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Phosphatidate degradation: Phosphatidate phosphatases (lipins) and lipid phosphate phosphatases

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Summary

Three lipid phosphate phosphatases (LPPs) regulate cell signaling by modifying the concentrations of a variety of lipid phosphates versus their dephosphorylated products. In particular, the LPPs are normally considered to regulate signaling by the phospholipase D (PLD) pathway by converting phosphatidate (PA) to diacylglycerol (DAG). LPP activities do modulate the accumulations of PA and DAG following PLD activation, but this could also involve an effect upstream of PLD activation. The active sites of the LPPs are on the exterior surface of plasma membranes, or on the luminal surface of internal membranes. Consequently, the actions of the LPPs in metabolizing PA formed by PLD1 or PLD2 should depend on the access of this substrate to the active site of the LPPs. Alternatively, PA generated on the cytosolic surface of membranes should be readily accessible to the family of specific phosphatidate phosphatases, namely the lipins. Presently, there is only indirect evidence for the lipins participating in cell signaling following PLD activation. So far, we know relatively little about how individual LPPs and specific phosphatidate phosphatases (lipins) modulate cell signaling through controlling the turnover of bioactive lipids that are formed after PLD activation.

Keywords

Diacylglycerol; lysophosphatidate; phosphatidate; phospholipase D; triacylglycerol synthesis

Identification of the phosphatidate phosphatases

Phosphatidate phosphatase activity (PAP) was first characterized in the mid 1950s as an enzyme involved in glycerolipid synthesis. PAP converts PA to the DAG that is the necessary precursor for the synthesis of triacylglycerols (TAG), phosphatidylcholine (PC) and phosphatidylethanolamine (PE). This pathway occurs mainly on membranes of the endoplasmic reticulum of mammalian cells, although some contribution may come from mitochondria. As expected at this time, PAP activity was found associated with membrane fractions, particularly microsomes, rather than the cytosol. However, when glycerolipid synthesis from fatty acids and glycerol phosphate was measured with microsomal membranes, there was relatively little production of TAG. The formation of TAG was greatly enhanced by addition of the cytosolic fraction and it was postulated that the cytosol contributed unknown stimulating factors for TAG synthesis [3,4]. After several years of investigation the heat-labile stimulating factor was identified as a soluble PAP [5,6].

This was unexpected since all of the other enzymes involved in glycerolipid synthesis were membrane-associated. There was a very active membrane-bound PAP activity that was readily detected in microsomal fractions and, therefore, no one had looked for a different PAP activity in the cytosolic fraction. The membrane-bound activity in these early experiments was probably catalyzed mainly by what were later called Type 2 phosphatidate phosphatases (PAP2) [7]. This PAP2 activity is not inhibited by N-ethylmaleimide and it does not require Mg^{2+} [7]. More detailed cell fractionation showed that a large proportion of this Mg^{2+} -independent PAP2 was located in plasma membranes rather than in the endoplasmic reticulum [7]. This led to the proposal that PAP2 was involved in signal transduction, particularly by acting in the PLD pathway to produce DAG that would activate classical and novel protein kinase C activities.

Lipid phosphate phosphatases

The enzymes that constitute the PAP2 (Mg^{2+} -independent) activity were purified and the cDNA for three different enzymes and a splice variant were identified [8,9]. These phosphatases hydrolyze a broad range of lipid phosphates including PA, LPA, ceramide 1-phosphate, S1P and DAG pyrophosphate [10]. The family of PAP2 enzymes was, therefore, renamed as lipid phosphate phosphatases (LPPs) because of this lack of clear substrate preference *in vitro* and uncertainty of their functions *in vivo* [10]. The LPPs belong to a phosphatase/phosphotransferase family that was first described by Stukey and Carman [11]. The family includes S1P phosphatases (SPPs), glucose 6-phosphatase and the sphingomyelin synthases [8,9]. LPPs possess six transmembrane domains, three conserved active site domains and a glycosylation site on an hydrophilic loop between the first and second active site domains [8,9,12]. The remaining members of this family are the lipid phosphatase-related proteins or plasticity-related genes (LPR/PRGs) and the type 2 candidate sphingomyelin synthases (CSS) [9,13]. CSS2b has now been identified as a presqualene diphosphate phosphatase [14]. Relatively little is known about the mechanisms of action of LRP/PRGs, which lack critical amino acids within the catalytic site [9]. Therefore, these proteins cannot use the conserved reaction mechanism that catalyses the phosphatase reactions of the LPPs. Despite this the LRP/PRGs appear to play critical roles in brain development and response to injury [13].

The LPPs appear to play a major role in regulating cell signaling by the bioactive lipid phosphates versus their dephosphorylated products e.g., DAG, ceramide and sphingosine [8, 9,15]. The LPPs are expressed on the plasma membrane as well as on internal membranes. The active site of the LPPs is on the outer surface of plasma membranes, or the luminal surface of internal membranes [8,16]. This topology is important since lipid phosphates do not readily cross membranes. Therefore, the access of the lipid phosphates to the LPPs, particularly in the intracellular compartment will be a major factor in determining the rate of degradation [15]. This review will concentrate on the roles of the LPPs in degrading intracellular lipid phosphates, especially PA formed by PLD activity. A previous article has reviewed the evidence for the LPPs acting as ecto-phosphatases and their roles in regulating cell signaling through the degradation of extracellular LPA and S1P [15].

