Rev Physiol Biochem Pharmacol (2006) 160:25–46
DOI 10.1007/112_0507

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Biological effects of lysophospholipids

Published online: 29 April 2006

Abstract Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) are potent biologically active lipid mediators that exert a wide range of cellular effects through specific G protein-coupled receptors. To date, four LPA receptors and five S1P receptors have been identified. These receptors are expressed in a large number of tissues and cell types, allowing for a wide variety of cellular responses to lysophospholipid signaling, including cell adhesion, cell motility, cytoskeletal changes, proliferation, angiogenesis, process retraction, and cell survival. In addition, recent studies in mice show that specific lysophospholipid receptors are required for proper cardiovascular, immune, respiratory, and reproductive system development and function. Lysophospholipid receptors may also have specific roles in cancer and other diseases. This review will cover identification and expression of the lysophospholipid receptors, as well as receptor signaling properties and function. Additionally, phenotypes of mice deficient for specific lysophospholipid receptors will be discussed to demonstrate how these animals have furthered our understanding of the role lysophospholipids play in normal biology and disease.

Introduction

Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) are two distinct biologically active lysophospholipid molecules capable of exerting dramatic effects on a wide variety of cell and tissue types. Although both serve as metabolites in membrane phospholipid synthesis, their ability to act as extracellular signaling molecules is their most intriguing function (Moolenaar 1995; Pages et al. 2001b; Spiegel and Milstien 2003). Early studies demonstrated that both LPA and S1P were capable of initiating a variety of cellular responses in diverse cell types. However, it was the identification and subsequent cloning of

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specific G protein-coupled receptors that provided invaluable insight into the biological activities of these molecules in vivo.

Activated platelets are a major source of LPA and S1P; however, these lysophospholipids are produced by many different cell types (Sano et al. 2002; Yatomi et al. 2000). Mature neurons, Schwann cells, adipocytes, and fibroblasts have been implicated in the production of LPA (Fukushima et al. 2000; Pages et al. 2001a, 2001b; Weiner et al. 2001). Likewise, in response to external stimuli, hematopoietic cells such as peripheral mononuclear cells, erythrocytes, and neutrophils can contribute to the level of S1P in the blood (Yang et al. 1999). Together, these multiple sources contribute to LPA and S1P levels in a range of biological fluids, including serum, saliva, and follicular fluid. Interestingly, ovarian cancer cells also produce S1P and LPA, and studies suggest that these molecules may contribute to tumor cell progression and metastasis (Eder et al. 2000; Yatomi et al. 2001).

S1P is produced by sphingosine kinase (SPHK) -mediated phosphorylation of sphingosine, a lipid synthesized through the metabolic conversion of ceramide. Two sphingosine kinase isoforms are known to exist in mice, SPHK1 and SPHK2 (Kohama et al. 1998; Liu et al. 2000a). Deletion of SPHK1 reduces serum and plasma levels of S1P to 50% of those observed in wild type mice, which indicates that SPHK2 or unidentified sphingosine kinases are responsible for the remaining SPHK catalytic activity (Allende et al. 2004). SPHK activity has been described in cytosolic, membrane, and extracellular compartments (Ancellin et al. 2002; Olivera et al. 1999). While SPHKs are involved in S1P production, lipid phosphate phosphatases (LPPs) and S1P lyase (SPL) degrade S1P. To date, two S1P phosphatases have been cloned, SPP1 and SPP2 (Mandala et al. 2000; Ogawa et al. 2003). The presence of these two enzymes in the endoplasmic reticulum (ER) indicates that these molecules dephosphorylate intracellular S1P. SPL is an evolutionarily conserved enzyme, also present in the ER, that catalyses the cleavage of S1P into hexadecanal and phosphoethanolamine (Van Veldhoven and Mannaerts 1994). Platelets lack SPL, which may partially account for their ability to produce large amounts of S1P. In *Dictyostelium discoideum*, *Caenorhabditis elegans*, and *Drosophila melanogaster*, loss of SPL results in abnormal development and differentiation (Herr et al. 2004; Li et al. 2001; Mendel et al. 2003). Mice deficient for SPL have not been generated; however, a targeted deletion of SPL in mouse F9 embryonic carcinoma cells causes an increase in endodermal differentiation in response to retinoic acid (RA) (Kihara et al. 2003). This effect is also observed in F9 cells stably expressing a SPHK (Kihara et al. 2003). Conversely, pretreatment of F9 cells with an S1P synthesis inhibitor blocks differentiation, indicating that an accumulation of S1P is responsible for this effect.

