGACAGAGATGGGGTACAGC
C V D W R R G S R T E Y T Q A S Y N T R V V G A E I A F L V Q V L S T E M G Y S
105
610      620      630      640      650      660      670      680      690      700      710      720
CCAGAGAAATGTTCACTCGGCCACAGCCTGGGCGCACAGTGGTTGGGAGGCAGGCAGGAGGCTGGAGGGCCATGGGCGAGGATACAGGGCTGGACCCCTGCAGAGCCCTGTTTC
P E N V H L I G H S L G A H V V G E A G R R L E G H V G R I T G L D P A E P C F
145
730      740      750      760      770      780      790      800      810      820      830      840
CAAGGCCTGCCAGAGGAAGTTCGGTTGGACCCATCGGATGCCATGTTTGTAGACGTGATCCACACAGATAGTGTCTCCATAAATCCCTATCTAGGTTTGGAAATGAGCCAAAAGGTCGGA
Q G L P E E V R L D P S D A M F V D V I H T D S A P I I P Y L G F G M S Q K V G
185
850      860      870      880      890      900      910      920      930      940      950      960
CATCTGGATTCTTCCCAATGGAGGGAAGGAAATGCCAGGATGTCAGAAAAACATCCCTTTCCACCATTGTTGATATCAACGGGATATGGGAAGGAACCAAAACTTTGTAGCCTGCAAC
H L D F F P N G G K E M P G C Q K N I L S T I V D I N G I W E G T Q N F V A C N
225
970      980      990      1000      1010      1020      1030      1040      1050      1060      1070      1080
CACCTGCGGAGCTACAAGTACTACGCCAGCAGCATCCTCAACCCCGAGGCTTCCCTGGGTACCCCTTCTCTTCTGAGAGATTCCAGCAGAATGACTGCTTCCCTTGTCCAGAAGAA
H L R S Y K Y Y A S S I L N P D G F L G Y P C S S Y E K F Q Q N D C F P C P E E
265
1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
GGGTGTCCCAAATGGGGCACTATGCTGATCAATTTGAGGGGAAAACCGCTACTGTGGAACAAACCGTTTATCTGAATACAGGAGATAGTGGTAACCTTACTCGTTGGAGATACAAAGTA
G C P K M G H Y A D Q F E G K T A T V E Q T V Y L N T G D S G N F T R W R Y K V
305
1210      1220      1230      1240      1250      1260      1270      1280      1290      1300      1310      1320
TCAGTCACACTGTCAGGAGCAAAGAAACTGAGTGGGTACATCTTGGTTGCTCTGTACGGAAATAATGGAAACTCAAAGCAGTATGAAAATTTCAAAGGATCCCTCAAACCGGAGCAAGG
S V T L S G A K K L S G Y I L V A L Y G N N G N S K Q Y E I F K G S L K P E A R
345
1330      1340      1350      1360      1370      1380      1390      1400      1410      1420      1430      1440
CATGTTCTGTCAGATGATGGACATCAATGTTGGAGAAATCCAGAAGGTTAAGTTCCTCTGGAACAACAAGGTGATAAATCTCTTCCGGCCACTCTGGGACGTTCCGAGATCACAGTG
H V R D I D V D I N V G E I Q K V K F L W N N K V I N L F R P T L G R S Q I T V
385
1450      1460      1470      1480      1490      1500      1510      1520      1530      1540      1550      1560
CAAAGTGGTGTAGACGGGAAAGAGTACAACCTTCTGTAGCAGCGACACTGTGCGTGAAGACGCTTTACAGTCGCTTTTCTCTGTTAAagatgtgccctgact
Q S G V D G K E Y N F C S S D T V R E D V L Q S L Y P C *
425
452

```

Fig. 2.
rPLRP-2 cDNA sequence and predicted protein sequence. Top line is nucleotide sequence, with untranslated region in lower-case letters and open reading frame in upper-case letters. The predicted amino acid sequence is given below in the single-letter amino acid code. Asterisk marks the stop codon. Putative active-site residues are in boldface; potential NH₂-glycosylation site is underlined.

