Journal of Cellular Biochemistry 92:900–912 (2004)

Lipid Phosphate Phosphatases and Related Proteins: Signaling Functions in Development, Cell Division, and Cancer

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Abstract Lipid phosphates initiate key signaling cascades in cell activation. Lysophosphatidate (LPA) and sphingosine 1-phosphate (S1P) are produced by activated platelets. LPA is also formed from circulating lysophosphatidylcholine by autotaxin, a protein involved tumor progression and metastasis. Extracellular LPA and S1P stimulate families of G-protein coupled receptors that elicit diverse responses. LPA is involved in wound repair and tumor growth. Exogenous S1P is a potent stimulator of angiogenesis, a process vital in development, tissue repair and the growth of aggressive tumors. Inside the cell, phosphatidate (PA), ceramide 1-phosphate (C1P), LPA, and S1P act as signaling molecules with distinct functions including the stimulation of cell division, cytoskeletal rearrangement, Ca^{2+} transients, and membrane movement. These observations imply that phosphatases that degrade lipid phosphates on the cell surface, or inside the cell, regulate cell signaling under physiological and pathological conditions. This occurs through attenuation of signaling by the lipid phosphates and by the production of bioactive products (diacylglycerol, ceramide, and sphingosine). Three lipid phosphate phosphatases (LPPs) and a splice variant dephosphorylate LPA, PA, CIP, and S1P. Two S1P phosphatases (SPPs) act specifically on S1P. In addition, there is family of four LPP-related proteins (LPRs, or plasticity-related genes, PRGs). PRG-1 expression in neurons has been reported to increase extracellular LPA breakdown and attenuate LPA-induced axonal retraction. It is unclear whether the LRPs dephosphorylate LPA directly, stimulate LPP activity, or bind LPA and S1P. Also, the importance of extra- versus intra-cellular actions of the LPPs and SPPs, and the individual roles of different isoforms is not firmly established. Understanding the functions and regulation of the LPPs, SPPs and related proteins will hopefully contribute to interventions to correct dysfunctions in conditions such as wound repair, inflammation, angiogenesis, tumor growth, and metastasis. J. Cell. Biochem. 92: 900–912, 2004.

Key words: ceramide 1-phosphate; lysophosphatidate; phosphatidate; phosphatase; plasticity-related genes; sphingosine 1-phosphate

FORMATION AND FUNCTION OF EXTRA-CELLULAR LYSOPHOSPHATIDATE (LPA) AND SPHINGOSINE 1-PHOSPHATE (S1P)

Before discussing the roles of phosphatases that degrade lipid phosphates in controlling cell activation it is important to provide a short description of the signaling actions of the lipid mediators themselves. Much fuller accounts of this topic are provided in the reviews that are cited and from other articles in this volume.

LPA and S1P are present in biological fluids and activate cells through families of G-protein coupled receptors as reviewed by Pyne and Pyne [2000], English et al. [2002], and Tigyi and Parrill [2003] and by other authors in this volume. These receptors are coupled through $G_{\alpha i}$ that decreases cAMP concentrations; $G_{12/13}$ that stimulates phospholipase D (PLD) and Rho leading to stress fiber formation; G_q that activates phospholipase C (PLC), Ca^{2+} transients, and protein kinase C isoforms. LPA and S1P receptors can also signal in a cell-specific manner through transactivation of EGF, or PDGF receptors as reviewed in Brindley et al. [2002]. This depends partly on the production of $\beta\gamma$-subunits, activation of Src, metalloprotein-

Grant sponsor: Canadian Institutes of Health Research; Grant number: MOP 10504.

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Received 27 November 2003; Accepted 26 February 2004
DOI 10.1002/jcb.20126

ases, or PLD2 [Wang et al., 2003] depending upon the cell type.

Extracellular LPA stimulates tissue growth and repair and it is produced by activated platelet, injured corneal tissue, neurones, and adipocytes as discovered by the Groups of Moolenaar, Chun, Tigyi, Goetzl, and others [reviewed by Tigyi and Parrill, 2003]. LPA is partly derived through an extracellular lysophospholipase D (lysoPLD) from a major circulating lipid, lysophosphatidylcholine (Fig. 1A). This lysoPLD has been identified as autotaxin, an ecto-phosphodiesterase that is widely implicated in tumor progression and metastasis [Tokumura, 2002; Umezu-Goto et al., 2002]. LPA can also be produced in inflammatory conditions through secretory phospholipase A_2 acting on PA [Fourcade et al., 1995]. LPA concentrations are high in ascites fluid of patients with ovarian tumors as described by the Groups of Mills, Xu, and others [see review by Mills and Moolenaar, 2003]. LPA promotes ovarian tumor development and protects against apoptosis caused by chemotherapeutic agents. LPA levels are also elevated in the blood of patients suffering from multiple myeloma.

