Lipid phosphate phosphatases (LPP1–LPP3) have been topographically modelled as monomers (molecular mass of 31–36 kDa) composed of six transmembrane domains and with the catalytic site facing the extracellular side of the plasma membrane. The catalytic motif has three conserved domains, termed C1, C2 and C3. The C1 domain may be involved in substrate recognition, whereas C2 and C3 domains appear to participate in the catalytic dephosphorylation of the substrate. We have obtained three lines of evidence to demonstrate that LPPs exist as functional oligomers. First, we have used recombinant expression and immunoprecipitation analysis to demonstrate that LPP1, LPP2 and LPP3 form both homo- and hetero-oligomers. Secondly, large LPP oligomeric complexes that are catalytically active were isolated using gel-exclusion chromatography. Thirdly, we demonstrate that catalytically deficient guinea-pig FLAG-tagged H223L LPP1 mutant can form an oligomer with wild-type LPP1 and that wild-type LPP1 activity is preserved in the oligomer. These findings suggest that, in an oligomeric arrangement, the catalytic site of the wild-type LPP can function independently of the catalytic site of the mutant LPP. Finally, we demonstrate that endogenous LPP2 and LPP3 form homo- and hetero-oligomers, which differ in their subcellular localization and which may confer differing spatial regulation of phosphatidic acid and sphingosine 1-phosphate signalling.

Key words: cell survival, lipid phosphate phosphatase (LPP), phosphatidic acid (PA), sphingosine 1-phosphate (S1P).

INTRODUCTION

LPPs (lipid phosphate phosphatases) are integral membrane proteins that catalyse the dephosphorylation of lipid phosphates [e.g. PA (phosphatidic acid), S1P (sphingosine 1-phosphate), LPA (lysophosphatidic acid) and C1P (ceramide 1-phosphate)] in vitro in a Mg2+-independent and N-ethylmaleimide-insensitive manner [1]. Four mammalian LPP isoforms have been cloned, termed LPP1, LPP1a, LPP2 and LPP3 [2–6], the last corresponding to the endoplasmic reticulum protein Dri42 that is up-regulated during differentiation of intestinal epithelial cells [7]. LPPs have been suggested to belong to a family of lipid phosphatases/phosphotransferases that also includes lipid phosphatase-related proteins (or plasticity-related genes 1–4) and sphingomyelin synthases [8]. Each LPP is predicted to have six transmembrane domains with the catalytic site, made of three conserved domains (C1–C3), facing the extracellular side of the plasma membrane or the luminal side of intracellular membranes (Figure 1) [9]. The plasma membrane location of LPP1, LPP1a, LPP2 and LPP3 has been detected with isoform-selective [2,10–13] and anti-(epitope tag) antibodies [13–16]. LPP1 and LPP3 have also been identified in caveolae [11,12], whereas LPP2 and LPP3 are present in cytoplasmic vesicles in CHO (Chinese-hamster ovary) cells [13]. LPP2 and LPP3 are constitutively co-localized with SK1 (sphingosine kinase 1), and LPP3–SK1 is re-localized to the Golgi apparatus upon induction of PLD1 (phospholipase D1) in CHO cells [13].

LPPs have the potential to influence physiological responses to the GPCR (G-protein-coupled receptor) agonists LPA and S1P. This may involve dephosphorylation of extracellular S1P and LPA via an ecto-LPP activity, which may limit bioavailability at their receptors, S1P1,5 and LPA1,3 [4,14–16]. In addition, overexpression of LPP2 or LPP3 reduces the S1P- and LPA-stimulated activation of p42/p44 MAPK (mitogen-activated protein kinase) in serum-deprived HEK-293 (human embryonic kidney) cells [10,13]. This effect is blocked by pre-treating HEK-293 cells with the caspase 3/7 inhibitor, Ac-DEVD-CHO (N-acetyl-Asp-Glu-Val-Asp aldehyde) [13]. Therefore LPP2 and LPP3 appear to regulate the apoptotic status of serum-deprived HEK-293 cells. In this regard, LPP2 reduced basal intracellular phosphatidic acid levels, whereas LPP3 reduced intracellular S1P in serum-deprived HEK-293 cells [13]. These data are consistent with an important role for LPP2 and LPP3 in regulating an intracellular pool of PA and S1P respectively that may govern apoptosis in response to cellular stress. In contrast, LPP1 has been implicated in reducing migration in response to PDGF (platelet-derived growth factor) in mouse embryonic fibroblasts [17] and LPA in Rat2 fibroblasts [18]. Therefore LPP1–LPP3 may exhibit different functions in cells.