<p>A</p> <p>1 ggtccatagaaccgacggatccgccacgATGCTGATGCTGTGGACATTTGCAGTGCTGC -17 M L M L W T F A V L</p> <p>61 TGGGAGCAGTAGCTGGAAAAGTGGTTGCTTTGACAACTCGGCTGCTTCAGTGATGATG -7 L G A V A G K E V C F D K L G C F S D D</p> <p>121 CCCGTTGGTACAGGAACCATAGACAGACCCCTGAAAGCACTGCCCTGGAGCCAGCAGACA 15 A P W S G T I D R P L K A L P W S P A Q</p> <p>181 TCAACACCGCTTTCTCCTCTACACCAACGAGAACCAGACAACCTACCAGAAAATTACTT 35 I N T R F L L Y T N E N Q D N Y Q K I T</p> <p>241 CAGATGCATCGAGTATCAGAAACTCCAATTTCAAACGAAACAGAAAACCCGCATCATT 55 S D A S S I R N S N F K T N R K T R I I</p> <p>301 TCCACGCTTCATTGACAAGGGAGGAGAACTGGCTATCTGCATGTGCAAGAATGT 75 I H G F I D K G E E N W L S D M C K N M</p> <p>361 TCAAAGTGGAGAGTGTGAAGTGCATCTGTGGACTGGAAAGTGGCTCCCGGGTACAT 95 F K V E S V N C I C V D W K G G S R A T</p> <p>421 ACACCCAGGCTACCCAGAACCTCCGGTCTGGTGGCAGAAAGTGGCATTATTGGTTAAG 115 Y T Q A T Q N V R V V G A E V A L L V N</p> <p>481 TTCTGAAGTCTGACCTCGGTCCACCTGACAACGTCACCTGATGGCCACAGCCTGG 135 V L K S D L G H P P D N V H L I G H S L</p> <p>541 GTTCCCACGTTGCTGGGGAGGCAGGAAAGAGACATTCCGAGCTATTGGGAGGATCACAG 155 G S H V A G E A G K R T F G A I G R I T</p> <p>601 GGTGGACGCGCTGAACCTTACTTCCAGGGCACACCTGAAGAAGTCCGCTCGGACCCCA 175 G L D A A E P Y F Q G T P E E V R L D P</p> <p>661 CCGATGCCAGTTTGTGGATGCAATTCACACAGATGCTGCACCCATCATCCCCAATTAG 195 T D A Q F V D A I H T D A A P I I P N L</p> <p>721 