Activated platelets [Yatomi et al., 1995; English et al., 2000], ovarian cancer cells [Hong

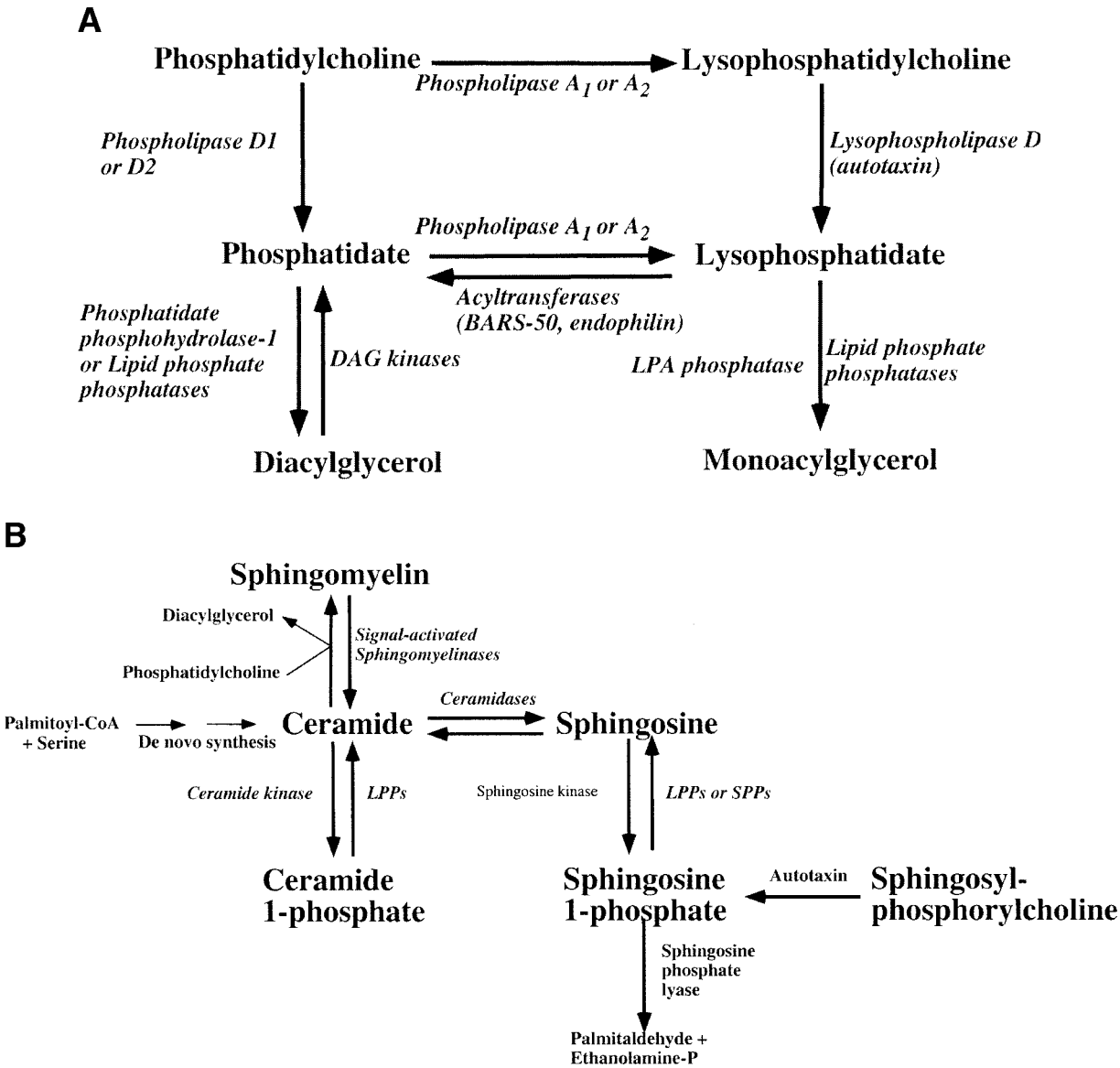

Fig. 1. **A**: Metabolism of phosphatidate and lysophosphatidate. **B**: Metabolism of some bioactive sphingolipids.

et al., 1999], and other cells secrete S1P. It can also be produced from sphingosylphosphorylcholine (Fig. 1B) by autotaxin [Clair et al., 2003]. External S1P is a potent stimulator of angiogenesis as described by the Groups of Hla, Spiegel, English, Garcia, and others [for review see English et al., 2002]. It accounts for the major chemotactic activity of clotted blood for endothelial cells (ECs) and for stimulating angiogenesis in vivo. Circulating S1P has also recently been implicated in immunosuppression [Mandala et al., 2002]. S1P is generated by sphingosine kinases (SK) (Fig. 1B). S1P can then be secreted and act in an autocrine paracrine mechanism on S1P receptors [Hobson et al., 2001]. In addition, cells can secrete SK, which can produce external S1P [Ancellin et al., 2002].

The work cited above and that described in other papers in this volume provides strong evidence that extracellular LPA and S1P are potent growth and chemotactic factors that increase wound healing, tumor growth, metastasis, and angiogenesis through various receptors. Excessive LPA and S1P production (e.g., through autotaxin), or defects in their removal (e.g., by phosphatases), could, therefore, lead to tumor progression and enhance metastasis.