Serum LPA levels are regulated by the secreted lysophospholipases PLA_1 and PLA_2, and lysophospholipase D (lysoPLD). PLA_1 and PLA_2 are also thought to be responsible for the de novo generation of lysophosphatidylcholine (LPC) (Fig. 1), a substrate for lysoPLD, which can then cleave LPC to produce LPA (Aoki et al. 2002; Sano et al. 2002).

The recent identification of lysoPLD as autotaxin (ATX) has been particularly helpful for understanding LPA pathways. ATX was first identified as a protein in melanoma cell culture medium that stimulated cancer cell motility (Murata et al. 1994; Stracke et al. 1992). ATX has a single transmembrane domain, two somatomedin B-like domains, and a catalytic domain (Stracke et al. 1997). ATX is most likely cleaved intracellularly, then released into the extracellular environment. ATX was initially proposed to function as a nucleotide phosphodiesterase, but the role of this activity in ATX function is currently unclear (Bollen et al. 2000; Goding et al. 1998). In addition to its ability to promote cancer cell motility, ATX has also been shown to promote angiogenesis in vivo (Nam et al. 2001). These results support the view that ATX is an important factor in cancer biology.

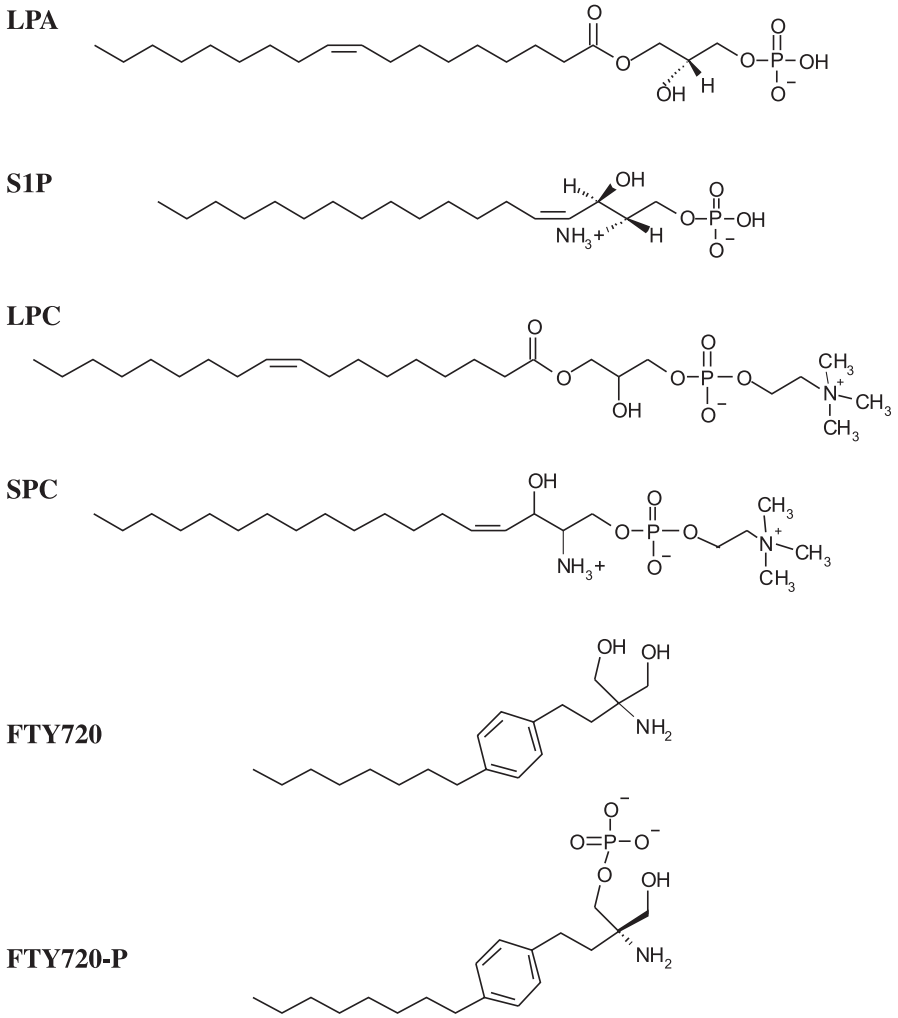

Fig. 1 Chemical structures of some of the bioactive phospholipids and FTY720/FTY720-P. *LPA* lysophosphatidic acid, *S1P* sphingosine 1-phosphate, *LPC* lysophosphatidylcholine, *SPC* sphingosylphosphorylcholine

