Molecular cloning and expression of pulmonary lipid phosphate phosphohydrolases

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Nanjundan, Meera, and Fred Possmayer. Molecular cloning and expression of pulmonary lipid phosphate phosphohydrolases. Am J Physiol Lung Cell Mol Physiol 281: L1484–L1493, 2001.—Pulmonary lipid phosphate phosphohydrolase (LPP) was shown previously to hydrolyze phosphatic acid and lyso phosphatic acid in purified rat lung plasma membranes. To better investigate the nature of pulmonary LPP isoforms and their role in the lung, LPPs were cloned by RT-PCR from both adult rat lung and type II cell RNA. The RT-PCR generated LPP1 (849 bp), up to three LPP1 variants, and LPP3 (936 bp) cDNAs. The three LPP1 variants include LPP1a (852 bp) and two novel isoforms, LPP1b (697 bp) and LPP1c (1004 bp). The pulmonary LPP1 and LPP3 isoforms are essentially identical to the previously cloned rat liver and intestinal LPPs, respectively, and the LPP1a isoform has 80% sequence identity to the human homolog. The LPP2 isoform was not detected in lung by RT-PCR. Northern analyses revealed that the mRNAs for LPP1 and LPP3 increase in fetal rat lung in late gestation to day 1 after birth. These mRNAs decrease somewhat during the neonatal period but increase slightly during postnatal development. Expression of LPP1, LPP1a, and LPP3 cDNAs in HEK 293 cells established that they encode functional LPP. In contrast, the novel isoforms LPP1b and LPP1c contain frameshifts that would result in premature termination, producing putative catalytically inactive polypeptides of 30 and 76 amino acids, respectively. Further investigation of the LPP1b isoform revealed that it was present across a variety of tissues, although at lower levels than LPP1a. Transient mammalian expression of LPP1b failed to increase phosphatidate phosphohydrolase activity in HEK 293 cells.

bound enzyme primarily localized to the plasma membrane (22, 23). Both phosphatidic acid (PA) and lyso phosphatidic acid (LPA) were excellent substrates for LPP in purified rat lung plasma membranes, whereas sphingosine 1-phosphate (S-1-P) was a relatively poor substrate. This contrasts with liver LPP, the activity of which hydrolyzes PA, LPA, ceramide 1-phosphate (C-1-P), and S-1-P to similar extents (30).

Recent studies indicate a potential role for LPP in signal transduction. LPP could act to hydrolyze PA arising in the plasma membrane from diacylglycerol (DAG) kinase (10) or phospholipase D (PLD; see Ref. 24), thereby regulating the levels of PA in the plasma membrane. It has been suggested that transient elevations in DAG levels arising through phospholipase C (PLC) degradation of phosphatidylinositol bisphosphate in alveolar type II cells can be extended through the stimulation of the PLD/LPP pathway (24). LPP activity exists in isolated type II cells, plasma membrane, and in plasma membrane detergent-resistant domain (caveolae) preparations (22, 23), and a proposed function is in the regulation of surfactant phospholipid secretion. The signaling pathway would involve a plasma membrane-localized LPP, which would act sequentially to PLD in the purinergic P2a receptor cascade where it would generate DAG from phosphatidylcholine-derived PA, thereby sustaining protein kinase C (PKC) activation and surfactant secretion (24). Another proposed function of LPP may be in controlling cell growth where recovery from lung injury would involve type II cell proliferation and migration to restore the damaged type I cell population (6). Subsequently, the type II cells undergo a transdifferentiation process to reestablish the alveolar epithelium. These latter processes may require elevated DAG levels, generated by LPP for PKC activation, leading to expression of specific genes required for this process.

To further our understanding of the role of LPP in the lung, pulmonary LPP isoforms were cloned by RT-PCR using RNA from adult rat lung and type II cells. The RT-PCR generated LPP1, three LPP1 variants, and LPP3 cDNAs. The three LPP1 variants include LPP1a.
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and two novel isoforms, LPP1b and LPP1c. The latter isoform was rare, but LPP1b was present at similar levels as LPP1/1a. A rat tissue profile was screened with isoform-specific primers to investigate whether the novel LPP1 variants are tissue specific. The developmental profiles for LPP1 and LPP3 were examined during fetal and neonatal development. Transient expression of LPP1, LPP1a, and LPP3 in HEK 293 cells was performed to verify that these cloned cDNAs encode Mg$^{2+}$-independent, NEM-insensitive PAP activity.