INTRACELLULAR ACTIONS OF PHOPHATIDATE (PA), LPA, CERAMIDE 1-PHOSPHATE (C1P), AND S1P

The intracellular signaling mechanisms for different lipid phosphates are not so clear in terms of understanding of a pattern of coordinated signaling, but there are diverse targets. Chemotactic peptides activate PLD in neutrophils and the resulting intracellular PA generation stimulates NADPH oxidase. Intracellular PA also stimulates protein kinase C-ζ, phosphatidylinositol 4-kinase, Raf, PLC-γ, increases Ras-GTP [see Brindley et al., 2002 for review] and inhibits protein phosphatase-1 [Jones and Hannun, 2002]. The effect of PA on Ras and Raf increases ERK activity and cell division. PA can increase cell division through mTOR and it stimulates stress fiber formation [see Brindley et al., 2002 for review]. The relative concentrations of LPA and PA in membranes control their curvature and vesicle budding [Huijbregts et al., 2000]. PLD1 and its activator, ARF, are involved in vesicle movement through PA production [Huijbregts et al., 2000]. PA generation through PLD is required for micro-vesicle budding from Golgi membrane. Ceramides inhibit PLD1 by preventing ARF association with membranes and this could contribute to the ceramide-inhibition of vesicle transport [Abousalham et al., 2002]. PA can also be formed by acylation of LPA through BARS-50, or endophilin. These actions stimulate COP-1 vesicle formation, and synaptic vesicle endocytosis, respectively [see Brindley et al., 2002 for review].

CIP is the sphingolipid analogue of PA. It probably plays a role in synaptic vesicle movement. C1P is also formed during neutrophil phagocytosis and it is involved in liposome fusion [see Brindley et al., 2002 for review]. Exogenous C1P stimulates the division of Rat2 fibroblasts [see Brindley et al., 2002 for review], but it does not increase PLD or ERK activities. More recent work has produced first evidence for intracellular targets of C1P. Interleukin-1β, or the Ca^{2+} ionophore, A23187, stimulated ceramide kinase activity in A549 lung carcinoma cells, and thereby increased the production of C1P [Pettus et al., 2003]. This stimulated the release of arachidonate and the synthesis of prostaglandin E_2. Conversely, decreasing ceramide kinase activity by RNA interference attenuated these effects, but not the response to C1P itself. These authors demonstrated a direct interaction and regulation of phospholipase A_2 by C1P and the involvement of C1P in inflammatory responses [Pettus et al., 2004]. Other work showed that C1P blocks the activation of the caspases-9/caspases-3 pathway and apoptosis in macrophages [Gómez-Muñoz et al., 2004]. Exogenous C1P also blocked acidic sphingomyelinase activity in intact macrophages as well as in cell homogenates. The authors proposed that there could be a direct interaction of C1P with acidic sphingomyelinase.

Recent work provided exciting evidence that intracellular LPA can signal through the PPAP-γ receptor [McIntyre et al., 2003]. Furthermore, alkylether analogues of LPA, or unsaturated acyl-forms of LPA promoted progressive neointima formation in a rat carotid artery model in vivo, effects that were mediated through PPAP-γ receptors [Zhang et al., 2004]. The authors concluded that these LPA effects could be involved in vascular remodeling caused by oxidized low-density lipoproteins. A further action of intracellular LPA could be mediated by a nuclear receptor, LPA_1 that regulates proinflammatory gene expression [Gobeil et al., 2003].

S1P is generated within cells by sphingosine kinases in response to PDGF and TNFα. S1P stimulates ERK giving a mitogenic response, it mobilizes intracellular Ca^{2+} and increases actin stress fiber formation [see Pyne and Pyne, 2000; English et al., 2002 for reviews].

The work described above presents a growing list of extra- and intra-cellular signaling effects of lipid phosphates. Therefore, enzymes that synthesize, or degrade lipid phosphates can control many aspects of signaling that are involved in cell division, apoptosis, inflammation, adhesiveness, and migration.

LIPID PHOSPHATE PHOSPHATASES AND S1P PHOSPHATASES

Two classes of phosphatidate phosphatases (PAP-1 and PAP-2) act on PA. PAP-1 is stimulated by Mg^{2+} and is inhibited by *N*-ethylmaleimide. It controls triacylglycerol and phosphatidylcholine synthesis by translocating to the cytosolic surface of internal membranes where PA is produced [Brindley and Waggoner, 1998]. PAP-1 could also regulate intra-cellular signaling: for example, PAP-1 is involved in cyclo-oxygenase expression and eicosanoid formation when WISH cells are activated through PKC [Johnson et al., 1999]. Also, PAP-1 co-immunoprecipitates with activated EGF receptors [Jiang et al., 1997]. PAP-1 should be able to access PA that is formed on the cytosolic surface of membranes and therefore, could be involved in regulating signal transduction processes as well as glycerolipid synthesis.

There is also a second class of phosphatidate phosphatases (PAP-2) that are not inhibited by *N*-ethylmaleimide and do not require Mg^{2+} [Brindley and Waggoner, 1998; Brindley et al., 2002]. PAP-2 is relatively non-selective for its lipid phosphate substrates since it dephosphorylates LPA, S1P, C1P, and diacylglycerol pyrophosphate in addition to PA. It was, therefore, renamed as lipid phosphate phosphatase (LPP) to reflect this substrate specificity and uncertainty as to the true substrates in vivo [Brindley and Waggoner, 1998]. The LPPs are predicted to regulate cell signaling through lipid phosphates versus their bioactive dephosphorylated counterparts (DAG, ceramide, and sphingosine) (Fig. 1).