ATX is important for LPA activity because it can access pools of lysophosphatidyl choline (LPC) that exists in comparatively high concentrations in many bodily fluids, to allow for the synthesis of LPA and subsequent activation of cellular responses through LPA receptors. ATX promotes the proliferation of several cancer cell lines and this increase in proliferation is enhanced in the presence of LPC, suggesting LPA activity is important for the effect (Hama et al. 2004; Umezu-Goto et al. 2002). Another feature shared between LPA and ATX is the ability to stimulate preadipocyte proliferation (Ferry et al. 2003; Pages et al. 2001a). ATX activity and subsequent LPA production also promotes the differentiation of primary preadipocytes and a preadipocyte cell line (Ferry et al. 2003). In vivo, ATX is expressed in mature adipocytes and is upregulated in genetically obese diabetic mice (Ferry et al. 2003). These results, together with the fact that LPA is present in the extracellular fluid of adipose tissue in vivo and released by adipocytes in vitro, implies that an LPA-dependent

paracrine control of adipose tissue is mediated by ATX (Valet et al. 1998). These data show that many LPA-mediated effects are paralleled by ATX, particularly effects mediated by the receptor LPA_1, and that ATX activity creates a microenvironment for LPA activity (Hama et al. 2004). We note that S1P is also generated by the hydrolysis of sphingosylphosphorylcholine (SPC) by ATX (Clair et al. 2003) (Fig. 1). However, this occurs at a lower efficiency than the generation of LPA from LPC, and is currently of uncertain biological significance.

Downregulation of lysophospholipid activity occurs in part, through the action of lipid phosphate phosphatases (LPPs). There are three members of this family of integral membrane glycoproteins, LPP-1, LPP-2, and LPP-3, also known as PAPs (Kai et al. 1996, 1997; Roberts et al. 1998). All three LPP proteins are capable of dephosphorylating and thus attenuating LPA and S1P activity. Byproducts of LPA and S1P degradation are monoacylglycerol and sphingosine, respectively. These LPPs can also dephosphorylate phosphatidic acid (PA) and ceramide 1-phosphate to produce diacylglycerol and ceramide. LPP proteins have six transmembrane domains and a conserved catalytic domain that faces the extracellular side of the plasma membrane or the luminal side of intracellular membranes (Zhang et al. 2000). Because of their ability to dephosphorylate extracellular LPA and S1P, LPPs are also known as "ecto" enzymes (Jasinska et al. 1999; Roberts et al. 1998; Roberts and Morris 2000; Smyth et al. 2003).

Treatment of platelets with an LPP-1 inhibitor results in an increase of LPA-dependent platelet responses, including Rho-dependent actin reorganization, morphological changes, and aggregation (Smyth et al. 2003). Introduction of LPPs into ovarian cancer cell lines increases apoptosis and decreases colony formation (Tanyi et al. 2003). Expression of LPP-3 in an ovarian cancer cell line severely reduces the ability of these cells to form tumors in mice (Tanyi et al. 2003). Interestingly, cells overexpressing LPPs were able to inhibit the growth of adjacent nontransfected cells (Tanyi et al. 2003). Thus, neighboring cells are rendered unresponsive to the proliferative effects of LPA by LPP-expressing cells in the immediate vicinity.

Plasticity related gene 1 (PRG-1) is a neuron-specific LPP that is localized to the membrane of outgrowing axons (Brauer et al. 2003). This protein is thought to inhibit lysophospholipid-induced axon collapse and thus permit axon outgrowth (Brauer et al. 2003). Like other LPPs, PRG-1 activity is dependent on an intact catalytic domain. PRG-1 and a related gene, PRG-2, are similar to other LPPs except they contain a long carboxy terminal tail that may be involved in cell signaling (Brauer et al. 2003). Some debate exists over the catalytic activity of some PRGs (McDermott et al. 2004).

S1P receptors

To date five S1P receptors have been cloned and are designated $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$. All of these receptors are responsive to S1P; however, the temporal and spatial pattern of receptor expression as well as the signaling molecules downstream of each receptor for a diverse variety of responses to S1P. Most receptors were initially cloned by multiple, independent groups as orphan receptors with various names, and the numbering system reflects their initial order in the literature.