MATERIALS AND METHODS

Materials. Trizol reagent, the Superscript preamplification kit, DNase I (amplification grade), isopropylthio-$\beta$-d-galactoside, X-gal, and tissue culture media were obtained from Gibco-BRL. PBS was obtained from CanSera. Porcine elastase was obtained from Worthington Biochemicals. RNase H and restriction enzymes were purchased from Pharmacia Biotech. The Advantage cDNA PCR kit was obtained from Clontech Laboratories. Mineral oil and herring sperm DNA were obtained from Sigma. pGEM-3Zf(+) was obtained from Promega. XL1-blue cells and QuikHyb solution were obtained from Stratagene. [\(\alpha\-32P\)]dCTP and [\(\gamma\-32P\)]ATP were purchased from Amersham. 1-Palmitoyl-2-oleoyl-phosphatidylcholine was purchased from Avanti Polar Lipids. DNA kinase from Escherichia coli was purchased from Calbiochem.

Cloning and sequencing of LPPs from rat lung and rat type II cells. The isolation of alveolar type II cells was followed according to Dobbs (7). Lungs were perfused with 0.9% saline as previously described (22). Total RNA was prepared using Trizol reagent. The RNA was DNase treated before first-strand cDNA synthesis. The reverse transcriptase reaction was performed using oligo(dT)12–18 as the primer and Superscript II reverse transcriptase. The cDNA was then RNase treated before the PCR reaction. PCR primers for amplification of LPP1, LPP2, and LPP3 were based on GenBank sequences (U90556, GAGGCCGCAGC (3'-primer) and GAGAGGA (5'-primer), and LPP1, the blots were washed in 0.1

Nucleotide sequencing of the various LPP clones was performed using fluorescent dye primer extensions with an automated DNA sequencer (Robarts Research Institute, DNA Sequencing Facility). The LPP sequences were analyzed using the BLAST and DiAlign2 programs.

Verification of LPP1 variants. The LPP variants were verified by using a combination of primers that were designed to be isoform specific. The PCR products were cloned into the pGEM-3Zf(+) vector and sequenced. The primers for LPP1/LPP1a, LPP1b, and LPP1c (primer set A) were 5'-GAGAGATCTGTGACCATGTTCGACAAAGCC (5'-primer); 3'-primer) and 5'-GAGGTTCTGCACGGCCGATGTCGTGCTTATAGCA (3'-primer); 383–365 bp). The primers for LPP1 and LPP1a (primer set B) were 5'-GCTGGCTTGGATTCCTTATATATA (5'-primer); 60–87 bp), 5'-AACAGCTGAAAATCCCTGATCC (5'-primer); 150–151 bp), and 5'-GAACTGGCCGTGGCAGTCGCCCTAATG (3'-primer); 386–365 bp). The primers for region II and III (see Fig. 1) of LPP1 (primer set B) were 5'-GCTGGCATGATCCCTTATATA (5'-primer); 68–81 bp) and 5'-TCCACCTAAATACGATAAGGG (3'-primer); 196–175 bp). The primers for region II and III (see Fig. 1) of LPP1a (primer set D) were 5'-TTCATTGTGGATACCTGTTTGA (5'-primer); 57–78 bp) and 5'-CAAGCCTCCATAGGACGATC (3'-primer); 186–165 bp).