Kai et al. [1996] first described mouse PAP2A (LPP-1). Other isoforms were rapidly identified (Fig. 2); [see Brindley et al., 2002 for review]. The LPPs belongs to a super-family that includes bacterial phosphatase, yeast diacylglycerolpyrophosphatase, yeast phytosphingosine phosphate phosphatase, and LPP, fungal haloperoxidase, mammalian glucose 6-phosphatase, and Wunen proteins in *Drosophila*. The LPPs possess three conserved active site domains and six transmembrane domains (Fig. 2). The conserved amino acids of the phosphatase family are shown in red in Figure 2 and they are essential for LPP-1 activity [Zhang et al., 2000b]. LPP-1 is partly expressed on the plasma membrane of cells with the C-terminal inside the cell [Jasinska et al., 1999]. This topology together with the identification of the *N*-glycosylation site [Zhang et al., 2000b] is compatible with the active site being outside the cell. This explains why LPP-1, LPP-2, and LPP-3 exhibit "ecto"-phosphatase activity in different cell types against PA, LPA, C1P, and S1P, which do not readily enter the cell [see Brindley et al., 2002 for review]. This conclusion that the conserved domains of the LPPs are located on the same side of the membrane as the *N*-glycosylation site is compatible with work on Dri42. This protein is expressed on the endoplasmic reticulum during differentiation of intestinal cells [Barilà et al., 1996]. Dri42 was subsequently identified as rat LPP-3.

These observations also indicate that the active sites of the LPPs should be expressed on the luminal surface of the endoplasmic reticulum, or Golgi membranes. Consequently, this may restrict the access of the lipid phosphates to the active sites of the LPPs if these lipids are generated on the cytosolic surface of membranes. One might, therefore, assume that this access to the active sites of the LPPs may be regulated by the rate of translocation of different lipid phosphates across membranes to the luminal surface. In addition, PAP-1 could be responsible for the degradation of PA on the cytoplasmic surface of the plasma membrane and internal membranes.

The identity of the signals that direct the LPPs to be expressed at the cell surface were recently determined by Kanoh's Group using polarized MDCK cells. These investigators observed that LPP-1 was located in the apical surface membrane, whereas LPP-3 was mainly in the basolateral membrane [Jia et al., 2003]. A novel apical sorting signal was found in the N-terminus of LPP-1, which consisted of FDKTRL. A dityrosine motif in the second cytoplasmic