$S1P_1$

$S1P_1$ was identified as a gene, edg-1, that was upregulated during phorbol 12-myristate 13-acetate (PMA) -induced differentiation of human endothelial cells in vitro (Hla and Maciag 1990). The $S1P_1$ receptor is expressed in a large number of cell types and tissues. By Northern blot analysis, high levels of $S1P_1$ expression are detected in mouse adult brain, heart, lung, liver, and spleen (Ishii et al. 2001, 2002; Liu and Hla 1997; McGiffert et al. 2002; Zhang et al. 1999). In the embryonic brain, high levels of $S1P_1$ expression are found in cerebellum, neocortical areas, intersomitic arteries, capillaries and developing blood vessels, aorta, and skeletal system (Liu and Hla 1997; McGiffert et al. 2002). $S1P_1$ expression has also been reported in a variety of immune system cells including $CD4^+$ and $CD8^+$ T cells, macrophages, dendritic cells, and NK cells (Goetzl et al. 2004; Graeler and Goetzl 2002; Jin et al. 2003; Matloubian et al. 2004; Wang et al. 2004).

Signaling via S1P and LPA receptors is mediated through at least three families of G proteins: G_i, G_q, and $G_{12/13}$. Co-immunopreciptation experiments using intracellular domains of $S1P_1$ and cell extracts showed that $S1P_1$ could couple to G_i proteins (Lee et al. 1996). It was shown in both Sf9 insect cells and in a *Xenopus* oocyte system that $S1P_1$ couples exclusively to the pertussis toxin (PTX) -sensitive G_i pathway (Ancellin and Hla 1999; Windh et al. 1999) (Fig. 2). Stimulation of the G_i-coupled $S1P_1$ receptor results in ERK activation, phospholipase C (PLC) activation, and inhibition of adenylyl cyclase (AC) (Lee et al. 1996; Okamoto et al. 1998; Zondag et al. 1998). S1P induces calcium mobilization in Chinese hamster ovary (CHO) cells but not in Sf9 insect cells, HEK293 cells, Cos-7 cells, or *Xenopus* oocytes overexpressing $S1P_1$ (Ancellin and Hla 1999; Okamoto et al. 1998; Zondag et al. 1998). S1P activates Rho in CHO cells expressing $S1P_1$ and Rho activation is required for S1P-induced migration and clustering of integrins into focal contact sites (Paik et al. 2001) (Fig. 2). S1P signaling and Rho activation through $S1P_1$ also mediate cell–cell contacts by upregulating cadherin expression and adherens junction assembly (Lee et al. 1998).

In endothelial cells, S1P-induced cortical actin assembly and cell migration requires the phosphorylation of $S1P_1$ (Lee et al. 2001). Interestingly, S1P induces PI-3-kinase-dependent activation of AKT in endothelial cells, and this activation may lead to phosphorylation of $S1P_1$ and subsequent activation of Rac (Lee et al. 2001).

The generation of $S1P_1$ receptor-deficient mice was essential in helping elucidate the in vivo function of $S1P_1$ (Liu et al. 2000b) (Table 1). Targeted deletion of the $S1P_1$ gene results in embryonic lethality between embryonic day 12.5 (E12.5) and E14.5 of development due to hemorrhaging (Liu et al. 2000b). $S1P_1$-deficient mice do not display defects in vasculogenesis or angiogenesis; however, vascular smooth muscle cells in the $S1P_1$-deficient mice fail to envelop completely the developing blood vessels (Liu et al. 2000b). Vascular maturation requires interactions between endothelial cells and vascular smooth muscle cells. Specific deletion of $S1P_1$ in endothelial cells showed that expression of the $S1P_1$ receptor in these cells is essential for proper vascular maturation (Allende et al. 2003). $S1P_1$ also mediates S1P-dependent migration of vascular smooth muscle cells. It is likely that without this receptor, recruitment of these cells into developing blood vessel walls is impaired. S1P-induced activation of Rac was ablated in fibroblasts derived from $S1P_1$-deficient mice, resulting in an inability of these $S1P_1$ null fibroblasts to migrate in response to S1P (Liu et al. 2000b).