Lipins (Mg^{2+} -dependent phosphatidate phosphatases)

The explanation for the initial failure to identify the soluble PAP lay in the methods used for determining the activity. Most assays used in this early work employed phosphatidate (PA) that was synthesized from phosphatidylcholine using plant phospholipase D in the presence of Ca^{2+} . This latter cation binds very strongly to PA and the Ca^{2+} salt of PA inhibits the soluble PAP activity [17,18]. Conversely, the soluble PAP activity was readily detected with PA produced on microsomal membranes after incubation with glycerol phosphate and an acyl-CoA generating system employing Mg^{2+} [5,6]. The resulting Mg^{2+} -salt form of PA is an ideal

substrate for the soluble PAP, which requires this cation for activity [18]. Realizing this made it possible to perform assays for the soluble PAP using chemically synthesized PA provided that it was depleted of Ca^{2+} [17,19].

The soluble PAP was subsequently named PAP1 after the characterization of the Mg^{2+} – dependent PAP2 [7]. PAP1 proved to be impossible to purify extensively from mammalian tissues because of instability and lack of clear resolution of activity during various separation techniques. However, the Mg^{2+} -dependent PAP was successfully purified from yeast [20]. The major breakthrough came in 2006 with a landmark paper by Carman and colleagues [21] in which they obtained sequence information from the pure PAP to identify the gene. They showed that yeast PAP1 (Pah1p/Smp2p) was the orthologue of the mammalian lipins and also that lipin-1 expressed in *E. coli* had PAP1 activity. This work was followed by studies by Donkor *et al.* demonstrating that the mammalian lipin-1A, -1B, -2 and -3 all possess Mg^{2+} -dependent PAP activity and that they exhibit tissue-specific expression [22]. The PAPs appear to be specific for PA since the yeast PAP does not dephosphorylate diacylglycerol pyrophosphate [21] and the mammalian lipins do not degrade LPA, ceramide 1-phosphate and S1P [22]. Lipin-1B has the highest specific activity for PAP1 followed by lipin-1A, lipin-2 and lipin-3. All of the mammalian lipins show cooperative kinetics with respect to PA concentrations (Fig. 1) when this is measured using a surface dilution kinetic model [23]. Similar cooperative kinetics were reported for the yeast PAP [21]. These combined kinetic results probably indicate that the lipins form higher order and interactive structures, but there is no direct evidence for this proposition at present.

Mg^{2+} -dependent PAP activity showed the appropriate properties to perform a regulatory function in glycerolipid synthesis. PAP1 activity in the liver is increased in starvation, diabetes, stress conditions and after ethanol consumption [24]. These are conditions in which the liver receives an increased supply of fatty acids, which often exceeds its ability for β -oxidation. The liver responds to this potentially toxic condition by increasing its capacity for TAG synthesis through PAP1 so that the excess fatty acids can be sequestered in fat droplets resulting in steatosis. Subsequent work demonstrated that the amount of physiologically active PAP1 on membranes depended on the translocation of the cytosolic PAP1 activity. This was caused by an accumulation of negatively charged lipids (e.g., fatty acids, acyl-CoA esters or phosphatidate) on the membranes [24,25]. This was interpreted as a feed-forward signal that enabled PAP1 to interact with its substrate and match the rate of glycerolipid synthesis to the fatty acid supply. Conversely, interaction of cationic amphiphiles with the membranes displaced PAP1 activity and decreased the flux of PA to DAG, TAG, PC and PE [26]. This work demonstrates that the ability of PAP1 to interact with membranes can control the rate of glycerolipid synthesis.

Indirect evidence was provided that this translocation of PAP activity was regulated by its phosphorylation [27]. Subsequent work, demonstrated that lipin-1 translocates to the endoplasmic reticulum and that this is controlled by its phosphorylation on multiple serine and threonine residues [28].

Physiological roles of lipin-derived PAP activity

Prior to their identification as PAP enzymes, the family of three mammalian lipin genes were identified through genetic studies in the mouse. The founding member, *Lpin1* (encoding lipin-1), was identified as the mutated gene in the fatty liver dystrophy (*fld*) mouse [29]. The *fld* mouse exhibits neonatal fatty liver, peripheral neuropathy, lipodystrophy, insulin resistance, and increased susceptibility to atherosclerosis [30–32]. Lipin-2 and lipin-3 were identified by sequence similarity to lipin-1, and the three represent a dispersed gene family with three

members in mammals, and single orthologues in most invertebrates and in single celled eukaryotes [29].

As described in a previous section, all three mammalian lipin proteins exhibit PAP activity, albeit at different specific activities [22,28]. PAP activity requires a conserved DIDGT motif that was originally described in the yeast PAH1 [21]. In addition to providing the DAG required for the synthesis of TAG, PC and PE in mammals and yeast, lipin-1 functions as a transcriptional coactivator [33,34]. In liver, lipin-1 binds to PGC-1 α and PPAR α to increase the expression of enzymes involved in β -oxidation [35]. Increased lipin-1 PAP activity enables the liver to sequester as TAG the increased fatty acids that are supplied to it in starvation and diabetes. Thus, under these conditions lipin-1 may act to coordinate the oxidation and storage of fatty acids as TAG to prevent the toxic effects resulting from fatty acid accumulation. Using mutant lipin-1 constructs in an *in vitro* assay, it was shown that lipin-1 coactivator activity proceeds even in the absence of PAP activity [35]. However, studies with the yeast PAH1-encoded PAP enzyme indicate that PAP activity is required for all roles of the yeast protein, including effects on cellular lipid composition, derepression of INO1 gene expression, and maintenance of ER/nuclear membrane structure [36]. Further studies are required to determine whether the mammalian lipin proteins differ from the yeast PAP in having a distinct role in gene regulation that is distinct from its PAP activity.