In the present study, we have investigated the structure–functional properties of the LPP isoforms and have established that these enzymes form homo- and hetero-oligomers and are localized in distinct intracellular compartments, where they may spatially regulate PA and S1P signalling.

EXPERIMENTAL

Materials

All biochemicals, including dioleoyl-PA, MβCD (methyl-β-cyclodextrin), FITC- and TRITC (tetramethylrhodamine β-isothiocyanate)-conjugated secondary antibodies, were from Sigma Chemical Co. Cell culture supplies and Lipofectamine™ 2000 were from Invitrogen. Anti-FLAG and anti-Myc antibodies were a generous gift from Dr. Tony Mason (University of Cambridge). Recombinant LPP proteins were produced as described previously [19]. The pCMV6-AC5-HA-LPP expression vector was a kind gift from Dr. Peter Seedorf (University of Cambridge). The pEG202-LPP1-HA and pEG202-LPP2-HA expression vectors were prepared by a PCR-based strategy, using the pCMV6-AC5-HA-LPP expression vector as template.

Abbreviations used: CHO, Chinese-hamster ovary; FCS, fetal calf serum; HEK-293, human embryonic kidney; LPA, lysophosphatidic acid; LPP, lipid phosphate phosphatase; MβCD, methyl-β-cyclodextrin; NP-40, Nonidet P40; PA, phosphatidic acid; PLD1, phospholipase D1; S1P, sphingosine 1-phosphate; SK1, sphingosine kinase 1; TRITC, tetramethylrhodamine β-isothiocyanate.

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were from Stratagene and Santa Cruz Biotechnology respectively. [γ-32P]ATP was purchased from GE Healthcare. [32P]Dioleoyl-PA, and affinity-purified anti-LPP2 and anti-LPP3 antibodies were prepared as described previously [10].

Cell culture

HEK-293 cells were maintained in MEM (minimum essential medium), supplemented with 10% (v/v) FCS (fetal calf serum) and penicillin/streptomycin. CHO cells were maintained in Ham’s F12 medium supplemented with 10% (v/v) FCS and penicillin/streptomycin.

LPP constructs

LPPs were separately amplified from pcDNA3.1 constructs encoding gpLPP1, hLPP2 and hLPP3 [10] using gene-specific primers encoding a C-terminal Myc epitope tag (EQKLISEEDL) or FLAG epitope tag (DYKDHDGDYKDHD) and inserted into pcDNA3.1. Myc-epitope-tagged R127K LPP1 and FLAG-tagged H223L LPP1 were generated by site-directed mutagenesis.

Transfection

HEK-293 cells were transiently transfected with LPP plasmid constructs as required. Cells at 75–95% confluence were placed in medium containing 1% FCS and transfected with 1 μg of plasmid construct following complex formation with Lipofectamine™ 2000, according to the manufacturer’s instructions. The cDNA-containing medium was removed after incubation for 24 h at 37°C, and the cells were incubated for a further 18 h in serum-free medium before experiments. Where indicated, cells were treated with 10 mM MβCD for 60 min before lysis and immunoprecipitation.