In the immune system, $S1P_1$ is expressed in a variety of cell types, including B and T cells (Goetzl et al. 2004; Graeler and Goetzl 2002; Jin et al. 2003; Matloubian et al. 2004; Wang et al. 2004). $S1P_1$ expression levels are highest on the most mature T cells in the thymus, and are downregulated on activated T cells that are normally retained in peripheral

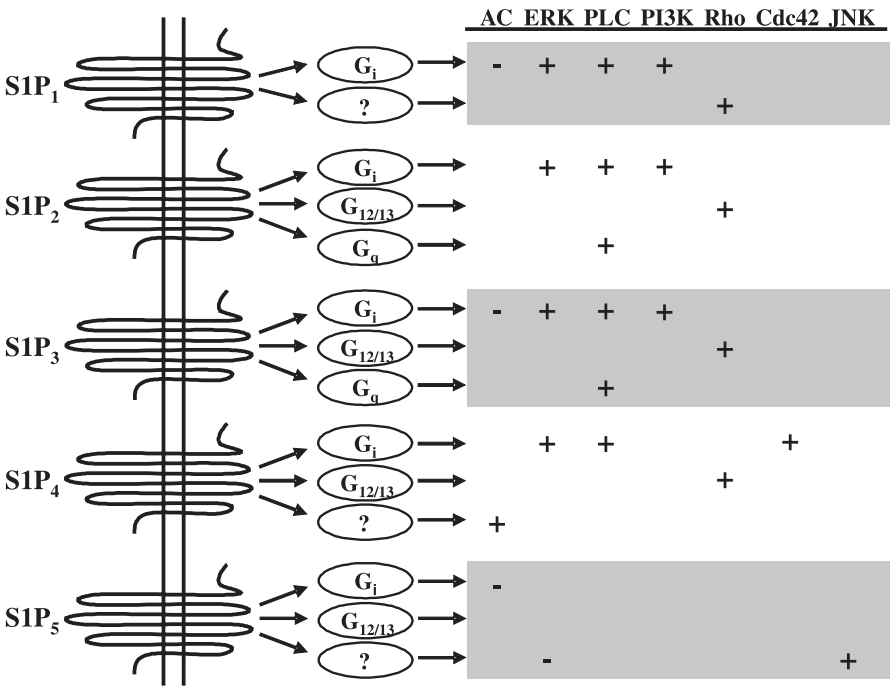

Fig. 2 Signaling pathways regulated by the S1P family of G protein-coupled receptors. The five known S1P receptors and the G proteins that couple to them are indicated. Cell signaling pathways downstream of receptor signaling are also indicated. *AC* adenylyl cyclase, *ERK* mitogen-activated protein kinase, *PLC* phospholipase C, *PI3K* phosphoinositol 3-kinase, *JNK* c-Jun N-terminal kinase

lymphoid organs (Matloubian et al. 2004). To ascertain the role of $S1P_1$ in hematopoietic cells, fetal liver cells derived from $S1P_1$-deficient mice were transferred into lethally irradiated hosts (Matloubian et al. 2004). Surprisingly, developing T cells from donor mice could not exit the thymus, and donor-derived B cells were present in peripheral lymphoid organs but dramatically reduced in blood and lymph. When donor-derived T cells were reintroduced into wild-type recipients, the T cells homed to secondary lymphoid organs; however, they could not recirculate to blood and lymph (Matloubian et al. 2004) (Table 1). These results were similar to the effects observed with FTY720, an agonist for $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$ receptors (Fig. 1) (Brinkmann et al. 2000, 2002). FTY720 downregulates $S1P_1$ receptor expression, thus mimicking an $S1P_1$-deficient state and inhibits developing T cells from exiting the thymus while retaining T cells in peripheral lymphoid organs (Graler and Goetzl 2004; Matloubian et al. 2004). It should be noted that other interpretations of these data exist and further studies are required to delineate the receptor mechanisms involved in these phenomena (Rosen and Goetzl 2005).

$S1P_2$

Two independent groups identified $S1P_2$ in screens of rat cDNA libraries (MacLennan et al. 1994; Okazaki et al. 1993). One group isolated $S1P_2$ from a hippocampal cDNA library while the second group identified $S1P_2$ by PCR of an aortic smooth muscle library with G protein-coupled receptor degenerate oligonucleotide primers. In the developing embryo,