The gene expression patterns of the three mammalian lipin proteins reveal distinct tissue distributions for each protein, suggesting unique physiological roles. Lipin-1 is expressed at highest levels in adipose tissue, skeletal muscle, and testis, and it is also present in liver, peripheral nerve and other tissues [22,29]. Lipin-2 expression is most prominent in liver, and is also present in several other tissues, while lipin-3 is expressed at low levels in most visceral tissues, but has substantial levels in small intestine [22].

The lipodystrophy in lipin-1 deficient *fld* mice reflects a key role for lipin-1 in adipocyte differentiation and lipid biosynthesis [37–39]. Interestingly, lipin-1 deficiency has recently been described in rare human patients, and it causes acute myoglobinuria in childhood [40]. In contrast to the mouse model of lipin-1 deficiency, there was no report of lipodystrophy in these individuals, which raises the possibility that another lipin protein is able to substitute for lipin-1 activity in human adipose tissue. In support of this possibility, lipin-2 has been detected in human adipose tissue [22], and is also expressed in a mouse preadipocyte cell line [41]. Thus, in the 3T3-L1 cell line, lipin-2 protein levels are highest in preadipocytes, but decline dramatically as adipocyte differentiation proceeds to become virtually undetectable in mature adipocytes, when lipin-1 is expressed at high levels [41]. Further work will establish whether there is a role for both lipin-1 and lipin-2 in adipose tissue *in vivo*, and whether there are species differences.

Rare patients with lipin-2 deficiency have also provided clues to its physiological function. These individuals have a complex phenotype known as Majeed syndrome, characterized by recurrent osteomyelitis, fever, and anemia [42,43]. Based on the tissue distribution of lipin-2, it is not clear how its deficiency causes these symptoms, but work in mouse models of lipin-2 deficiency will likely be informative.

The study of lipin deficiencies has revealed that, in addition to the impaired production of DAG, the accumulation of phosphatidate within cells likely accounts for some of the detrimental effects observed. Indeed, the peripheral neuropathy in *fld* mice is associated with PA accumulation in Schwann cells, which may in turn activate signaling through the MEK/ Extracellular-signal Regulated Kinase (ERK) cascade [44]. Furthermore, a muscle sample obtained from one patient with lipin-1 deficiency revealed elevated phosphatidate levels [40]). These findings suggest that the lipin proteins serve an important role in regulating the

balance of lipid intermediates, including PA and DAG, and maintenance of cellular lipid homeostasis.

Measurement of PAP and LPP activities

The different properties exhibited by PAP (lipins) and LPPs provide the basis for performing assays that distinguish between these two major groups of enzymes in tissue extracts. A simple way to determine the PAP activity of the lipins is to measure total PAP activity. The measurement is repeated in parallel assays, but in the presence of excess NEM that is sufficient to react with any dithiothreitol used to preserve PAP activity [19]. This latter measurement gives the LPP activity and the difference between the assays is the NEM-sensitive activity i.e., lipin PAP. Mixing with PA with PC favors the activity of the lipin PAP compared to LPP activity [7]. A more detailed kinetic requirement of PAP and LPP2 activities can be obtained by presenting PA in micelles of Triton-X100 (Fig. 1) and applying a surface dilution kinetic model to establish the substrate affinities and $V_{\max}$ values [23].

LPP activity can be measured with PA or another suitable lipid phosphate substrate. An ideal way to disperse the PA is by using Triton X-100 [45]. If PA is chosen as the substrate then N-ethylmaleimide can be used to inhibit lipin PAP activity.

A further consideration when using PA is whether is to measure the formation of DAG, or inorganic phosphate [17,19]. Measuring DAG is reliable if the activity of DAG lipase is low in the sample, or if the latter activity is inhibited [19]. Care should be taken when determining the release of water soluble phosphate from ^{32}P -labeled PA since this can include glycerol [^{32}P]phosphate and [^{32}P]phosphate formed through phospholipase A activities followed by the action of acid or alkaline phosphatases [17,19].

Intracellular functions of the lipid phosphate phosphatases

Activation of G-protein coupled receptors (e.g., LPA and S1P receptors) and receptor tyrosine kinases stimulate the production of bioactive lipid phosphates within the cell. These lipids and their dephosphorylated products regulate cell signaling. It has, therefore, been accepted for many years that the LPPs can regulate cell signaling by decreasing the concentration of the lipid phosphates and increasing signaling through the products. For example, activation of PLD1 or PLD2 produces PA, which is assumed to be metabolized by the LPPs to yield DAG (Fig. 2). This hypothesis is supported by several reports that increased LPP activity decreases PA concentrations in cells (see [8] for review) and increases the concentration of DAG [46–48]. PA itself activates a variety of intracellular signaling targets including ERK, mTOR and sphingosine kinase-1 thus increasing S1P formation [15] (Fig. 2). S1P activates ERK and it stimulates cell division and protects against apoptosis. S1P also stimulates the mobilization of intracellular Ca^{2+} . The dephosphorylation of S1P could also be mediated by the intracellular actions of the LPPs as well as by two specific S1P phosphatases [15].