Immunoprecipitation

The medium was removed, and cells were lysed in 1 ml of ice-cold immunoprecipitation buffer containing 20 mM Tris/HCl (pH 8.0), 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 1% (v/v) NP-40 (Nonidet P40), 10% (v/v) glycerol, 1 mg/ml BSA, 0.5 mM sodium orthovanadate, 0.2 mM PMSF, and protein inhibitors leupeptin, antipain, pepstatin and aprotinin (all at 10 μg/ml) for 75 min at 4°C. The material was harvested, centrifuged at 22000 g for 10 min at 4°C, and 200 μl of cell lysate supernatant (equalized for protein, 0.5–1 mg/ml) was taken for immunoprecipitation with anti-FLAG or anti-Myc antibodies (2 μg of antibody and 20 μl of 1:1 immunoprecipitation buffer/Protein A–Sepharose CL4B) as required. After agititation for 2 h at 4°C, the immune complex was collected by centrifugation at 22000 g for 15 s at 4°C. Immunoprecipitates were washed twice with buffer A containing 10 mM Hepes (pH 7), 100 mM NaCl, 0.2 mM PMSF, 10 μg/ml leupeptin, 20 μg/ml aprotinin and 0.5% NP-40 and once in buffer A without NP-40. The immunoprecipitates were then combined with boiling sample buffer and subjected to SDS/PAGE and Western blotting.

SDS/PAGE and Western blotting

Cell lysates were prepared using sample buffer containing 62 mM Tris/HCl (pH 6.7), 1.25% (w/v) SDS, 10% (v/v) glycerol, 3.75% (v/v) 2-mercaptoethanol and 0.05% Bromophenol Blue, and proteins were resolved by SDS/PAGE (10% gels). Western blotting with specific antibodies was used to identify proteins of interest [10]. Immunoreactive proteins were visualized using enhanced chemiluminescence detection.

Immunofluorescence

Cells were grown on 12 mm glass coverslips to 60–90% confluence and were transfected as described above. Cells were fixed in 3.7% (v/v) formaldehyde in PBS for 10 min, then permeabilized in 0.1% Triton X-100 in PBS for 1 min. Non-specific binding was reduced by pre-incubating cells in blocking solution containing 5% (v/v) FCS and 1% (w/v) BSA in PBS for 1 h. Cells were incubated in primary antibody (1:100 dilution in blocking solution) for 1 h at room temperature (20°C) (or overnight at 4°C) and then incubated with the appropriate TRITC- or FITC-conjugated secondary antibody (1:100) for 1 h.
Cells were mounted on glass slides using Vectashield mounting medium (Vector Laboratories) and visualized using a Nikon E600 epifluorescence microscope.

LPP activity

Membranes of HEK-293 cells, which had been transiently transfected with LPP plasmid constructs, were prepared by homogenization in ice-cold buffer (containing 50 mM Tris/maleate, pH 7.0, 1 mM EDTA, 150 mM NaCl and 10 mM 2-mercaptoethanol) and centrifugation at 22 000 g at 4 °C for 10 min. Pellets were resuspended in homogenization buffer (at 20–200 mg of protein/ml) and stored at −20 °C. Membrane LPP activity was measured as the liberation of [32P]Pi from [32P]-labelled PA (500 μM; 1000 d.p.m./pmol–625 d.p.m./nmol) in the presence of Triton X-100 (fixed lipid/detergent ratio of 1:10), 37.5 mM Tris/maleate, 7.5 mM 2-mercaptoethanol and 0.2 mg/ml BSA at 30 °C for 5 min. Incubations were stopped by the addition of 5 vol. of chloroform/methanol/10 mM HCl (15:30:2, by vol.). Organic and aqueous phases were resolved by the addition of 1.25 vol. each of chloroform and 0.1 M HCl. Liberated [32P]Pi, was measured by counting radioactivity in the upper phase. All assays were performed under conditions where less than 10% of the substrate was dephosphorylated. Anti-FLAG antibody immunoprecipitates were assayed similarly.