GATTGGAAATGAGCCAGACTGTGGGTCACTAGATTTCTTTCTAATGGAGGATGGAAA 215 G F G M S Q T V G H L D F F P N G G M E</p> <p>781 TGCTGGATGTCAGAAGAATATCCTTTCCCAAATCGTTGACATCGACGGGATCGGGAAG 235 M P G C Q K N I L S Q I V D I D G I W E</p> <p>841 GAACTCGGACTTTGCTGCCTGTAACCACTTGAAGCTACAATATTACACCGACAGTA 255 G T R D F A A C N H L R S Y K Y Y T D S</p> <p>901 TCGTCAACCCGACTGGCTTCTGCTGCTCTCCTGTAGCTCTTACAATGTTTTTCTGCAA 275 I V N P T G F S G F S C S S Y N V F S A</p> <p>961 ATAAGTCTTCCATGTGGGAGTGAAGGATGCCACAGATGGTCACTATGCTGACAAAT 295 N K C F P C G S E G C F Q M G H Y A D K</p> <p>1021 ACCCTGGGAAGACAAAAGAACTTACCAGAAATTTTACCTGAACACTGGTGACAAGAGCA 315 Y P G K T K E L Y Q K F Y L N T G D K S</p> <p>1081 ACTTCGACGTTGGCGGTACCAGGTGACTGTCCCTCTCTGGACAGAAGGTCACTGGGC 335 N F A R W R Y Q V T V T L S G Q K V T G</p> <p>1141 ACATTCTAGTTTCTTTGTTGGAAAATGGAGGAACTTAAACAGTATGAAGTTTCAAGG 355 H I L V S L F G N G G N S K Q Y E V F K</p> <p>1201 GCTCTGCATCCAGGTGACACTCATGTCAAGGAATTTGACTCTGATATGGATGTTGGAG 375 G S L H P G D T H V K E F D S D M D V G</p> <p>1261 ATTTGCAGAAAGTTAAATTTATTTGGTACAACAATGTGATCAACCAACTCTACCCAAAG 395 D L Q K V K F I W Y N N V I N P T L P K</p> <p>1321 TGGGAGCATCAAGGATTTCAAGTGAACGAAATGACGGCAGAGTGTCAACTTCTGTAGTC 415 V G A S R I S V E R N D G R V F N F C S</p> <p>1381 AAGATACAGTGAGGAGGATGCTCTGCTTACACTGTCTGCATGTtagaaggctgctgatg 425 Q D T V R E D V L L T L S A C</p> <p>1441 