The DAG that is produced from PA can theoretically activate classical and novel protein kinase Cs and RasGRP [15]. However, generation of DAG through the PLD pathway did not result in the activation of protein kinase Cs in porcine aortic endothelial cells [49]. These authors proposed that the saturated/monounsaturated fatty acid composition of the DAGs derived from PC do not activate the protein kinase Cs compared to the polyunsaturated DAGs that are produced from phosphatidylinositol 4,5-bisphosphate.

PA can also be metabolized to LPA by phospholipase A activities. The role of the LPPs in decreasing PA concentrations should decrease this LPA production (Fig. 2) and thereby the activation of nuclear LPA₁ receptors that regulate pro-inflammatory gene expression [50]. Polyunsaturated LPA can also stimulate PPAR γ receptors [51–53].

Evidence for intracellular actions of the LPPs on cell signaling came from the Pyne Group [54,55] who demonstrated that the LPPs control ERK activation by extracellular thrombin as well as by S1P, PA and LPA. These effects correlated with decreased intracellular PA and this could not be explained by ecto-LPP activity since thrombin signaling was affected. The effects of LPP2 and LPP3 on intracellular PA and S1P concentrations, respectively, control cell survival [54].

Increased LPP1 activity also attenuates Ca^{2+} -transients and the production of the inflammatory cytokine, IL-8, downstream of LPA receptor activation [56]. HEK 293 cells that over-express LPP3 exhibited greater DAG formation following the stimulation of phospholipase D and consequent PA formation. The metabolic association of PLD2 and LPP3 is supported by the observation that both of these enzymes in caveolin-1-enriched micro-domains [48]. It was proposed that chronic increases in the concentrations of DAG following over-expression of LPP1 decrease the expression of some PKCs and thereby ERK activation. This could explain the decrease in cell division [54] and PDGF-induced cell migration [47].

Although, the LPPs are normally thought to metabolize PA formed after PLD action [8], our recent work with fibroblasts showed that a major effect of LPP1 in decreasing PA accumulation is upstream of PLD activation [45]. Over-expression of LPP1 attenuates the effects of exogenous LPA and PDGF in stimulating PLD activation and PA formation [57]. This indicates that LPP1 alters cell signaling upstream of PLD activation in addition to converting PA to DAG (Fig. 2). The stimulation of cell migration by LPA requires the activation of PLD2. Increased expression of LPP1 attenuates this stimulation and consequent fibroblast migration probably by decreasing PA formation [45]. In support of this conclusion, recent work shows that the S1P-induced migration of human pulmonary artery endothelial cells also involves activation of PLD2. The formation of PA through PLD2 leads to the stimulation of PKC- ζ and Rac-1, which is required for the cells to migrate [58].

Do the lipins metabolize PA formed by PLD1 or PLD2?

So far, we have discussed evidence that the LPPs are the enzymes that metabolize PA formed by the PLDs. However, there are some problems in understanding how this could happen from a theoretical point of view. For example, as described above, the active sites of LPPs are outside the cell, or on the luminal surface of ER, or Golgi membranes [8]. By contrast, the PLDs should produce PA on the cytosolic surface of membranes. Thus, there is a barrier to the hydrolysis of PA formed on the cytosolic surface of membranes unless the PA is efficiently transported to the active sites of the LPPs.

A further possibility is that the lipins could metabolize PA that is formed to regulate cell signaling by the PLDs or some DAG kinases. The cytosolic distribution of the lipins would seem to be ideal for metabolizing PA that is formed on the cytosolic surfaces of cell organelles. At present, there is no conclusive evidence to support this hypothesis, but there are indications that the PAP activity of the lipins could be involved in regulating cell signaling.

PAP activity appears to regulate EGF signaling since it co-immunoprecipitates with EGF receptors [59]. This association decreases upon activation of the receptor while PAP activity associated with protein kinase-C- ϵ (PKC- ϵ), a DAG-dependent PKC [59]. This work was performed before the identity of the lipins was established, but the major PAP activity was sensitive to N-ethylmaleimide and it was Mg^{2+} -dependent, consistent with PAP rather than LPP activity.

PAP activity is also involved in cyclo-oxygenase expression and eicosanoid formation when WISH cells are activated through PKC [60,61]. It appears that activation of cytosolic phospholipase A_2 depends on the activation of PLD and the generation of DAG through PAP

activity. The stimulation of cyclooxygenase-2 by lipopolysaccharide in macrophages also depends on the generation of DAG through PAP activity [62]. Most of the evidence for this involvement in PAP (lipin) activity relies on the use of the relatively non-specific PAP inhibitors, bromoenol lactone and propanolol. However, it is significant, that lipopolysaccharide increased DAG formation within 2 min and this was accompanied by the translocation of Mg^{2+} -dependent PAP activity from the cytosol to the membrane fraction. Our own work also shows that activation of rat fibroblasts by LPA causes a translocation of Mg^{2+} -dependent PAP activity to the membrane fraction within 2 min when the production of PA and DAG is increased by LPA and PDGF (C. Pilquil and DN Brindley, unpublished results). These observed translocations of PAP1 activity probably resulted from the increased presence of PA in membranes as described above.

As described above, recent work [44] establishes a novel role for PA and lipin-1 in regulating Schwann cell function. Deletion of the *Lpin1* gene in Schwann cells produced pronounced peripheral neuropathy characterized by myelin degradation, Schwann cell dedifferentiation and proliferation, and a decrease in nerve conduction velocity [44]. Demyelination was caused by the accumulation of PA, likely resulting from decreased PAP activity. PA was shown to activate the MEK-ERK pathway in Schwann cells and this is required for PA-induced demyelination [44].