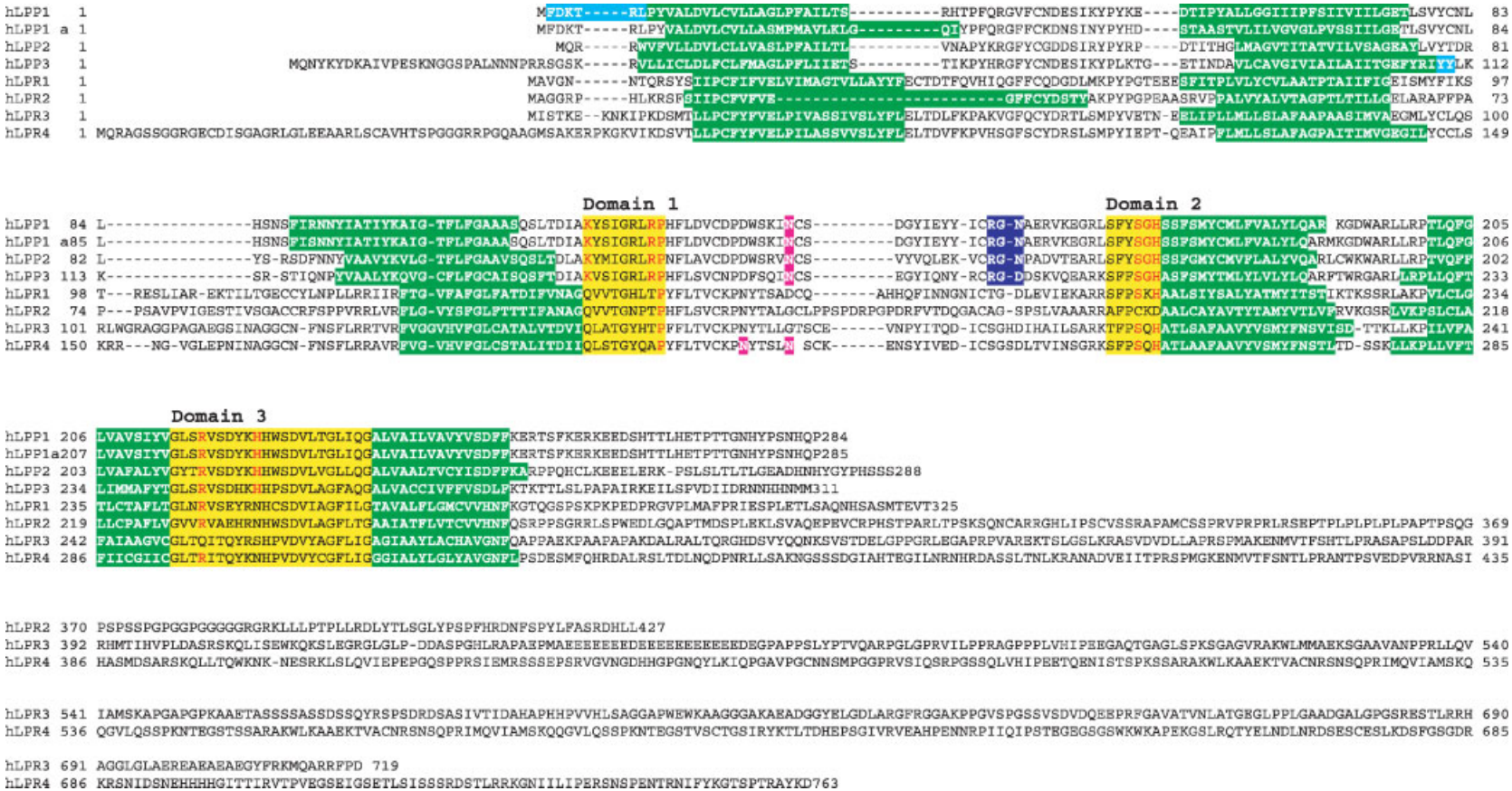

Fig. 2. Alignment of human lipid phosphate phosphatases (LPPs) and plasticity-related genes (PRGs), also known as LPP-related proteins (LPRs). Predicted transmembrane regions (established by using HMMTOP V2.0) are in green, regions surrounding the three conserved domains that constitute the active sites of the LPPs are in yellow. The red residues conserved amino acids that are required for LPP activity. The blue region is where putative integrin binding domains are located on an external loop and in magenta are the established, or putative *N*-glycosylation sites. The residues in the N-terminus of LPP-1 and the second cytosolic domain of LPP-3 that are highlighted in turquoise are sequences that have been shown to target these LPPs to apical and basolateral membranes of MDCK cells [Jia et al., 2003]. The sequences of the PRGs, or LPRs that are shown were obtained from GenBank as follows: LPR-1 (AY304515) = PRG-3 (AY337718), LPR-2 (AY304516) = PRG-4 (AY339628), LPR-3 (AY304517) = PRG-2 (AF541282), LPR-4 (AY304518) = PRG-1 (AF541281).

portion of LPP-3 was responsible for targeting to the basolateral membrane. These sequences are highlighted in turquoise in Figure 2.

Mandala et al. [2000] cloned cDNA for a mammalian phosphatase (SPP) that is specific for S1P and a second form has been discovered [Ogawa et al., 2003] (Fig. 3). The SPPs possess three conserved domains similar to the LPPs, but the transmembrane domains are different (Fig. 3). The active site of the SPPs should be expressed in the lumen of the endoplasmic reticulum, or Golgi [Kihara et al., 2003].

Hiroyama and Takenawa [1999] purified an LPA-specific phosphatase that has homology to prostate acid phosphatase. This enzyme could help to regulate the concentration of intracellular LPA.

LIPID PHOSPHATE PHOSPHATASE-RELATED PROTEINS (LPRs), OR PLASTICITY-RELATED GENES (PRGs)

Recently, a family of four LPP-related proteins (LPRs), or plasticity-related genes (PRGs) that show structural similarity to the LPPs has been identified (Fig. 2). These proteins, like the LPPs, all contain six putative transmembrane regions. Unlike other members of the LPP family, PRG-1 and PRG-2 (LPR-4 and -3, respectively) have very long hydrophilic C-terminal tails. The PRGs were so named since they are expressed highly during development in the brain and PRG-1 expression is increased in the hippocampus after injury [Bräuer et al., 2003]. The PRGs, or LPRs lack conserved amino acids in positions that correspond to the active sites of the LPPs (shown in red in Fig. 2) and which were shown to be essential for phosphatase activity for LPP-1 [Zhang et al., 2000b]. For example, all of the LPRs (PRGs) lack the conserved lysine and arginine residues in Domain 1 and the histidine in Domain 3. In the case of LPR2, or PRG4, the only conserved residues are a proline in Domain 1 and an arginine in Domain 3. The conserved residues of the phosphatase family are involved in substrate binding, or the catalytic mechanism as demonstrated for chloroperoxidase and glucose 6-phosphatase [Messerschmidt et al., 1997; Ghosh et al., 2004]. From these considerations, the PRGs (LPRs) cannot participate in a dephosphorylation reaction using the same mechanism that has been proposed for the LPPs, chloroperoxidase, and glucose 6-phosphatase.

Despite this, over-expression of PRG-1 was reported to increase the dephosphorylation of exogenous LPA in N1E-115 cells [Bräuer et al., 2003]. This effect was not seen with a PRG-1 mutant in which the histidine in Domain 2 was mutated to lysine. The increased ecto-LPA phosphatase activity with wild-type PRG-1 need not indicate that PRG-1 is a phosphatase itself. It could have resulted indirectly if PRG-1 (LPR-4) were to have stimulated the activity of an existing LPP. By contrast, to PRG-1, PRG-3 (LPR-1) expression did not increase ecto-phosphatase activity [Savaskan et al., 2004]. Expression of PRG-3 in neuronal and COS-7 cells promoted cell spreading and membrane protrusions [Savaskan et al., 2004]. Also in fibroblasts, expression of PRG-3 [Morris, personal communication], that was called lipid phosphate phosphatase-related protein-1 (LPR-1) by this Group caused the dramatic formation of extensive actin containing filopodia and spreading in fibroblasts. These effects of PRG-3 (LPR-1) were obtained in the absence of a phosphatase activity against lipid phosphates. Consequently, the PRGs (LPRs) appear to exert significant effects on cell signaling and cell structure, but it is still too early to have a definite explanation for the mechanisms that are responsible for these effects.