Transient expression of LPPs in HEK 293 cells. HEK cells were maintained in DMEM (high glucose) containing 10% FBS. LPP1 and LPP1b were first digested from pGEM-3Zf(+) with Sal I, blunt ended with Klenow fragment, and Kpn I digested. LPP1a was digested from pGEM-3Zf(+) with Hind III, blunt ended with Klenow fragment, and Kpn I digested. LPP3 was digested from pGEM-3Zf(+) with BamHI and blunt ended with Klenow fragment. These LPP1/1a/1b inserts were then subcloned into the Kpn I and Xba I (blunt-ended) sites and, for LPP3 cDNAs, into the Xba I (blunt-ended) site of the mammalian expression vector pTracer-CMV2 (Invitrogen). The orientation was verified by a combination of restriction enzyme digestion and sequencing. Transfection-quality plasmid DNA was obtained using a calf intestinal phosphatase after overnight restriction enzyme digestion. Competent XL1-blue cells were transformed with the ligation products, and positive colonies were selected by performing blue-white colony screening.

Southern analysis of tissue profile. RNA was isolated from tissues obtained from 150- to 200-g Sprague-Dawley rats. As described above, RT-PCR was performed, and the products were run on a 2% agarose gel. The gel was denatured in denaturing buffer (0.5 M NaOH and 1.5 M NaCl) for 30 min at room temperature. Subsequently, the gel was transferred to neutralizing buffer (0.5 M Tris-HCl (pH 7.0) and 1.5 M NaCl) and slowly shaken for 30 min at room temperature. The gel was soaked in 20 x saline-sodium citrate (SSC) transfer buffer for 30 min and then transferred to a nylon membrane using the TurboBlotter Rapid Downward Transfer System (Schleicher & Schuell). Blots were probed with LPP1.

Northern analysis. A 1% agarose-RNA formaldehyde gel containing total RNA from tissues (20 μg/lane) was transferred to nitrocellulose membranes. Prehybridization for 20 min at 68°C in QuikHyb solution was followed by hybridization with the appropriate probe with 100 μl of denatured herring sperm DNA for 1 h at 68°C. For LPP3, the blots were washed for 1 h at 60°C in 2 x SSC and 0.1% SDS followed by a 30-min wash in 0.1 x SSC and 0.1% SDS at room temperature. For LPP1, the blots were washed in 0.1 x SSC and 0.1% SDS at room temperature for 6 h. LPP1 and LPP3 probes were prepared by random prime labeling using (\(\alpha\-32P\))dCTP. The membranes were exposed to X-ray film between 24 and 72 h for the LPP3 probe and up to 1 wk for the LPP1 probe.

Transient expression of LPPs in HEK 293 cells. HEK cells were maintained in DMEM (high glucose) containing 10% FBS. LPP1 and LPP1b were first digested from pGEM-3Zf(+) with Sal I, blunt ended with Klenow fragment, and Kpn I digested. LPP1a was digested from pGEM-3Zf(+) with Hind III, blunt ended with Klenow fragment, and Kpn I digested. LPP3 was digested from pGEM-3Zf(+) with BamHI and blunt ended with Klenow fragment. These LPP1/1a/1b inserts were then subcloned into the Kpn I and Xba I (blunt-ended) sites and, for LPP3 cDNAs, into the Xba I (blunt-ended) site of the mammalian expression vector pTracer-CMV2 (Invitrogen). The orientation was verified by a combination of restriction enzyme digestion and sequencing. Transfection-quality plasmid DNA was obtained using a

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Maxi Plasmid Preparative Kit (Qiagen). Transient expression of LPP1, LPP1b, and LPP3 was obtained using 1 μg of DNA and Effectene transfection reagent (Qiagen). Transient expression of LPP1a was obtained using 2 μg of DNA. Cells were harvested 36–48 h posttransfection, sonicated for 10 s (3 bursts), and then centrifuged at 100,000 g for 1 h at 4°C in a Ti70.1 rotor to obtain total membranes.

**LPP assays.** The activity was assayed using pure PA as substrate. Unlabeled PA and [32P]PA were prepared as described previously (22). Reaction mixtures contained 100 mM Tris-maleate buffer, pH 6.5, 0.6 mM PA (0.4 μCi/mmol), 0.5 mM EDTA, 0.5 mM EGTA, pH 7.0, 0.2 mg of essentially fatty acid-free albumin, and 1 mM dithiothreitol in a final reaction volume of 0.1 ml. Incubation times were 60 min in duration at 37°C. The protein was preincubated at 37°C for 10 min with 4.2 mM NEM. Reactions were terminated by the addition of 1.5 ml of chloroform-methanol (1:1). The phases were broken with 0.75 ml of 0.1 N HCl, and a sample of the upper aqueous phase was taken for scintillation counting to determine the [32P]Pi released (22).