The combined work described in this section provides strong evidence that the mammalian PAPs (lipins) regulate signal transduction by PA and DAG (Fig. 2). This conclusion is also supported by work with yeast where PAP regulates PA concentrations in nuclear/endoplasmic reticulum membranes [34]. PAP activity controls the structure of these membranes by regulating PA accumulation. The *pah1Δ* mutation causes aberrant expansion of the nuclear/endoplasmic reticulum membranes and derepression of genes for phospholipids synthesis that contain a UAS_{INO} in their promoters [34].

Conclusions

In this review we have used terms such as PAP1 and PAP2 to distinguish between two different families of enzymes that were given these names before their structures were known. We now know that these two different types of PAP activity are catalyzed by two structurally distinct families of proteins. We, therefore, recommend that the protein products encoded by the *Lpin1*, *Lpin2* and *Lpin3* genes should be called lipin-1, -2 and -3, respectively, and that PAP should be used to designate the enzymatic activity of these lipins. With respect to the yeast PAP enzyme, it should be referred to as the *PAH1*-encoded PAP. The PAP2 enzymes that are encoded by the *Pap2a*, *Pap2B* and *Pap2c* genes should be called LPP1, LPP3 and LPP2, respectively.

The LPPs and the lipins are involved in regulating signaling following activation of PLD1 and PLD2. It was originally thought that the LPPs convert the PA formed by the PLDs to DAG and that this modifies the balance of these bioactive lipids in regulating cell signaling. This is probably true, but the location of the active sites of the LPPs on the luminal surface of internal membranes means that they may not readily access PA formed on the cytosolic surface. More recent work implicates LPP1 in controlling PA formation upstream of PLD activation. The action of the LPPs in controlling PA production by the PLDs, or converting it to DAG could indirectly decrease the conversion of PA to LPA, which is also bioactive. Additionally, LPA is a substrate for the LPPs (Fig. 2).

The role of the lipins in regulating signaling by the PLDs is inferred mainly through inhibitor studies. The lipins use PA specifically as a substrate and their cytosolic location would put them in an ideal position to participate in the PLD pathways. The agonist-induced translocation

of cytosolic PAP to membranes, which presumably depends on PA formation, is also compatible with a function for the lipins in the PLD pathway.

Future studies using cells and mouse models with genetic alterations in the expression of each of the LPPs and lipins will allow further elucidation of the mechanisms by which they interact to control cell signaling through PLD1 and PLD2.

Abbreviations

DAG	diacylglycerol
LPA	lysophosphatidate
LPP	lipid phosphate phosphatase
LPR/PRGs	lipid phosphatase-related proteins or plasticity-related genes
MAG	monoacylglycerol
PAP	phosphatidate phosphatase
PC	phosphatidylcholine
PE	phosphatidylethanolamine
S1P	sphingosine 1-phosphate
TAG	triacylglycerol

References

1. Kates M. Hydrolysis of lecithin by plant plastid enzymes. *Can J Biochem Physiol* 1955;33:575–589. [PubMed: 13240529]
2. Smith SW, Weiss SB, Kennedy EP. The enzymatic dephosphorylation of phosphatidic acids. *J Biol Chem* 1957;228:915–922. [PubMed: 13475370]
3. Stein Y, Shapiro B. The synthesis of neutral glycerides by fractions of rat liver homogenates. *Biochim Biophys Acta* 1957;24:197–198. [PubMed: 13426221]
4. Hübscher G, Brindley DN, Smith ME, Sudgwick B. Stimulation of biosynthesis of glyceride. *Nature* 1967;216:449–453.
5. Johnston JM, Rao GA, Lowe PA, Schawrz BE. *Lipids* 1967;2:14. [PubMed: 17805715]
6. Smith ME, Sedgwick B, Brindley DN, Hübscher G. The role of phosphatidate phosphohydrolase in glyceride biosynthesis. *Eur J Biochem* 1967;3:70–77. [PubMed: 4295052]
7. Jamal Z, Martin A, Gómez-Muñoz A, Brindley DN. Plasma membrane fractions from rat liver contain a phosphatidate phosphohydrolase distinct from that in the endoplasmic reticulum and cytosol. *J Biol Chem* 1991;266:2988–2996. [PubMed: 1993672]
8. Brindley DN. Lipid phosphate phosphatases and related proteins: signaling functions in development, cell division, and cancer. *J Cell Biochem* 2004;92:900–912. [PubMed: 15258914]
9. Sigal YJ, McDermott MI, Morris AJ. Integral membrane lipid phosphatases/phosphotransferases: common structure and diverse functions. *Biochem J* 2005;387:281–293. [PubMed: 15801912]
10. Brindley DN, Waggoner DW. Mammalian lipid phosphate phosphohydrolases. *J Biol Chem* 1998;273:24281–24284. [PubMed: 9733709]
11. Stuke J, Carman GM. Identification of a novel phosphatase sequence motif. *Protein Sci* 1997;6:469–472. [PubMed: 9041652]
12. Pyne S, Kong KC, Darroch PI. Lysophosphatidic acid and sphingosine 1-phosphate biology: the role of lipid phosphate phosphatases. *Semin Cell Dev Biol* 2004;15:491–501. [PubMed: 15271294]
13. Brindley DN, Bräuer AU. Lipid mediators and modulators of neural function: Lysophosphatidate and lysolipids. In: Lajtha, A.; Gorraci, G.; Tettamanti, G., editors. *Handbook of Neurochemistry and Molecular Biology: Lysophosphatidate and lysolipids*. Springer; New York: 2009. in press