Chromatography

Approx. 10⁷ HEK-293 cells (separately transiently transfected with plasmid constructs encoding FLAG-tagged LPP1, LPP2 or LPP3) were lysed in 1 ml of buffer containing 50 mM Hepes (pH 7.5), 100 mM NaCl, 1 mM EDTA, 1 mM PMSF, 46 mM β-octylglucopyranoside (or 1% Triton X-100) and 10 μg/ml each of leupeptin and aprotinin and were mixed end-over-end for 60 min. A high-speed supernatant was prepared by ultracentrifugation at 30 000 g for 60 min in a Type 50.2 Ti rotor. Then, 100 μl of this was applied to a Superose 12 column and eluted at 30 °C/min using 50 mM Hepes (pH 7), 150 mM NaCl and 1 mM EDTA. Samples of 50 μl of the resulting 0.5 ml fractions were assayed for LPP activity as described above, except that samples were incubated for 60 min. Samples were also subjected to acid precipitation and Western blotting with anti-FLAG antibody.

RESULTS AND DISCUSSION

LPPs form both homo- and hetero-oligomers

We investigated the possibility that LPPs form oligomeric complexes and have assessed whether this molecular arrangement is necessary for catalytic competency. The rationale for this approach was provided by several studies. For instance, Siess and Hofstetter [19] have reported that the Stokes radius of LPP is consistent with a hexameric arrangement of subunits. Additionally, X-ray analysis of a related non-specific acid phosphatase from Escherichia blattae has demonstrated that this enzyme exists as hexamer of three identical dimers [20]. Wunen, a Drosophila LPP homologue, has also been demonstrated to form dimers [21].

HEK-293 cells were transiently transfected with plasmid constructs encoding C-terminally Myc-tagged and/or FLAG-tagged LPPs and were subjected to immunoprecipitation with respective antibodies to test whether LPP1–LPP3 can form homo- and hetero-oligomers in intact cells. Western blot analysis of cell lysates established that FLAG-tagged and Myc-tagged LPP1 were expressed in HEK-293 cells as three major proteins with molecular masses of 31, 34.5 and 35 kDa (Figure 2a), suggesting that the recombinant enzyme undergoes post-translational modification. FLAG-tagged and Myc-tagged LPP2 was expressed as a smeared protein on SDS/PAGE with a molecular mass of 33–36 kDa (Figure 2a), whereas FLAG-tagged and Myc-tagged LPP3 was expressed as three major proteins with molecular masses of 32, 34 and 36 kDa in HEK-293 cells (Figure 2a).

In the present study, we have demonstrated that Myc-tagged LPP1 can be co-immunoprecipitated with FLAG–LPP1 using anti-FLAG antibody (Figure 2b, upper left-hand panels). We also used immunoprecipitation analysis to demonstrate that LPP2

![Figure 2](image-url)
and LPP3 form homo-oligomers (Figure 2b, upper left-hand panels) and that LPP1, LPP2 and LPP3 form hetero-oligomers with each other (Figure 2b, upper middle and right-hand panels). Similar results were obtained using anti-Myc antibody (Figure 2b, lower panel), confirming the formation of LPP1, LPP2 and LPP3 homo-oligomers and LPP2–LPP3 hetero-oligomers. Formation of oligomers of epitope-tagged LPPs requires their co-expression in an intact cell system. This conclusion was based on evidence showing that combining lysates from cells separately transfected with each plasmid construct did not result in formation of FLAG-tagged LPP–Myc-tagged LPP oligomers (Figure 2c). We also considered the possibility that LPPs may be localized to discrete lipid rafts in close proximity, but are not physically associated. If this were the case, then anti-FLAG or anti-Myc antibody might ‘pull down’ the lipid raft containing both tagged forms. However, we rejected this possibility, as cholesterol depletion from cell membranes using MJ/CD to destroy lipid rafts did not prevent co-immunoprecipitation of Myc-tagged LPP1 with FLAG-tagged LPP3 using anti-FLAG antibody (Figure 2d).

Additional evidence for an interaction between LPPs was evident from immunofluorescent cell imaging experiments. These experiments demonstrated that recombinant Myc–LPP1–FLAG–LPP2, FLAG–LPP1–Myc–LPP3 and Myc–LPP2–FLAG–LPP3 are co-localized at the plasma membrane and in a perinuclear region of HEK-293 cells (as shown by yellow immunofluorescence in the merged panels of Figure 3).