**RESULTS**

**Cloning of pulmonary LPPs and variants.** LPP isoforms were cloned from perfused rat lung and freshly isolated alveolar type II cells that were maintained on tissue culture plastic overnight. Primers were designed to amplify the entire coding sequence of LPP1, LPP3, and a partial region of LPP2. RT-PCR products were cloned and sequenced revealing the presence of mRNA for LPP1 and its variants in lung (LPP1a, -1b, and -1c) and type II cells (LPP1b). LPP2 mRNA was not detected in lung or type II cells but was detected in RNA from rat brain, indicating that LPP2 mRNA is not expressed by lung. LPP3 was present in whole lung and type II cells.

**Other assays.** Protein was determined by the method of Lowry et al. (19) in the presence of 1% SDS using BSA as a standard.

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The LPP1 PCR products, when sequenced, revealed multiple products, including LPP1, LPP1a, LPP1b, and LPP1c. Figures 1 and 2, respectively, show the nucleotide sequence alignment and a graphical view of the LPP1 variants. The LPP1 products (LPP1, -1a, and -1b) were confirmed using other combinations of primers whose products were verified by sequencing. The LPP1 and LPP3 isoforms proved essentially identical to the previously cloned rat liver (5, 9) and intestinal isoforms (3, 10), respectively. Predicted amino acid sequences were identical to those reported previously. The LPP1a isoform is homologous to the human (11, 18) and guinea pig (12, 28) isoforms. The LPP1b and LPP1c isoforms are novel but contain nucleotide insertions/deletions (as indicated in bold in Figs. 1 and 2) that result in a frameshift leading to early termination of the polypeptide. A homolog of the LPP1b isoform was recently identified in the database of expressed sequence tags (dbEST) that was cloned from Bovine Taurus cartilage fetus (Genbank accession no. AV595831).

Sequence analysis of pulmonary LPP isoforms. Hydrophathy plot analysis of LPP1, LPP1a, and LPP3 based on the Kyte and Doolittle algorithm using the TMHMM program suggests that each has six membrane-spanning regions, whereas the predicted truncated isoforms could have only one (LPP1b) or two (LPP1c) transmembrane domains (data not shown). The full-length proteins (LPP1, LPP1a, and LPP3) contain an active site comprised of three domains (Fig. 3), which is part of a novel phosphatase superfamily that includes glucose 6-phosphatase, certain acid phosphatases, and chloroperoxidase (5, 27). They each contain a potential NH2-linked glycosylation site at amino acid positions 142, 143, and 171 (LPP1, LPP1a, and LPP3, respectively). The principal divergent regions among these LPPs include the NH2- and COOH-termini. Regions of divergence between LPP1 and LPP1a include the end of transmembrane region I and the beginning of transmembrane region II as well as certain residues in the first extracellular loop (Fig. 3). The LPP1 and LPP1a isoforms are most probably derived...
from alternative splicing. The expected molecular weights of the full-length LPPs are 31.9 kDa (LPP1), 32.0 kDa (LPP1a), and 35.2 kDa (LPP3). The LPP1b isoform contains a G nucleotide insertion at the 58th base, which would predict a peptide of 30 amino acids (3.61 kDa). Likewise, the LPP1c isoform contains a nucleotide deletion at the 214th base, which would predict a peptide of 76 amino acids (8.55 kDa).

If proteins are expressed, these isoforms would be catalytically inactive, since they would not possess the active site. Furthermore, they would both lack the NH2-linked glycosylation site. Three different preparations of lung RNA were used for the RT-PCR, resulting in the consistent appearance of LPP1b, whereas LPP1c was much rarer. The LPP1b isoform was not generated in reactions without cDNA. PCR of LPP1 or LPP1a cDNAs did not result in the formation of LPP1b cDNA.