tgtgaccaccaagtcccactgtaataaagtctagtatcaaagccaccggaa-poly (dA)</p>	<p>B</p> <p>10 tgccatectggtcgcgccagctccatccacccccagcgtaccagtcaccATGAAGGTC -17 M K V</p> <p>61 CTTGTTGTTCTGCTTGTAAACCTCGTTGCCGTGGCCTATGCAGCTCCTGGACCCCGGGT -14 L V V L L V T L V A V A Y A A P G P R G</p> <p>121 CTTTITATCAACCTGGAGGACGGTGAGATCTCGTAAACAGTATGCAGTGTAGAGCAGA 7 L F I N L E D G E I C V N S M Q C K S R</p> <p>181 TGCTGCCAATGACACCATCCTGGGATCGCCGGTGCACACACAAGCCATGGAGAAC 27 C C Q H D T I L G I A R C T H K A M E N</p> <p>241 AGCGAGTCTCCCCAAAGACCTCTATGGGATCTACTACAGGTGTCCCTGTGAGCGGGC 47 S E C S P K T L Y G I Y R C P C E R G</p> <p>301 CTGACCTGTGAGGGGACAGGAGCATCATTGGCGGCATACCAACACCAACTACGGCGTC 67 L T C E G D R S I I G A I T N T N Y G V</p> <p>361 TGCCCTGACTCCACCCGCTCCAAGCAGTgagatcgtgacgtgagctgggcccactctccc 87 C L D S T R S K Q</p> <p>421 tttcccttactcgcccactctgagtcacccattggcaattaagcccattgcaacctta -poly (dA)</p>
--	---

Fig. 3. Nucleotide sequences and predicted amino acid sequences for rat procolipase and rPL. *A*: complete nucleotide sequence and predicted amino acid sequence for the lipase clone. *B*: the same information for the colipase clone. Top line of each panel shows the nucleotide sequence, with untranslated regions in lowercase letters and coding region in capital letters. The predicted amino acid sequence is given in the single-letter code below the nucleotide sequence.

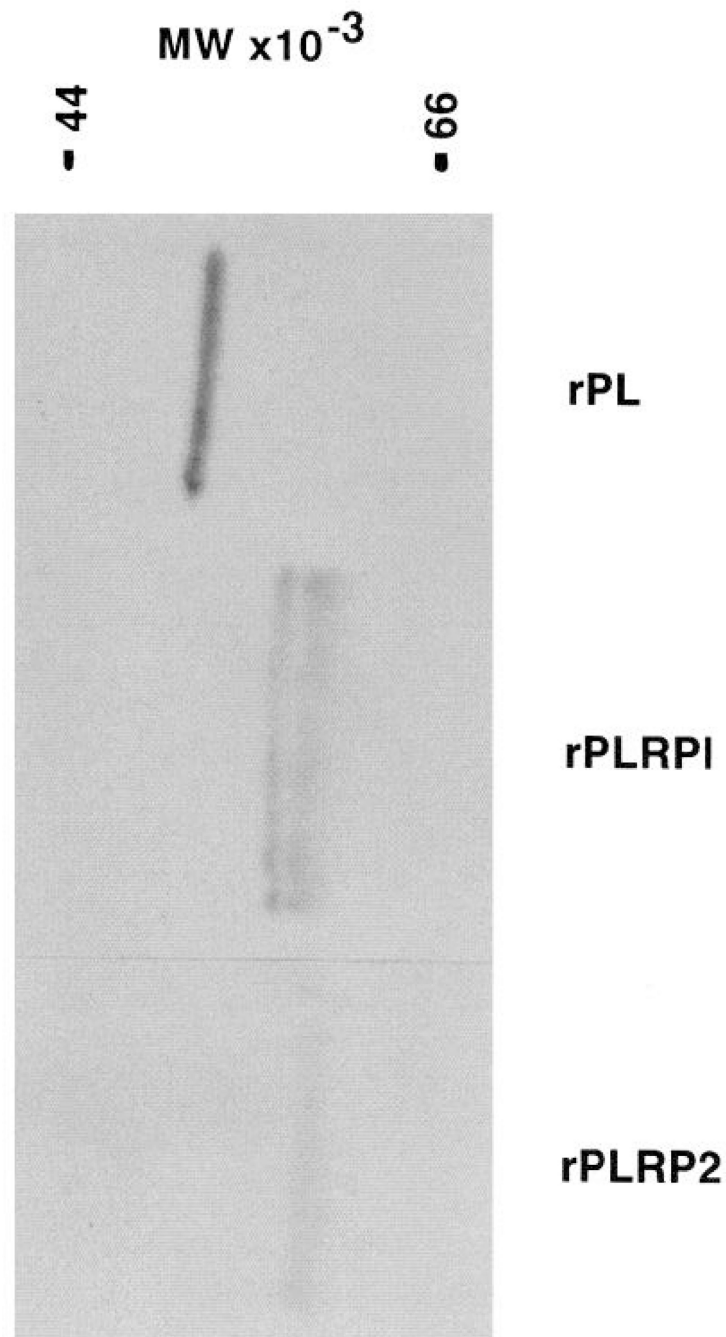