**Elution properties of LPP isoforms**

Oligomerization will increase the apparent molecular mass above the monomeric molecular mass of LPPs, determined by cloning (31–36 kDa). One method to establish this is by using gel-exclusion chromatography. Therefore we specifically assessed whether we could separate both oligomeric and monomeric LPP species using Superose 12 chromatography. Membranes from HEK-293 cells transiently transfected with plasmid constructs encoding FLAG-tagged LPP1, LPP2 or LPP3 were subjected to solubilization using β-octylglucopyranoside (Figures 4a–4c).

Immunoblotting fractions eluted from Superose 12 with anti-FLAG antibody revealed the presence of 31, 34.5 and 35 kDa forms of FLAG-tagged LPP1 in fractions 13–16 eluted after Blue Dextran (fraction 12, molecular mass of 2000 kDa) (Figure 4a). FLAG-tagged 34.5 kDa LPP1 was also detected in fractions 28–30 that correspond to a molecular size consistent with monomeric LPP1 (Figure 4a). LPP1 monomers might therefore exist in equilibrium with oligomeric LPP1. In addition, the 31 and 35 kDa forms of LPP1 do not appear to exist as monomers. These 31 and 35 kDa forms of LPP1 might therefore be sequentially modified and produced from 34.5 kDa LPP1 when this latter form is initially assembled as an oligomer. LPP1 activity was associated with the high-molecular-mass complex (Figure 4a), with no activity being detected in fractions containing the 34.5 kDa monomer. This might suggest that the monomeric LPP1 is inactive or that it is inactivated during the gel-filtration step.

We also detected 33–36 kDa FLAG-tagged LPP2 in fractions corresponding to a high-molecular-mass complex and which co-eluted with LPP activity. A 34 kDa form was also present in the low-molecular-mass fraction and was devoid of LPP activity (Figure 4b). Furthermore, 32, 34 and 36 kDa forms of FLAG-tagged LPP3 and activity were also detected in fractions corresponding to a high molecular mass, whereas the 34 kDa form was present in the low-molecular-mass fraction and was devoid of activity (Figure 4c). We also considered the possibility that recombinant LPPs might behave differently compared with endogenous LPP and that oligomerization might be a property unique to recombinant enzyme. However, endogenous LPP activity (from vector-transfected cells) was also detected in high-molecular-mass fractions isolated from Superose 12 chromatography (Figure 4d).

Western blots of elution profiles of FLAG-tagged LPP1, LPP2 or LPP3 (Figure 4e) and activity measurements (results not shown) were similar when a different detergent, Triton X-100, was used. This suggests that micellar size (90 kDa for Triton X-100 and 30 kDa for β-octylglucopyranoside) does not account for elution of LPP activity in high-molecular-mass fractions, i.e. in large micelles containing monomeric LPP.

These results suggest that oligomerization and/or subsequent post-translational modification, such as glycosylation, might result in formation of competent catalytic sites. Phosphorylation of LPPs has proved difficult to demonstrate, and glycosylation has been excluded as a requirement for catalytic activity, as site-directed mutagenesis of the glycosylation site in LPP1 has been shown previously to have no effect on activity [9]. Therefore we focused on the possibility that the oligomerization itself might confer competency on the catalytic site(s).

**Catalytic activity and oligomerization**

Three highly conserved domains (C1–C3) that constitute the catalytic site have been identified in the phosphatase superfamily that includes bacterial acid phosphatase, yeast and bacterial diacylglycerol pyrophosphatase, fungal chloroperoxidase, mammalian glucose-6-phosphate phosphatase and the Drosophila protein Wunen [22]. The C1–C3 domains have been mapped to the extramembrane loops 3 and 5 of LPP1 (Figure 1).