Overexpression of rat pulmonary LPPs in HEK 293 cells. Transient transfection of rat lung LPP1, LPP1a, and LPP3 cDNAs in HEK cells resulted in an 8.7-, 2.4-, and 16.9-fold increase in membrane-bound LPP enzyme activity, respectively (Fig. 5). The activity of the three LPP isoforms was assayed using phosphatidate as a substrate in the presence of NEM and without Mg2+.

Developmental profiles for pulmonary LPPs. To obtain insight into the potential functions of the cloned LPP isoforms, their mRNA expression patterns were investigated across rat lung development. The profiles for the mRNA levels of LPP1 and LPP3 were determined by Northern analysis (Fig. 7). The mRNA levels were low at 17 days of gestation but increased slightly
in the fetal period (21 and 22 days gestation). There was a further increase in LPP1 mRNA expression in the newborns that remained high until day 4. The mRNA expression increased further on day 16, although remaining low compared with that in the newborn. The mRNA levels for LPP3 were low at 17 days of gestation and increased at 19 days gestation. The mRNA increased in the early neonate (1 or 2 days) and remained fairly constant during the first week of life. The mRNA expression increased during the second week of life (16 days) and in the adult.

**DISCUSSION**

LPP isoforms in rat lung and type II cells. In the lung, we previously observed that PA and LPA were excellent substrates in plasma membranes, whereas S-1-P was a relatively poor substrate (22). To investigate the possible presence of novel pulmonary isoforms, LPPs were cloned by RT-PCR from both adult rat lung and type II cell RNA that generated LPP1, three LPP1 variants (LPP1a, LPP1b, and LPP1c), and LPP3 cDNAs. The LPP2 isoform was absent in lung tissue but present in brain, as determined by RT-PCR, consistent with previous studies demonstrating the presence of human LPP2 mRNA in brain (9).

The cDNAs for all three major LPP isoforms (LPP1, LPP2, and LPP3) code for proteins with six transmembrane domains and a potential NH₂-linked glycosylation site located between transmembrane domains III and IV. All LPPs examined to date appear to be glycosylated, and altering the potential sites abolishes glycosylation of LPP1 (34). Comparison of LPP sequences with other members of the novel phosphatase family, such as glucose 6-phosphatase, chloroperoxidase, and acid phosphatase, revealed three highly conserved domains (5, 27). Conserved domains I and II flank the glycosylation site, whereas conserved domain III lies between transmembrane domains V and VI. Site-directed mutagenesis studies show that certain amino acids within these three conserved domains are essential for enzymatic activity. Taken together with recent evidence showing that the COOH-terminus of LPP1 is cytosolic (12), this would position the active site of LPP1, and apparently the other major isoforms, on the external side of the plasma membrane. In keeping with the above, coexpression of LPP1 in the readily transfecible rat2 fibroblast cell line resulted in an enhanced capacity for hydrolyzing exogenously administered LPA, PA, and C-1-P. LPA, C-1-P, and S-1-P are biologically active lipids that have been implicated in eliciting various biological responses, including cellular proliferation, differentiation, migration, and inhibition of apoptosis (1, 17). LPP1 was recently shown to be capable of hydrolyzing exogenously presented LPA (33) and to attenuate LPA-mediated effects on cellular proliferation through the endothelial differentiation gene-2 (EDG-2) receptor (32). Ras-transformed rat2 fibroblasts, which exhibit lower LPP-specific activities compared with their parental cell line, possess higher PA-to-DAG ratios, suggesting that the ectoenzyme is able to access endogenous PA generated within the cell (21). Consistent with this suggestion, Sciorra and Morris (25) have provided evidence that LPP3 can generate DAG sequentially to PLD in HEK 293 cells in caveolin-
enriched domains. The manner by which PA, apparently generated on the cytosolic leaflet of the plasma membrane, becomes available to LPP active sites on the cell’s external surface is not known. In addition, the substrate specificity of LPP1a, its localization, and its possible functions require further investigation.