14. Fukunaga K, Arita M, Takahashi M, Morris AJ, Pfeffer M, Levy BD. Identification and functional characterization of a presqualene diphosphate phosphatase. *J Biol Chem* 2006;281:9490–9497. [PubMed: 16464866]
15. Brindley DN, Pilquil C. Lipid phosphate phosphatases and signaling. *Journal of Lipid Research*. 2008 Papers in press.
16. Zhang QX, Pilquil CS, Dewald J, Berthiaume LG, Brindley DN. Identification of structurally important domains of lipid phosphate phosphatase-1: implications for its sites of action. *Biochem J* 2000;345(Pt 2):181–184. [PubMed: 10620492]
17. Sturton RG, Brindley DN. Problems encountered in measuring the activity of phosphatidate phosphohydrolase. *Biochem J* 1978;171:263–266. [PubMed: 206266]
18. Martin A, Hales P, Brindley DN. A rapid assay for measuring the activity and the Mg^{2+} and Ca^{2+} requirements of phosphatidate phosphohydrolase in cytosolic and microsomal fractions of rat liver. *Biochem J* 1987;245:347–355. [PubMed: 2822021]
19. Martin, A.; Gómez-Muñoz, A.; Jamal, Z.; Brindley, DN. Characterization and assay of phosphatidate phosphatase. In: Dennis, EA., editor. *Methods in Enzymology*. Vol. 197. Academic Press Inc; California: 1991. p. 553–563.
20. Lin YP, Carman GM. Purification and characterization of phosphatidate phosphatase from *Saccharomyces cerevisiae*. *J Biol Chem* 1989;264:8641–8645. [PubMed: 2542283]
21. Han GS, Wu WI, Carman GM. The *Saccharomyces cerevisiae* Lipin homolog is a Mg^{2+} -dependent phosphatidate phosphatase enzyme. *J Biol Chem* 2006;281:9210–9218. [PubMed: 16467296]
22. Donkor J, Sariahmetoglu M, Dewald J, Brindley DN, Reue K. Three mammalian lipins act as phosphatidate phosphatases with distinct tissue expression patterns. *J Biol Chem* 2007;282:3450–3457. [PubMed: 17158099]
23. Carman GM, Deems RA, Dennis EA. Lipid signaling enzymes and surface dilution kinetics. *J Biol Chem* 1995;270:18711–18714. [PubMed: 7642515]
24. Brindley, DN. Phosphatidate phosphohydrolase: its role in glycerolipid synthesis. Brindley, DN., editor. CRC Press Inc; Boca Raton: 1988.
25. Martin-Sanz P, Hopewell R, Brindley DN. Long-chain fatty acids and their acyl-CoA esters cause the translocation of phosphatidate phosphohydrolase from the cytosolic to the microsomal fraction of rat liver. *FEBS Lett* 1984;175:284–288. [PubMed: 6090213]
26. Martin A, Hopewell R, Martin-Sanz P, Morgan JE, Brindley DN. Relationship between the displacement of phosphatidate phosphohydrolase from the membrane-associated compartment by chlorpromazine and the inhibition of the synthesis of triacylglycerol and phosphatidylcholine in rat hepatocytes. *Biochim Biophys Acta* 1986;876:581–591. [PubMed: 3011104]
27. Gómez-Muñoz A, Hatch GM, Martin A, Jamal Z, Vance DE, Brindley DN. Effects of okadaic acid on the activities of two distinct phosphatidate phosphohydrolases in rat hepatocytes. *FEBS Lett* 1992;301:103–106. [PubMed: 1451777]
28. Harris TE, Huffman TA, Chi A, Shabanowitz J, Hunt DF, Kumar A, Lawrence JC Jr. Insulin controls subcellular localization and multisite phosphorylation of the phosphatidic acid phosphatase, lipin 1. *J Biol Chem* 2007;282:277–286. [PubMed: 17105729]
29. Peterfy M, Phan J, Xu P, Reue K. Lipodystrophy in the fld mouse results from mutation of a new gene encoding a nuclear protein, lipin. *Nat Genet* 2001;27:121–124. [PubMed: 11138012]
30. Langner CA, Birkenmeier EH, Ben-Zeev O, Schotz MC, Sweet HO, Davisson MT, Gordon JI. The fatty liver dystrophy (fld) mutation. A new mutant mouse with a developmental abnormality in triglyceride metabolism and associated tissue-specific defects in lipoprotein lipase and hepatic lipase activities. *J Biol Chem* 1989;264:7994–8003. [PubMed: 2722772]
31. Langner CA, Birkenmeier EH, Roth KA, Bronson RT, Gordon JI. Characterization of the peripheral neuropathy in neonatal and adult mice that are homozygous for the fatty liver dystrophy (fld) mutation. *J Biol Chem* 1991;266:11955–11964. [PubMed: 2050689]
32. Reue K, Xu P, Wang XP, Slavin BG. Adipose tissue deficiency, glucose intolerance, and increased atherosclerosis result from mutation in the mouse fatty liver dystrophy (fld) gene. *J Lipid Res* 2000;41:1067–1076. [PubMed: 10884287]
33. Reue K, Brindley DN. Thematic Review Series: Glycerolipids. Multiple roles for lipins/phosphatidate phosphatase enzymes in lipid metabolism. *J Lipid Res* 2008;49:2493–2503. [PubMed: 18791037]