Fig. 4. Expression of human PL (hPL), rPL, and rPLRP-2 in baculovirus-infected Sf9 cells. Aliquots (20 μ l) of media from infected cells were collected 4 days postinfection and analyzed by SDS-PAGE and immunoblot with a polyclonal antibody against hPL. First lane, rPL; second lane, rPLRP-1; last lane, rPLRP-2.

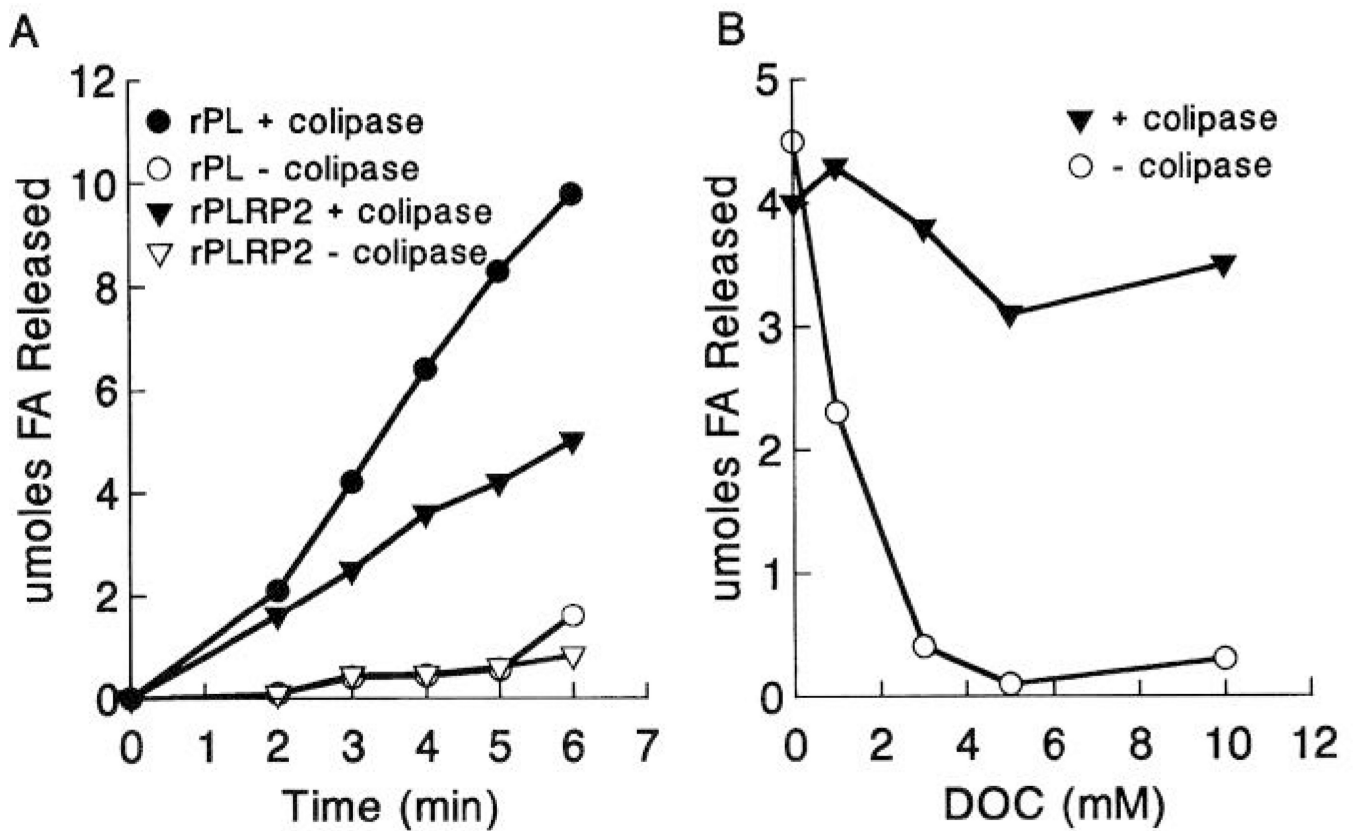
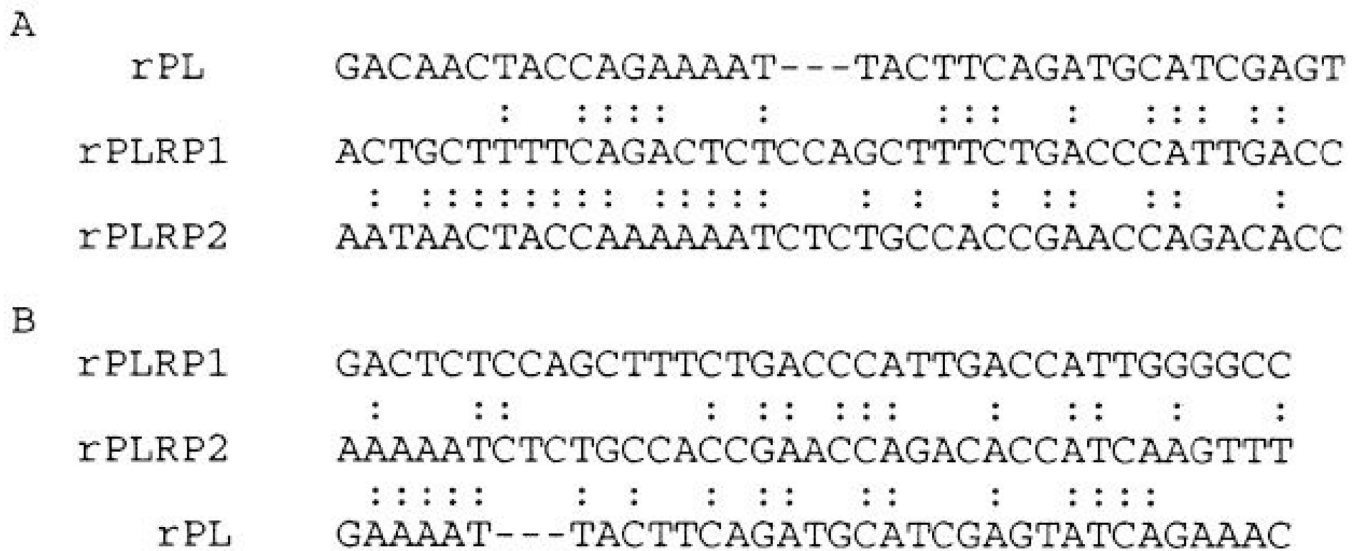


Fig. 5. Activity of rPL and rPLRP-2 against triolein. Each protein (100 ng) was assayed in 50- μ l volumes with the standard assay system (10). *A*: time course of the reaction in presence of deoxycholic acid (DOC) with and without human procolipase. *B*: inhibition of rPLRP-2 by various concentrations of DOC with and without human procolipase. Assay was done with 2.5% gum arabic; FA, fatty acid.

**Fig. 6.**

Nucleotide sequence of the specific probes for PL and PLRP-1. *A*: sequence of the PL probe compared with the homologous region of PLRP-1 and PLRP-2. Double dots show bases that match the PL sequence. Calculated median temperature (T_m) of PL is 10°C for PLRP-1 and 17°C for PLRP-2 (14). Calculated T_m of PL for hybridization to itself is 73°C. *B*: PLRP-1 probe sequence compared with homologous sequence in PL and PLRP-2. Double dots show bases that are identical. Calculated T_m of PL is 20°C for PLRP-1, 17°C for PLRP-2, and 79°C for itself.