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Conserved amino acid mutants K120R, R127K and P128I (C1 domain), S169T and H171L (C2 domain), and R217K and H223L (C3 domain) of murine LPP1 substantially reduce catalytic activity [9]. C2 and C3 domains are involved in LPP/lipid interaction [9]. C2 and C3 domains are involved in LPP/lipid interaction [9].
phosphotransferase activity, whereas the C1 domain might be a substrate-recognition site [8]. The latter is based on the fact that this region is replaced in sphingomyelin synthase, which functions as a phosphotransferase and displays a different substrate preference compared with that of LPP [8].

We considered the simplest model in which dimers are formed and these are then organized in an oligomeric arrangement. In this model, it is possible that the C1, C2 and C3 domains in each LPP monomer form two competent catalytic sites by domain swapping between the monomers. This can be achieved if the two catalytic sites are formed in a ‘back to front’ orientation (e.g. C1 from monomer A, conferring substrate binding, is shared with C2 and C3 from monomer B, conferring catalytic activity, and vice versa). Therefore we created catalytically deficient Myc-tagged R127K LPP1 (C1 mutagenesis) and FLAG-tagged H223L LPP1 (C3 mutagenesis) mutants (cell membrane LPP1 fold activity increase above mock-transfected: FLAG-tagged wild-type LPP1, 79.5 ± 6.3-fold; Myc-tagged wild-type LPP1, 30.2 ± 2-fold; FLAG-tagged H223L LPP1, 2.3 ± 0.25-fold; Myc-tagged R127K LPP1, 2 ± 0.48-fold). We predicted that if dimerization involves domain swapping between the monomers to form two catalytic sites then co-expression of the two catalytically deficient LPP1 forms, mutated in C1 and C3 domains respectively, should theoretically form one competent catalytic site and reconstitute ∼50% of the wild-type LPP1 activity at equivalent expression level. However, when these mutants were co-expressed (and co-immunoprecipitated with anti-FLAG antibody), we detected negligible LPP1 activity (Figure 5), i.e. ∼2% of the activity measured in immunoprecipitates derived from cells co-expressing FLAG-tagged and Myc-tagged wild-type LPP1 (Figure 5, inset shows approximate equivalent amounts of wild-type and mutant LPP1 recovered in anti-FLAG immunoprecipitates).

Myc-tagged wild-type LPP1 was co-immunoprecipitated with catalytically deficient FLAG-tagged H223L LPP1 (from cells

Figure 4  Gel-exclusion chromatography of LPP
Superose 12 gel-exclusion chromatography of (a–d) β-octylglucopyranoside- or (e) Triton-X100-solubilized membranes from HEK-293 cells that have been transiently transfected with plasmid constructs encoding: (a) FLAG-tagged LPP1, (b) FLAG-tagged LPP2, (c) FLAG-tagged LPP3 or (d) vector. Chromatographic fractions were subjected to LPP activity assays (a–d) using dioleolyl-PA and Western blotted (a–c and e) with anti-FLAG antibody. (a–c) show FLAG–LPP activity, i.e. from which endogenous LPP activity of vector-transfected cells (d) has been subtracted. Markers were Blue Dextran, 2000 kDa; albumin, 67 kDa.
co-expressing both forms) using anti-FLAG-tag antibody (Figure 5, inset). Moreover, significant LPP1 activity was detected in the immunoprecipitate (Figure 5, ~40% of the activity measured in anti-FLAG immunoprecipitates derived from cells co-expressing FLAG-tagged wild-type LPP1 and myc-tagged wild-type LPP1). As a control, we found that anti-FLAG immunoprecipitates prepared from lysates of cells overexpressing wild-type Myc-tagged LPP1 alone had negligible LPP1 activity (results not shown) and no detectable Myc-tagged wild-type LPP1 on Western blots (Figure 2b, left panel). The findings therefore suggest that in an oligomeric arrangement, the catalytic site of the wild-type enzyme can function independently of the catalytic site of the mutant. If dimers are formed between wild-type and mutant enzyme, we can conclude from these data that dimerization is not required for catalytic competency. However, we cannot definitively rule out that there is a close arrangement of separate wild-type and mutant LPP dimers and that these are ‘pulled down’ together by anti-FLAG antibody. In this case, we cannot exclude dimerization as a mechanism for formation of catalytically competent sites, except that it is unlikely that these are formed using domain swapping in a ‘back to front’ orientation.