34. Carman GM, Han GS. Phosphatidic acid phosphatase, a key enzyme in the regulation of lipid synthesis. *J Biol Chem*. 2008
35. Finck BN, Gropler MC, Chen Z, Leone TC, Croce MA, Harris TE, Lawrence JC Jr, Kelly DP. Lipin 1 is an inducible amplifier of the hepatic PGC-1 α /PPAR α regulatory pathway. *Cell Metab* 2006;4:199–210. [PubMed: 16950137]
36. Han GS, Siniossoglou S, Carman GM. The cellular functions of the yeast lipin homolog pah1p are dependent on its phosphatidate phosphatase activity. *J Biol Chem*. 2007
37. Phan J, Peterfy M, Reue K. Biphasic expression of lipin suggests dual roles in adipocyte development. *Drug News Perspect* 2005;18:5–11. [PubMed: 15753971]
38. Peterfy M, Phan J, Reue K. Alternatively spliced lipin isoforms exhibit distinct expression pattern, subcellular localization, and role in adipogenesis. *J Biol Chem* 2005;280:32883–32889. [PubMed: 16049017]
39. Zhang P, O'oughlin L, Brindley DN, Reue K. Regulation of lipin-1 gene expression by glucocorticoids during adipogenesis. *J Lipid Res* 2008;49:1519–1528. [PubMed: 18362392]
40. Zeharia A, Shaag A, Houtkooper RH, Hindi T, de Lonlay P, Erez G, Hubert L, Saada A, de Keyzer Y, Eshel G, Vaz FM, Pines O, Elpeleg O. Mutations in *LPIN1* cause recurrent acute myoglobinuria in childhood. *Am J Hum Genet* 2008;83:489–494. [PubMed: 18817903]
41. Grimsey N, Han GS, O'Hara L, Rochford JJ, Carman GM, Siniossoglou S. Temporal and spatial regulation of the phosphatidate phosphatases lipin 1 and 2. *J Biol Chem*. 2008
42. Majeed HA, Al-Tarawna M, El-Shanti H, Kamel B, Al-Khalaileh F. The syndrome of chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anaemia. Report of a new family and a review. *Eur J Pediatr* 2001;160:705–710. [PubMed: 11795677]
43. Ferguson PJ, Chen S, Tayeh MK, Ochoa L, Leal SM, Pelet A, Munnich A, Lyonnet S, Majeed HA, El-Shanti H. Homozygous mutations in *LPIN2* are responsible for the syndrome of chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anaemia (Majeed syndrome). *J Med Genet* 2005;42:551–557. [PubMed: 15994876]
44. Nadra K, de Preux Charles AS, Medard JJ, Hendriks WT, Han GS, Gres S, Carman GM, Saulnier-Blache JS, Verheijen MH, Chrast R. Phosphatidic acid mediates demyelination in *Lpin1* mutant mice. *Genes Dev* 2008;22:1647–1661. [PubMed: 18559480]
45. Pilquill C, Dewald J, Cherney A, Gorshkova I, Tigyi G, English D, Natarajan V, Brindley DN. Lipid phosphate phosphatase-1 regulates lysophosphatidate-induced fibroblast migration by controlling phospholipase D2-dependent phosphatidate generation. *J Biol Chem* 2006;281:38418–38429. [PubMed: 17057224]
46. Yue J, Yokoyama K, Balazs L, Baker DL, Smalley D, Pilquill C, Brindley DN, Tigyi G. Mice with transgenic overexpression of lipid phosphate phosphatase-1 display multiple organotypic deficits without alteration in circulating lysophosphatidate level. *Cell Signal* 2004;16:385–399. [PubMed: 14687668]
47. Long JS, Yokoyama K, Tigyi G, Pyne NJ, Pyne S. Lipid phosphate phosphatase-1 regulates lysophosphatidic acid- and platelet-derived-growth-factor-induced cell migration. *Biochem J* 2006;394:495–500. [PubMed: 16356167]
48. Sciorra VA, Morris AJ. Sequential actions of phospholipase D and phosphatidic acid phosphohydrolase 2b generate diglyceride in mammalian cells. *Mol Biol Cell* 1999;10:3863–3876. [PubMed: 10564277]
49. Pettitt TR, Martin A, Horton T, Liossis C, Lord JM, Wakelam MJ. Diacylglycerol and phosphatidate generated by phospholipases C and D, respectively, have distinct fatty acid compositions and functions. Phospholipase D-derived diacylglycerol does not activate protein kinase C in porcine aortic endothelial cells. *J Biol Chem* 1997;272:17354–17359. [PubMed: 9211874]
50. Gobeil F Jr, Bernier SG, Vazquez-Tello A, Brault S, Beauchamp MH, Quiniou C, Marrache AM, Checchin D, Sennlaub F, Hou X, Nader M, Bkaily G, Ribeiro-da-Silva A, Goetzl EJ, Chemtob S. Modulation of pro-inflammatory gene expression by nuclear lysophosphatidic acid receptor type-1. *J Biol Chem* 2003;278:38875–38883. [PubMed: 12847111]
51. Siess W, Tigyi G. Thrombogenic and atherogenic activities of lysophosphatidic acid. *J Cell Biochem* 2004;92:1086–1094. [PubMed: 15258894]