REGULATION OF CELL SIGNALING BY LPPs AND RELATED PROTEINS LPRs: EXTRACELLULAR FUNCTIONS

The demonstration that LPP-1 is an ecto-phosphatase [Jasinska et al., 1999] indicated that the LPPs could control exogenous LPA and S1P concentrations. Over-expression of LPP-1 attenuated the activation of ERK, cell division, PLD, and Ca^{2+} transients by exogenous LPA [Jasinska et al., 1999; Pilquil et al., 2001]. Dephosphorylation of LPA yields monoacylglycerol that has little biological activity, except for 2-arachidonoylglycerol, which is a cannabinoid receptor ligand [Sugiura and Waku, 2000]. LPP action on PA, C1P, and S1P yields DAG, ceramide or sphingosine, respectively, which can more readily enter the cell and are bioactive [Roberts and Morris, 2000]. Consequently, the effects of LPPs on signaling by exogenous lipid phosphates depend upon the intracellular effects of the products in addition to attenuation of signaling through surface receptors. Other work demonstrates that the expression of

Fig. 3. Alignment of human sphingosine-1 phosphate phosphatases (hSPPs). Predicted transmembrane regions (established by using HMMTOP V2.0) are in green, conserved regions of the active site are in yellow and in red are residues that are presumed to be required for activity. The blue region is where an integrin binding domain is possibly located depending on the orientation and subcellular distribution of the protein. The model proposed by Kihara et al. [2003] indicates that the sixth hydrophobic helix does not span the membrane. The magenta highlighting shows putative *N*-glycosylation sites (determined by using NetNGlyc 1.0), but this again depends upon the orientation of the proteins. The accession numbers for hSPP1 and hSPP2 are NM 030791 and AF542512, respectively.

ecto-activity is regulated. For example, a gonadotropin releasing hormone (GnRH) agonist increases plasma membrane expression of LPPs in ovarian cancer cells [Imai et al., 2000]. It was concluded [Imai et al., 2000] that the increased ecto-LPP activity explained the anti-proliferative effects of GnRH on ovarian carcinomas. Also, over-expression of LPP-3 decreases the growth, survival, and tumorigenesis of ovarian cancer cells by increasing exogenous LPA degradation [Tanyi et al., 2003]. Exogenous LPA increases the expression of ecto-LPP-1 activity in platelets [Smyth et al., 2003]. Increased ecto-LPP-1 activity also decreased net LPA production by platelets and LPA-induced shape changes and aggregation [Smyth et al., 2003]. Hooks et al. [2001] also used hydrolysable and non-hydrolysable LPA analogues and showed that attenuation of signaling by LPP-1 correlated with dephosphorylation. The ligand specificity for platelet aggregation was similar to that for mitogenesis, but this did not correlate with LPA receptor activation. Ecto-LPP activities also regulate net extracellular LPA production and proliferation of preadipocytes [Simon et al., 2002].

As discussed above, PRG-1 was shown by Bräuer et al. [2003] to increase extracellular LPA dephosphorylation. This protected neurons against LPA-mediated axon collapse and promoted outgrowths in the hippocampus [Bräuer et al., 2003]. Consequently, the PRGs (LRPs) could modify signaling through LPA by stimulating LPP activities, binding lipid phosphates, or perhaps by exhibiting phosphotransferase activity.

Other work suggests that the LPPs themselves could act structurally in addition to having phosphatase activity. For example, hLPP-3 contains an RGD cell adhesion sequence that is exposed outside of the cell (high-lighted in blue in Fig. 2). Retroviral over-expression of hLPP-3 in endothelial cells induced cell aggregation plus cell/cell interactions, it increased p120 catenin expression and resulted in activation of focal adhesion kinase, Akt, and GSK3β [Humtsoe et al., 2003]. Expression of recombinant hLPP-3 promoted cell adhesion, spreading, and tyrosine phosphorylation of proteins including focal adhesion kinase and paxillin. GST-LPP-3 bound to a specific subset of integrins and anti-$\alpha_v\beta_3$ and anti-$\alpha_5\beta_1$ integrin antibodies blocked binding. These effects were not observed with recombinant LPP-3 in which RGD was mutated to RGE. Furthermore, in human platelets, LPP-3 co-localized with $\alpha_{IIb}\beta_3$ and it was postulated that the interaction could control the lateral organization of LPP-3 in platelet membranes [Smyth et al., 2003]. This combined work, therefore, provides evidence for a role of LPP-3 in cell attachment and integrin signaling.

However, it is important to note that this RGD sequence of human LPP-3 is not conserved in mouse and rat LPP-3 [see Waggoner et al., 1999 for review], which contain RGE. This sequence did not alter cell/cell interactions [Humtsoe et al., 2003]. hLPP-1, hLPP1a, and hLPP2 have RGN instead of RGD in hLPP-3 (Fig. 2). Peptides with RGD and RGN sequences antagonize the binding of T-lymphocytes to fibronectin [Preciado-Patt et al., 1994]. hSPP-1 contains an RGD sequence that could also be involved in cell/cell contacts depending upon its orientation and if it can be expressed in the plasma membrane (Fig. 3). These combined observations require further investigation since they could be significant in regulating cell adherence and migration, processes that are intimately involved angiogenesis, tumor formation, and metastases.