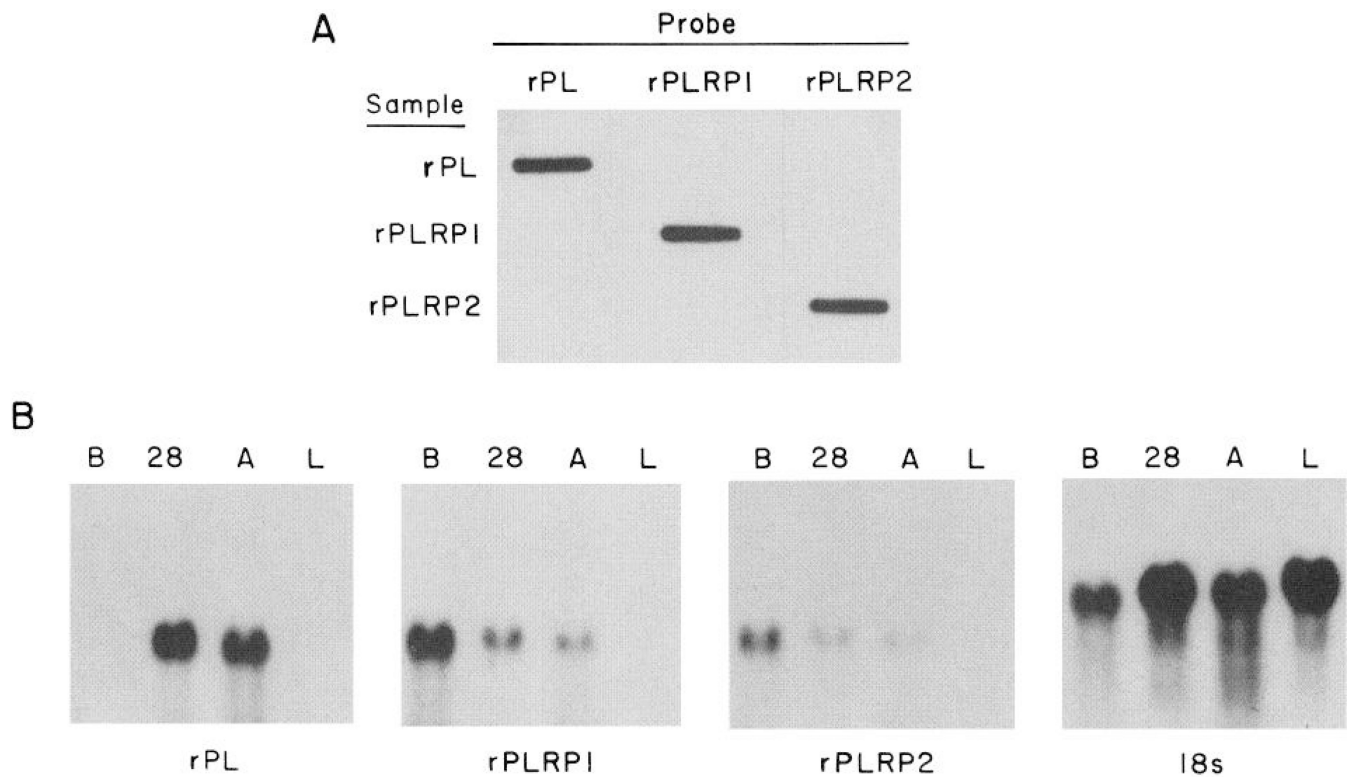


Fig. 7. RNA blots of rat pancreas RNA hybridized with specific probes. *A*: 3 slot blots containing cDNA for rPL, rPLRP-1, and rPLRP-2 hybridized with the specific probes described in Fig. 6. One hundred nanograms of each cDNA insert restricted from pGEM with *EcoR* I was denatured with 0.2 M NaOH and applied to a slot-blot apparatus containing a Hy-Bond N⁺ membrane. DNA was cross-linked to the membrane with ultraviolet light and hybridized to the probe indicated in the figure under conditions for dot blot given in METHODS. *B*: RNA blots of rat pancreas total RNA hybridized with each specific probe and an 18S RNA probe. RNA (25 µg) was separated on an agarose gel in formaldehyde (14) and transferred to Hy-Bond N⁺ membranes. Hybridization conditions were identical to those described in METHODS. Rat age is given above each line. B, 12 h after birth; 28, 28 days of age; A, adult; L, adult liver. Probe is given under each autoradiograph.