Table 1 LPA and S1P receptor null mutantmice phenotypes

Receptor	In vivo phenotype	Cell signaling defects
$S1P_1$	Embryonic lethality between E12.5 and 14.5 Hemorrhage due to defective blood vessel formation Failure of null thymocyte egress in radiation chimeras	Reduced migratory response of MEFs in response to S1P
$S1P_2$	Slightly reduced viability Seizures in certain genetic backgrounds Neuronal hyperexcitability	Decreased S1P-induced Rho activation in MEFs
$S1P_3$	Slightly reduced viability Severe lethality in $S1P_2$-null background	Decreased S1P-induced PLC activation and AC inhibition in MEFs In $S1P_2/S1P_3$ double-null MEFs, S1P-induced Rho activation abolished, reduction of PLC activation
$S1P_5$	No obvious phenotype	Diminished S1P-induced pre-oligodendrocyte process retraction
LPA_1	Partial neonatal lethality due to impaired suckling Craniofacial deformities Frontal hematoma Increased apoptosis of Schwann cells of sciatic nerve	Reduced PLC activation and complete block of AC inhibition in MEFs treated with LPA; reduced morphological changes and proliferation of neuronal cells in response to LPA
LPA_2	No obvious phenotype	Reduction of MEF PLC activation in response to LPA Loss of PLC, JNK, and Akt activation, as well as proliferative responses in LPA_1/LPA_2 double-null MEFs in response to LPA Reduced stress fiber formation in LPA_1/LPA_2 double-null MEFs in response to LPA
LPA_3	No obvious phenotype Reduced fertility in female null mice due to implantation abnormalities	

Abbreviations: *MEF*, mouse embryo fibroplast; *JNK*, c-Jun kinase; *PLC*, phospholipase C; *AC*, adenylyl cyclase

$S1P_2$ RNA is present at high levels in the embryonic brain (MacLennan et al. 1994). In situ hybridization localized $S1P_2$ expression specifically to the midbrain and choroids plexus (McGiffert et al. 2002). Immunohistochemistry analysis suggested that $S1P_2$ protein was present in young differentiating neurons and in axons during a period of outgrowth, although the specificity of the antisera employed is not clear (MacLennan et al. 1997). $S1P_2$ RNA expression is high in a variety of human and rodent adult tissues, including lung, heart, kidney, thymus, and spleen (Ishii et al. 2001, 2002; MacLennan et al. 1994; McGiffert et al. 2002; Okazaki et al. 1993; Yamaguchi et al. 1996; Zhang et al. 1999). High $S1P_2$ RNA levels in the thymus are likely due to a relatively high level of $S1P_2$ expression in double- and single-positive thymocytes (Matloubian et al. 2004). The expression of $S1P_2$ in monocytes and dendritic cells may account for high $S1P_2$ levels in the spleen (Goetzl et al. 2004).

When co-expressed in Sf9 cells, $S1P_2$ was found to couple to G_i, G_q, and $G_{12/13}$ protein families (Windh et al. 1999) (Fig. 2). When overexpressed in *Xenopus* oocytes, $S1P_2$ mobilizes calcium in response to S1P stimulation (An et al. 1997a; Ancellin and Hla 1999). An $S1P_2$-dependent calcium response to S1P was also observed in human and rat cell lines (An et al. 1999). The calcium response was partially PTX-sensitive, indicating that $S1P_2$

coupling to G_i in part mediates this effect. In Jurkat cells overexpressing $S1P_2$, S1P induced activation of a serum response element (SRE) promoter (An et al. 1997a). Activation of the SRE requires the G_i-Ras-Raf-ERK pathway and the GTPase Rho. S1P-induced rounding of HEK293 and PC12 cell lines transfected with $S1P_2$ provided further evidence that $S1P_2$ is coupled to $G_{q/o}$ and $G_{12/13}$ proteins (Van Brocklyn et al. 1999) (Fig. 2).

Three independent groups generated $S1P_2$-deficient mice (Ishii et al. 2002; Kono et al. 2004; MacLennan et al. 2001) (Table 1). $S1P_2$-deficient mice from two groups were found to be viable, fertile, and displayed no obvious defects (Ishii et al. 2002; Kono et al. 2004). However, when mice deficient in both $S1P_2$ and $S1P_3$ were crossed together, the number of pups obtained was dramatically reduced compared to the number of viable pups obtained from wild-type crosses (Ishii et al. 2002; Kono et al. 2004). The high incidence of embryonic death observed in $S1P_2$/$S1P_3$-double-null embryos may be due to endothelial cell abnormalities that lead to hemorrhage (Kono et al. 2004). Interestingly, the loss of $S1P_2$ also leads to an exacerbation of the $S1P_1$-null phenotype. $S1P_1$/$S1P_2$-double-null mice have a vasculature that appears less mature than that observed in $S1P_1$ null mutant mice, and this defect may account for the slightly earlier incidence of embryonic lethality of $S1P_1$/$S1P_2$-null mutant mice compared to mice deficient for $S1P_1$ only (Kono et al. 2004). A few weeks after birth, $S1P_2$-null mice from the third group developed severe and sometimes lethal seizures (MacLennan et al. 2001). Neurons derived from these $S1P_2$-null mutant mice were hyperexcitable in vitro (MacLennan et al. 2001). It is likely that genetic background strain differences or differences in embryonic stem cells used to create the mice account for the differences between the two $S1P_2$-deficient mice.