Function of LPP1b. LPP1b is a novel isoform that predicts an inactive truncated protein of 30 amino acids. The possibility that LPP1b could be generated by PCR through chance was considered. PCR was performed using three separate preparations of RNA from lung tissue, and this isoform was generated in all reactions. Bidirectional sequencing was performed on numerous clones showing the consistent presence of the “G” nucleotide in exactly the same position in all clones sequenced. PCR of LPP1 and LPP1a clones
consistently produced the original cDNAs without the “extra” G nucleotide. Tissue profiles showed that LPP1b was expressed at relatively high levels in the lung and brain and varied across tissues different from that of the LPP1/LPP1a isoforms. Furthermore, LPP1b mRNA, determined by RT-PCR, was present in some human lung epithelial cells, namely human bronchial epithelial cells and an adenocarcinoma lung cell line, A549 (J. Faulkner and F. Possmayer, unpublished observations), and preliminary results suggest the presence of LPP1b mRNA in mouse lung cells. Thus the mRNA for this LPP1 variant is present in species other than the rat.

Whether the LPP1b isoform is produced or remains present in cells is unknown, and such a protein would be inactive. Precedent exists for transcription of mRNAs coding for truncated isoforms of PLD, DAG kinase, and PLC-δ, which would lack catalytic activity. It has been proposed that such isoforms might possess regulatory functions at either the mRNA or the protein level.

For example, Steed et al. (26) have observed that human PLD-2 cDNAs can contain a 56-bp splicing insert that results in premature termination during translation. This PLD-2c variant was detected in a large number of tissues and was relatively high compared with PLD-2a (active) in liver and heart but relatively low in skeletal muscle and brain. Splice variants of PLD-1a, such as PLD-1 and PLD-1b, show 114-bp deletions that would result in the generation of inactive proteins because they lack the essential transphosphatidylation motif contained in PLD-1a. It has been suggested that proteins corresponding to PLD-1, PLD-1b, PLD-2a, and PLD-2c may have functional roles, for example, by acting as modulators of PLD activators, but this has not been demonstrated definitively (26).

Kai et al. (15) have observed that human retina exhibits abundant expression of a phosphatidylserine-dependent DAG kinase isoform that appears to function in phosphatidylinositol regeneration from DAG arising through PLC activation. Compared with the retina, other human tissues contain very low levels of DAG kinase-γ RNA but express a catalytically inactive form of DAG kinase-γ in varying amounts. A low level of the full-length DAG kinase, but not the truncated form, is expressed in the brain. Although the mechanism is unknown, these results indicate regulated expression of the different DAG kinase isoforms at the mRNA level. The authors propose that truncation through mRNA splicing could represent a physiological mechanism for downregulating expression of this enzyme (15).

Rat testes express an alternate splice form of PKC, designated PKC-δIII, with an 83-nucleotide insertion within the caspase-3 recognition domain that results in premature truncation (29). The resulting protein possesses regulatory segments but lacks the catalytic domain. When expressed in Chinese hamster ovary-K1 cells, PKC-δIII was predominantly localized to plasma membranes, whereas the parental PKC-δI isoform was homogeneously dispersed throughout the cytoplasm. Phorbol ester stimulation promoted only a small further translocation of PKC-δIII but a more prominent translocation of PKC-δI. The authors propose that PKD-δIII could act as a dominant-negative modulator of PKC activation (29).

PLD and DAG kinase generate PA at the plasma membrane, whereas PKC-δ is activated by DAG. We consider it possible that the generation of LPP1b mRNA could provide a means of downregulating LPP1 or LPP1a mRNA transcription. It is also possible that LPP1b protein could function in a modulatory capacity, for example, acting as a dominant-negative type during up- or downregulation. It is stressed that no direct evidence is yet available, and the presence of LPP1b peptide must still be demonstrated in cells. Overexpression of LPP1b in HEK 293 cells did not affect LPP activity assayed in vitro. Further studies using pulmonary type II or other cells are required to test these suggestions.