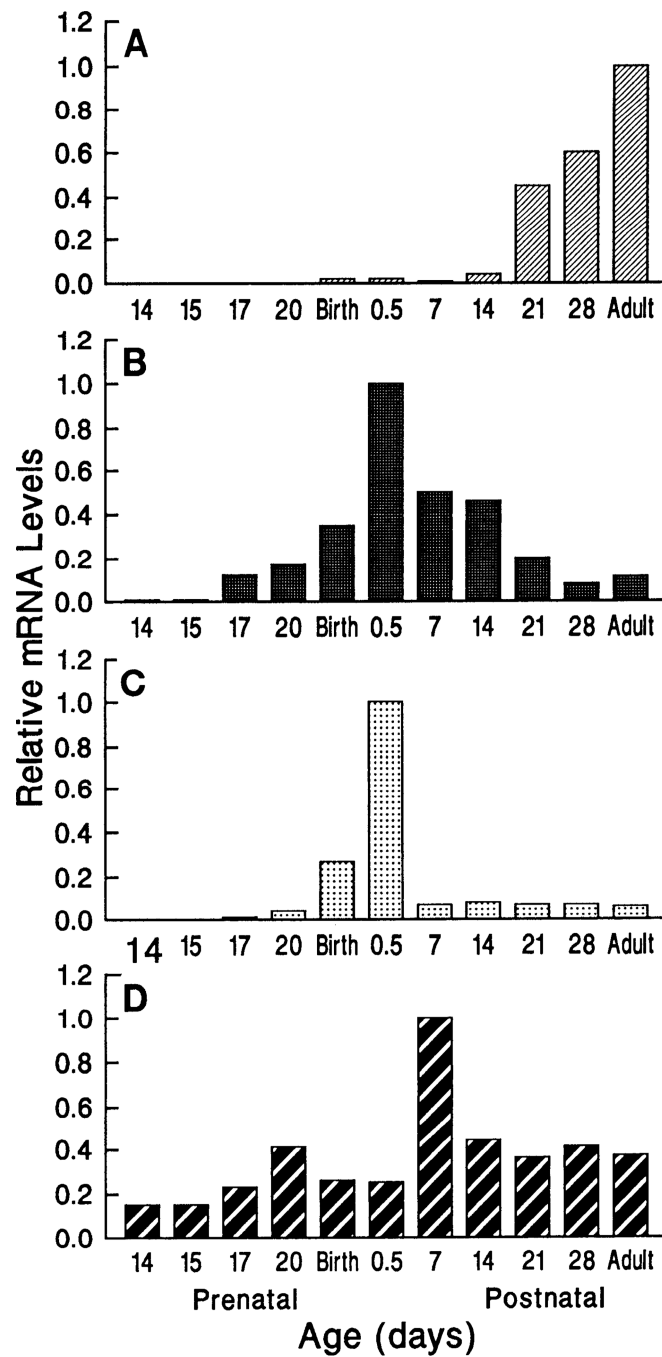


Fig. 8. Developmental patterns of colipase, PL, PLRP-1, and PLRP-2 mRNA in the rat. Dot blots containing RNA isolated from rat pancreas at various embryonic and postnatal ages were hybridized to oligonucleotide probes as described in METHODS. Relative mRNA levels are given at each age. *A*: results for PL. *B*: results for PLRP-1. *C*: results for PLRP-2. *D*: results for colipase. The birth, 0.5-day, 28-day, and adult points are averages of 3 separate determinations. All others are averages of 2 determinations.

Table 1

Lipase activity of rPL and related proteins

	DOC		TDC	
	-Colipase	+Colipase	-Colipase	+Colipase
rPL	0.38 ± 0.14	8.0 ± 0.90	ND	7.4 ± 0.47
rPLRP-1	0.28 ± 0.07	0.22 ± 0.11	0.23 ± 0.05	0.29 ± 0.04
rPLRP-2	0.20 ± 0.10	4.4 ± 0.45	ND	4.9 ± 0.29

Results are means ± SD of 7–8 measurements. Values did not vary if media or partially purified protein was assayed or if procolipase or colipase was added; values are given for the lipase protein as determined by immunoblot. The bile salt in the assay is indicated by deoxycholic acid (DOC) and taurodeoxycholic acid (TDC). Activity was measured at 2, 3, 4, and 5 min for rat pancreatic lipase (rPL) and rPL-related protein 2 (rPLRP-2) and at 5, 10, 30, and 60 min for rPLRP-1. Initial velocity was determined from the slope of the line. Assays are linear for at least 60 min.