INTRACELLULAR FUNCTIONS OF THE LPPs AND SPPs

In addition to being expressed on the plasma membrane the LPPs are also located on internal membranes and therefore, could control the relative concentrations of lipid phosphates inside the cell. This in turn could modify signal transduction by modulating the balance of internal signaling by lipid phosphates and their products. Alderton et al. [2001] showed diminished activation of ERK by extracellular LPA, S1P, and PA in cells that over-expressed LPP-1, -1A, and -2, but not LPP-3. This was related to intracellular PA concentrations. Also over-expression of LPP-1, -1A, and -2 attenuated the activation of ERK by thrombin for which signaling does not depend upon extracellular LPA [Alderton et al., 2001]. Previous work showed that over-expression of LPP-1 diminished PA concentrations in cells [Leung et al., 1998]. HEK 293 cells that over-express LPP-3 exhibited greater DAG formation subsequent to PLD stimulation and PLD2 and LPP-3 are both present in caveolin-1-enriched micro-domains [Sciorra and Morris, 1999]. The DAG could

modify signaling provided that its fatty acid composition is relatively unsaturated [Pettitt et al., 1997]. LPP activity is also enriched in caveolin-enriched domains isolated from rat lung, isolated alveolar type II cells and the type II mouse cell lines (MLE12 and MLE15) [Nanjundan and Possmayer, 2001]. LPP-3 was localized predominantly in caveolin-enriched domains from rat lung. The subcellular localizations of the LPPs are cell type specific. Phorbol ester treatment of MLE15 cells caused a 3.8-fold increase in LPP activity caveolin-enriched domains.

Expression of mSPP-1 in fibroblasts decreased the intracellular S1P concentration and increased that of ceramide [Mandala et al., 2000]. This change diminished cell survival and induced characteristic traits of apoptosis.

ANIMAL MODELS

Other evidence for the differential involvement of the LPPs in signaling comes from work with transgenic mice. Mice that over-express LPP-1 have a 50% decrease in birth weight, and abnormalities in fur growth and disrupted hair structure with decreased numbers of hair follicles [Yue et al., 2004]. The males showed decreased fertility with a severe impairment of spermatogenesis. Female mice that over-expressed LPP-1 also showed defects in fertility since implantation of LPP-1 over-expressing, or wild-type embryos into pseudopregnant LPP-1 mothers yielded a decreased litter size. The role of LPP-1 over-expression in signaling was measured in immortalized fibroblasts. There was an increased accumulation of diacylglycerol in fibroblasts from over-expressing mice following phorbol ester-induced stimulation of PA production. In contrast to previous studies, there was no significant difference in the activation of ERK1/2 after stimulating the fibroblasts from the LPP-1 over-expressing mice with LPA, S1P, thrombin, EGF, or PDGF. Surprisingly, there were also no significant differences in the basal concentrations of five major acyl-species of LPA in the blood of the LPP-1 over-expressing mice. Consequently, LPP-1 over-expressing mice displayed several unexpected phenotypes without showing obvious changes in several aspects of LPA-induced signaling [Yue et al., 2004].

By contrast, LPP-2 knockout mice [Zhang et al., 2000a] are fertile and viable, whereas LPP-3 knockout mice showed a very severe phenotype [Escalante-Alcalde et al., 2003]. Mouse embryos deficient for LPP-3 failed to form a chorio-allantoic placenta and yolk sac vasculature. In addition, a subset of embryos showed a shortening of the anterior–posterior axis that was similar to that in *axin* deficiency, a critical regulator of Wnt signaling. The loss of LPP-3 resulted in a decreased β-catenin-mediated TCF transcription, whereas elevated levels of LPP-3 had the opposite effect. Escalante-Alcalde et al. [2003] provided evidence that LPP-3 could normally function as a Wnt signaling antagonists in vivo, but the mechanisms for this interaction were not established. This action did not appear to depend totally upon the phosphatase activity of LPP-3 since mutant LPP-3 that lacked activity were partially effective in inhibiting TCF/β-catenin transcription in HEK293 cells. Additional support for a role of LPP-3 in axis patterning was provide from the observations that ectopic expression of LPP-3 in dorsal blastomeres of *Xenopus* embryos caused a mild ventralizing effect [Escalante-Alcalde et al., 2003]. Also, axis duplication induced by injection of *Xwnt8* or *3a* mRNA was inhibited by co-injection of LPP-3 mRNA. Although the link between these observations and the catalytic effect of LPP-3 is not clear, the authors did shown that embryonic fibroblasts from the LPP-3 knockout mice contained increased concentrations of PA and decreased diacylglycerol. The latter change was parallel by a decrease in the phosphorylation of protein kinase C as demonstrated with a phospho-pan PKC antibody. Also the extracellular concentration of LPA extracted from cultures of the fibroblast from the LPP-3 knockout mice was increased relative to the wild-type.

Other work that confirms a role for LPPs in controlling cell migration comes from studies with the two *Drosophila* Wunen proteins. These are homologous to LPP-3 and negatively regulate the migration of primordial germ cells [Zhang et al., 1997; Starz-Gaiano et al., 2001; Burnett and Howard, 2003]. Significantly, introduction of mouse LPP-1 has no effect on an endogenous *Drosophila* germ-cell-specific factor in vivo, whereas human LPP-3 causes aberrant migration and germ cell death [Burnett and Howard, 2003]. These results demonstrate that individual LPPs have distinct functions that cannot be replaced by other family members. This work also demonstrates an under-

lying signaling system for cell migration that may be conserved between flies and human beings.

Starz-Gaiano et al. [2001] identified *Wun2*, showed it maps within 5 kb of *wun* and established that the two mRNAs have the same expression pattern. Both mRNAs were affected in the original *wun* mutants. Disruption of either gene alone does not have a severe effect on germ cell migration, suggesting that the two Wunen proteins act redundantly. The authors showed that mutations in the catalytic domains of Wunen-2 eliminate the repellent signal. They concluded that spatially restricted hydrolysis of phospholipids creates a gradient for the migration and survival of germ cells.