The results showing that catalytically deficient Myc-tagged R127K LPP1 and FLAG-tagged H223L LPP1 can form oligomers (Figure 5) might suggest that full catalytic activity is not required for oligomerization. However, in the case of Wunen, there is evidence to indicate that catalytic activity is required for dimerization, but not for in vivo function [21].

Endogenous LPP2 and LPP3 form a complex

We have shown here that endogenous LPP is eluted as a high-molecular-mass oligomer from Superose 12 chromatography. However, we sought additional evidence that the endogenous LPPs undergo oligomerization. To this end, we found that the endogenous forms of LPP2 and LPP3 are present in complexes in CHO cells (Figure 6). The enzymes exhibit different patterns of post-translational modification and subcellular localization compared with recombinant LPPs expressed in HEK-293 cells. LPP3 is expressed as two major proteins in CHO cells with molecular masses of 32 and 34 kDa, whereas LPP2 has a molecular mass of 33 kDa. Co-immunoprecipitation analysis using anti-LPP2 or anti-LPP3 antibodies (which are specific for each LPP isoform respectively [10,13]) demonstrated that LPP2 forms a complex with the 32 kDa form of LPP3, but not with the 34 kDa form (Figure 6).

The presence of the endogenous LPP2–LPP3 (32 kDa) hetero-oligomer in CHO cells suggests a functional role for this oligomer. In this respect, we have drawn on results obtained previously in our laboratory concerning the subcellular distribution of LPP2 and LPP3 in CHO cells. We have shown previously that endogenous LPP2 or LPP3 is localized in cytoplasmic vesicles in CHO cells. However, in CHO cells stimulated with PMA (which activates Golgi-apparatus-associated PLD1), a discrete pool of LPP3 is re-localized to the Golgi apparatus [13]. We have also obtained similar results in CHO cells expressing inducible PLD1 (PLD1 is induced by doxycycline) and ectopically expressing recombinant LPP3 [13]. Thus induction of PLD1 is associated with a redistribution of LPP3 (which is normally co-localized with SK1 in cytoplasmic vesicles) to the Golgi apparatus. We do not know whether this involves increased movement of LPP3 and SK1 to the Golgi apparatus or whether trafficking of these proteins away from the Golgi apparatus is reduced. Nevertheless, this re-localization appears to be important, as overexpression of LPP3 reduces intracellular S1P levels and this leads to the onset of apoptosis in HEK-293 cells [13]. We have also shown that SK1 interacts with PLD-derived PA (via a PA-binding domain in SK1) [23]. Therefore the redistribution of LPP3 might represent a physiological desensitization mechanism for controlling the PLD1–SK1 interaction and S1P formation in the Golgi apparatus. This discrete pool of LPP3 may comprise the 34 kDa and/or 32 kDa forms.

Additionally, we have previously demonstrated that endogenous LPP2 does not traffic to or from the Golgi apparatus upon PMA stimulation of CHO cells or upon induction of PLD1, although it is also co-localized with SK1 in cytoplasmic vesicles [13] and, as we show in the present study, can form a complex with 32 kDa LPP3 (Figure 6). We have shown that overexpression of LPP2 reduces intracellular S1P and have suggested that this might prevent its movement to or entrapment with SK1 at the Golgi apparatus upon PLD1 activation [13]. Under these conditions, this may involve predominantly LPP2 homo-oligomers. Endogenous LPP2–LPP3 (32 kDa) hetero-oligomers may regulate PA and/or S1P concentrations in cytoplasmic vesicles, given their co-localization with SK1 [13]. In conclusion, we suggest that LPP oligomers might regulate compartmentalized pools of S1P and...
PA and contribute to the spatial signalling by these lipids within cells.

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Functional studies of lipid phosphate phosphatases