52. McIntyre TM, Pontsler AV, Silva AR, St Hilaire A, Xu Y, Hinshaw JC, Zimmerman GA, Hama K, Aoki J, Arai H, Prestwich GD. Identification of an intracellular receptor for lysophosphatidic acid (LPA): LPA is a transcellular PPARgamma agonist. *Proc Natl Acad Sci U S A* 2003;100:131–136.
53. Zhang C, Baker DL, Yasuda S, Makarova N, Balazs L, Johnson LR, Marathe GK, McIntyre TM, Xu Y, Prestwich GD, Byun HS, Bittman R, Tigyi G. Lysophosphatidic acid induces neointima formation through PPARgamma activation. *J Exp Med* 2004;199:763–774. [PubMed: 15007093]
54. Long J, Darroch P, Wan KF, Kong KC, Ktistakis N, Pyne NJ, Pyne S. Regulation of cell survival by lipid phosphate phosphatases involves the modulation of intracellular phosphatidic acid and sphingosine 1-phosphate pools. *Biochem J* 2005;391:25–32. [PubMed: 15960610]
55. Alderton F, Darroch P, Sambhi B, McKie A, Ahmed IS, Pyne N, Pyne S. G-protein-coupled receptor stimulation of the p42/p44 mitogen-activated protein kinase pathway is attenuated by lipid phosphate phosphatases 1, 1a, and 2 in human embryonic kidney 293 cells. *J Biol Chem* 2001;276:13452–13460. [PubMed: 11278307]
56. Zhao Y, Usatyuk PV, Cummings R, Saatiyan B, He D, Watkins T, Morris A, Spannhake EW, Brindley DN, Natarajan V. Lipid phosphate phosphatase-1 regulates lysophosphatidic acid-induced calcium release, NF-kappaB activation and interleukin-8 secretion in human bronchial epithelial cells. *Biochem J* 2005;385:493–502. [PubMed: 15461590]
57. Pilquill C, Ling ZC, Singh I, Buri K, Zhang QX, Brindley DN. Co-ordinate regulation of growth factor receptors and lipid phosphate phosphatase-1 controls cell activation by exogenous lysophosphatidate. *Biochem Soc Trans* 2001;29:825–830.
58. Gorshkova I, He D, Berdyshev E, Usatyuk P, Burns M, Kalari S, Zhao Y, Pendyala S, Garcia JG, Pyne NJ, Brindley DN, Natarajan V. Protein Kinase C- $\{\epsilon\}$ Regulates Sphingosine 1-Phosphate-mediated Migration of Human Lung Endothelial Cells through Activation of Phospholipase D2, Protein Kinase C- $\{\zeta\}$, and Rac1. *J Biol Chem* 2008;283:11794–11806. [PubMed: 18296444]
59. Jiang Y, Lu Z, Zang Q, Foster DA. Regulation of phosphatidic acid phosphohydrolase by epidermal growth factor. Reduced association with the EGF receptor followed by increased association with protein kinase Cepsilon. *J Biol Chem* 1996;271:29529–29532. [PubMed: 8939878]
60. Balboa MA, Balsinde J, Dennis EA. Involvement of phosphatidate phosphohydrolase in arachidonic acid mobilization in human amnionic WISH cells. *J Biol Chem* 1998;273:7684–7690. [PubMed: 9516474]
61. Johnson CA, Balboa MA, Balsinde J, Dennis EA. Regulation of cyclooxygenase-2 expression by phosphatidate phosphohydrolase in human amnionic WISH cells. *J Biol Chem* 1999;274:27689–27693. [PubMed: 10488110]
62. Grkovich A, Johnson CA, Buczynski MW, Dennis EA. Lipopolysaccharide-induced cyclooxygenase-2 expression in human U937 macrophages is phosphatidic acid phosphohydrolase-1-dependent. *J Biol Chem* 2006;281:32978–32987.

Figure 1. All mammalian lipin proteins exhibit PAP1 activity

Different lipins were overexpressed in HEK 293 cells and PAP1 activity was determined in cell lysates two days after transfection with the constructs [22]. Mg^{2+} -dependent PAP activity was determined essentially as before except that the concentration of PA was kept constant at 200 μ M and that of Triton X-100 was adjusted to provide the mol% of PA in Triton X100 that is quoted. The specific activity of PAP (relative to protein) in cells transfected with the empty vector was <1% of that for cells expressing lipin-1A and it was subtracted from all other values. The relative specific activity of the lipins was then calculated by normalizing to the corresponding Western blot of the V5 tagged [22]. The results demonstrate that each lipin has PAP activity and that the relative activities for lipin-1A and -1B are higher than for lipin-2 and

lipin-3. Each lipin shows cooperative kinetics as expected from the yeast Pah1 [21]. We have not given kinetic constants since the kinetic analysis was performed with overexpressed, but impure enzymes, which are intrinsically labile.

Figure 2. Proposed roles of LPPs and lipins in the PLD signaling cascades

Green arrows indicate the stimulation of signaling targets and red lines indicate inhibition of signaling. cPLA₂, cytosolic PLA₂; PKC, protein kinase C; PI 4K, phosphatidylinositol 4-kinase; PLC, phospholipase C; SK-1, sphingosine kinase-1, SPP, specific sphingosine 1-phosphate phosphatases. The authors gratefully acknowledge support from the Canadian Institutes of Health Research to D.N.B (BC039) and the National Heart, Lung, and Blood Institute (HL-28481 and HL-90553) to K.R. D.N.B. is a recipient of a Medical Scientist Award from the Alberta Heritage Foundation for Medical Research.