Expression of pulmonary LPPs. Across the variety of rat tissues examined, lung showed a high expression of LPP1 mRNA and relatively high levels of LPP1a and LPP1b mRNAs. LPP3 mRNA levels were also high in the lung, although lower than the liver and brain. The LPP2 isoform was detected in the rat brain but was absent in the lung. Hooks and colleagues (9) have described high tissue-specific expression of human LPP2 detected in brain, pancreas, and placenta. The profiles obtained in this study compare similarly with those obtained for LPP1 and LPP3 in the mouse and human (16, 17). Leung and colleagues (18) have indicated higher expression of the human LPP1a isoform in certain tissues, including the heart and pancreas. In rats, LPP1 appears to be predominant over LPP1a or LPP1b in most tissues.

LPP1 and LPP3 mRNA levels increased in fetal rat lung in late gestation to the first day of life, declined slightly in the neonatal period, but tended to increase during later development. LPP1b levels, as indicated by semiquantitative RT-PCR, declined relative to LPP1/1a during late gestation and postnatal development. LPP1a mRNA expression was low in fetal lung and increased after birth.

Transient expression of the pulmonary LPP cDNAs in HEK 293 cells confirms that the full-length pulmonary LPPs are, indeed, catalytically active members of the LPP/PAP2 superfamily. Meanwhile, expression of the novel LPP1b isoform did not modulate LPP enzyme activity, consistent with the catalytically inactive, truncated 30-residue protein. The reason for our inability to overexpress LPPs in the MLE12 cell line is unknown but may be the result of toxic effects arising from the high constitutive expression. Overexpression of lipid signaling enzymes may lead to changes in cell morphology because of membrane damage, which may eventually lead to cell death from the strong cytoxic alovirus promoter in the pTracer-CMV2 expression vector. It was reported that stable ECV 340 and HEK
293 cell lines overexpressing LPP1, LPP1a, and LPP3 had a significant decrease in PA content compared with control cell lines (18, 25).

Other potential LPPs. It is possible that there exist other LPPs that have yet to be cloned from lung. A S-1-P phosphohydrolase recently cloned by Mandala et al. (20) had high homology to the active site of the LPPs but was proposed to contain 8–10 transmembrane domains. Boudker and Futerman (4) have identified an NEM-insensitive, Mg$^{2+}$-independent activity in rat liver plasma membranes that demonstrates specificity toward C-1-P. There also exists a nuclear LPP activity characterized by Baker and Chang (2) in neuronal nuclei that appears to be NEM insensitive and Mg$^{2+}$ independent. It displayed specificity toward LPA but was inhibited only by S-1-P and not C-1-P. Furthermore, Imai and collaborators (11) have recently described an activity in ovarian cancer cells that is NEM insensitive and Mg$^{2+}$ independent with activity against LPA that was not inhibited by S-1-P or C-1-P. Hence, the enzymological properties of these activities, including their substrate specificity, distinguish them from the presently cloned LPPs.

The cloning of the NEM-sensitive, Mg$^{2+}$-dependent PAP1 and its substrate specificity has not been reported yet. This enzyme may also be implicated in signaling, as reported by various investigators (13, 14). Other NEM-sensitive enzymes may exist, including an activity characterized by Frank and Waechter (8) that is unaffected by Mg$^{2+}$ and catalyses the hydrolysis of polyisoprenyl phosphate and PA with similar efficiencies.

We have shown previously that pulmonary LPP activity exists in alveolar type II cells and hydrolyzes PA and LPA in purified lung plasma membranes. Thus various LPPs were cloned by RT-PCR from both adult rat lung and type II cell RNA generating LPP1, up to three LPP1 variants, and LPP3 cDNAs. The three LPP1 variants include LPP1a and two novel truncated isoforms, LPP1b and LPP1c. The full-length rat lung cDNAs encode functional NEM-insensitive phosphatidate phosphohydrolases. These pulmonary LPPs are proposed to be involved in regulating surfactant phospholipid secretion in alveolar type II cells and in controlling cell growth during lung development and injury. The LPP1b and LPP1c isoforms contain frameshifts that would result in premature termination, producing putative catalytically inactive polypeptides of 30 and 76 amino acids, respectively. Further investigation of the LPP1b isoform mRNA across a tissue profile revealed that it exists in a number of tissues and is relatively abundant in lung. At present, the significance of the LPP1b isoform is not known.

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