Mouse embryonic fibroblasts (MEFs) derived from $S1P_2$ null mutant mice proved quite useful in further understanding the role $S1P_2$ plays in S1P signaling. In response to S1P, MEFs derived from $S1P_2$ null mutant mice showed a significant decrease in Rho activation but had relatively normal PLC, calcium mobilization, and AC responses (Ishii et al. 2002).

$S1P_3$

$S1P_3$ was isolated by PCR of human genomic DNA with degenerate primers to cannabinoid receptors (Yamaguchi et al. 1996). In human tissues, high levels of $S1P_3$ RNA are found in the heart, lung, kidney, and pancreas (Yamaguchi et al. 1996). The pattern of tissue expression overlaps with adult mouse tissues, where high $S1P_3$ levels are also seen in the spleen and embryonic brain (McGiffert et al. 2002; Zhang et al. 1999). $S1P_3$ expression during embryonic development was determined using in situ hybridization (McGiffert et al. 2002). In the embryonic brain, $S1P_3$ localizes to the olfactory epithelium, choroids plexus, and developing blood vessels (Ishii et al. 2001; McGiffert et al. 2002). $S1P_3$ RNA was also detected in the embryonic lung, kidney, intestine, submandibular gland, and cartilaginous regions (Ishii et al. 2001; McGiffert et al. 2002; Zhang et al. 1999).

Similar to $S1P_2$, $S1P_3$ was also found to couple to the G_i, G_q, and $G_{12/13}$ G protein families in Sf9 co-expression experiments (Windh et al. 1999) (Fig. 2). When expressed in Jurkat T cells or *Xenopus* oocytes, activation of $S1P_3$ by S1P induces calcium mobilization (An et al. 1997a, 1999; Ancellin and Hla 1999). In rat hepatoma cells overexpressing $S1P_3$, S1P stimulation initiates a PLC-mediated sustained calcium mobilization response and IP_3 production (An et al. 1999). The calcium response and IP_3 production were partially inhibited by PTX, indicating that these responses are mediated in part by coupling to G_i proteins (An et al. 1999). Stable $S1P_3$ expression in these cells also inhibited forskolin-induced cAMP accumulation (An et al. 1999). This AC inhibition was abolished when the

cells were treated with PTX, indicating that this is a G_i-mediated response (An et al. 1999). G_i-mediated coupling of $S1P_3$ to the Ras-Raf-ERK pathway was also demonstrated in Jurkat T cells in response to S1P (An et al. 1997a).

S1P also induces activation of Rho in $S1P_3$-expressing CHO cells, and this activation is thought to mediate CHO cell migration (Paik et al. 2001). Downregulation of $S1P_3$ with antisense oligonucleotides inhibits activation of Rho, and Rho-dependent endothelial cell migration in response to S1P (An et al. 1997a). S1P also induces activation of Rho in $S1P_3$-expressing CHO cells, and this activation is thought to mediate CHO cell migration (Paik et al. 2001).

Similar to mice deficient for $S1P_2$, $S1P_3$-deficient mice displayed no obvious phenotype (Ishii et al. 2001; Kono et al. 2004) (Table 1). Mice deficient for $S1P_3$ are viable, fertile, and develop normally. When $S1P_3$-null mice were bred together, resultant litter sizes were slightly smaller than those obtained from wild-type crosses (Ishii et al. 2001). However, when mice deficient for both $S1P_2$ and $S1P_3$ were bred together, litter sizes were severely reduced (Ishii et al. 2002; Kono et al. 2004) (Table 1). MEFs generated from both $S1P_3$-deficient mice and $S1P_2$/$S1P_3$-double-null mice were used to analyze the roles of these receptors in transducing S1P signals (Ishii et al. 2001, 2002). PLC activation in response to S1P treatment was severely reduced in $S1P_3$-deficient MEFs (Ishii et al. 2001). Residual PLC activity was blocked by PTX treatment. In contrast, when $S1P_3$-deficient MEFs were infected with $S1P_3$-expressing retrovirus, PTX treatment did not block S1P-induced PLC activation (Ishii et al. 2001). This result shows that the S1P-induced PLC activity in MEFs occurs largely through $S1P_3$ and couples to PTX-insensitive G proteins. $S1P_3$-deficient MEFs also have a slight reduction in G_i-mediated inhibition of cAMP accumulation (Ishii et al. 2001). The loss of $S1P_3$ in MEFs did not have an effect on S1P-induced Rho activation (Ishii et al. 2001). However, in MEFs deficient for $S1P_2$, S1P-induced activation of Rho was severely reduced, and in $S1P_2$/$S1P_3$-null MEFs, Rho activation was abolished (Ishii et al. 2002). This indicates that $S1P_3$ has a minor role in Rho activation in MEFs. An increasing number of studies have revealed roles for this receptor in cardiovascular and respiratory systems (Gon et al. 2005; Levkau et al. 2004; Nofer et al. 2004; Sanna et al. 2004; Tolle et al. 2005).