Burnett et al. [2004] recently demonstrated that Wunen dimerizes. This depends upon the last 35 amino acids at the C-terminus and the presence of an intact catalytic domain. Murine LPP-1 and human LPP-3 also form associations, but do not form interactions with each other. Wunen also did not interact with Wunen-2. The formation of complexes did not appear to be required for phosphatase activity, or the effects of Wunen in vivo.

The distribution of the LPPs in mammalian cells is tissue-specific. mRNAs for LPP-1/1a are expressed to a high extent in human prostate, aorta, bladder, uterus, kidney, lung, and heart [see Waggoner et al., 1999 for review]. hLPP-1a is predominant in heart, whereas hLPP-1 is predominant in kidney, lung, placenta, and liver. mRNA for LPP-2 was found mainly in brain, pancreas, and placenta. These combined results indicate that different LPPs have important and distinct functions that cannot be replaced by other LPPs.

REGULATION OF LPP AND PRG (LPR) ACTIVITY

Most of out knowledge of the regulation of LPP activity comes from experiments where their synthesis has been modified. Little is known about more rapid effects on activity other than changes in subcellular distribution (see above).

LPP-3 mRNA is increased by about threefold after treating quiescent HeLa cells with EGF whereas mRNA for LPP-1 was not affected [Kai et al., 1997]. LPP-1 mRNA is increased in human prostatic adenocarcinoma cells by androgens [Ulrix et al., 1998]. Expression of Dri42 (LPP-3) in rat intestinal mucosa is increased during epithelial differentiation [Barilà et al., 1996]. LPP-1 mRNA was decreased in keratinocytes and endothelium from healing rabbit cornea [Wang et al., 2002]. Differentiation of 3T3F442A preadipocytes into adipocytes leads to an 80% decrease in ecto-LPP activity as a result of decreased mRNA expression for LPP-1, LPP-2, and LPP-3 [Simon et al., 2002]. LPP-3 activity in endothelial cells is increased by VEGF and this may play a role in regulating angiogenesis [Humtsoe et al., 2003]. This combined work demonstrates differential turnover and regulation of the LPPs.

In the case of PRG-1 (LPR-4), the expression of this protein is increased in the hippocampus during development. PRG-1 facilitates axonal outgrowths and regenerative sprouting [Bräuer et al., 2003]. PRG-3 (LPR-1) also shows a specific expression pattern during embryonic and postnatal brain development where its expression is predominantly in neuronal cell layers. In mature brain, the strongest PRG-3 expression occurs in the hippocampus and cerebellum. Over-excitation of neurons induced by kainic acid leads to a transient down-regulation of PRG-3.

CONCLUSIONS

The work described above shows that LPA and S1P are powerful extracellular signaling molecules that control a variety of cell activities including division and cell movement. These effects are cell-type specific, depending upon the relative expression of the different LPA and S1P receptors and their coupling to different heterotrimeric G-proteins. Inside the cell, lipid phosphates such as LPA, PA, S1P, and C1P are involved in regulating processes such as the activation of ERK, mTOR, PPAR-γ receptors, cell division, Ca^{2+} transients, membrane movement, etc. Consequently, enzymes that modify the turnover of the lipid phosphates can play key roles in regulating signal transduction by extra- and intra-cellular lipid phosphates.

The phosphatases can control signaling by attenuating the effects of the lipid phosphates and by forming products such as DAG, sphingosine, and ceramide that are also bioactive. The ecto-phosphatase activities of the LPPs could decrease the activation of the LPA and S1P receptors and can also promote the uptake into

the cell of the dephosphorylated products. Consequently, intracellular signaling by DAG, sphingosine and ceramides can be increased by the ecto-LPPs. Furthermore, these products can also be re-phosphorylated inside the cells to promote additional signaling cascades, which in turn can be modulated by internal actions of the LPPs and SPPs. These phosphatases could also control the release of LPA and S1P from cells thereby regulating their effects in autocrine/paracrine signaling [Johnson et al., 2003]. It is difficult in many publications to decide at which level the phosphatases are exerting their effects on cell signaling. The discovery that the LPPs alter cell activation independently of the catalytic activity also adds a further level of complexicity. At present, the relative importance of different LPPs and SPPs in regulating these intra- and extra-cellular signaling actions, the preferred substrates and the exact functions of these enzymes are not well established. Available evidence indicates that the different LPPs and SPPs have distinct signaling functions that cannot be substituted by other isoforms.

The PRGs (LRPs) also add a new level of complexity. PRG-1 has been reported to increase the dephosphorylation of exogenous LPA and attenuate LPA-induced neurite retraction [Bräuer et al., 2003]. By contrast, PRG-3 does not increase LPA dephosphorylation [Savaskan et al., 2004; Morris, personal communication]. The PRGs have profound effects on cell signaling and morphology. The exact mechanisms of action of the different PRGs (LRPs) need to be established to understand their individual effects on cell activation

At present, there is a rapid increase in our knowledge of the varied role of different lipid mediators as regulators of cell division, death, and movement. The LPPs, SPPs, and PRGs clearly modulate these signaling events, and we need to understand in far greater detail how these effects are mediated. In the longer-term, these advances in knowledge will hopefully shed light on how to intervene and treat medical conditions that involve derangements of cell adhesion, chemotaxis, angiogenesis, wound healing, tumor development, and metastasis.

ACKNOWLEDGMENTS

Work on lipid phosphates and the phosphatases in my laboratory was financed by grants form the Canadian Institutes of Health Research (MOP 10504).

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