$S1P_4$

$S1P_4$ was originally cloned from in vitro differentiated human dendritic cells using chemokine G-protein-coupled receptor (GPCR) degenerate primers (Graler et al. 1998). The $S1P_4$ receptor expression is unique compared to other S1P receptors, as its expression is confined to lymphoid cells and tissues. High levels of $S1P_4$ RNA are present in the thymus, spleen, lymph nodes, as well as peripheral blood lymphocytes (Contos et al. 2002b; Graler et al. 1998; Ishii et al. 2001). In thymus, $S1P_4$ transcripts are detected in immature CD4 and CD8 double-positive as well as mature single-positive thymoctyes (Matloubian et al. 2004). $S1P_4$ is expressed in an incredibly broad range of lymphoid cells, including $CD4^+$ and $CD8^+$ T cells, B cells, NK cells, dendritic cells, and macrophages (Goetzl et al. 2004; Graeler and Goetzl 2002; Jin et al. 2003; Matloubian et al. 2004; Wang et al. 2004). Although high $S1P_4$ RNA levels are also detected in the lung, it is not clear if this is due to expression in alveolar macrophages (Ishii et al. 2001, 2002; McGiffert et al. 2002; Zhang et al. 1999).

S1P-mediated cell signaling occurs, in part, through G protein subunits G_i and $G_{12/13}$ that have been shown to couple directly to the $S1P_4$ receptor (Graler et al. 2003) (Fig. 2). In

cell lines overexpressing $S1P_4$, PTX-sensitive responses to S1P include activation of PLC, the mitogen-activated protein kinase signal transduction pathway, and the activation of the small Rho family GTPase Cdc42 (Graler et al. 2003; Kohno et al. 2003; Van Brocklyn et al. 2000; Yamazaki et al. 2000). This indicates that G_i coupling to $S1P_4$ mediates all of these responses.

In CHO cells overexpressing $S1P_4$, S1P induces cell rounding, stress fiber formation and cell motility (Graler et al. 2003; Kohno et al. 2003). The S1P-induced migratory response of CHO cells overexpressing $S1P_4$ is abolished by a dominant negative Cdc42 or PTX treatment (Kohno et al. 2003). Interestingly, S1P also induces Rho activation in CHO cells overexpressing $S1P_4$ (Graler et al. 2003). However, it is not clear if Rho activation is required for cell migration, stress fiber formation, and morphological changes in these cells. In response to S1P, $S1P_4$-overexpressing Jurkat T cells also undergo a cell motility response (Graler et al. 2003).

In response to S1P, MEFs infected with $S1P_4$-expressing retrovirus show a robust AC response (Ishii et al. 2001). This is in contrast to $S1P_1$, $S1P_3$, and $S1P_5$, which all inhibit AC activity in response to S1P (Im et al. 2000a, 2001; Ishii et al. 2001; Malek et al. 2001; Niedernberg et al. 2002). It has not been determined which G protein subunit couples with $S1P_4$ to activate this response.

Mice deficient for $S1P_4$ have not yet been reported. However, given the lymphoid-specific expression pattern of this receptor, defects in lymphocyte development and/or function can be expected. Based on expression patterns and experiments done in vitro, it is likely that the loss of $S1P_4$ will have an effect on T cell proliferation, chemotaxis, and immune response localization. In support of this hypothesis, $S1P_1$ and $S1P_4$ are expressed on $CD4^+$ and $CD8^+$ splenic T cells, which undergo chemotaxis in response to S1P (Graeler and Goetzl 2002). RNA and protein levels of these receptors are both downregulated upon treatment with the anti-T cell receptor (TCR) antibodies CD3 and CD28 (Graeler and Goetzl 2002). Downregulation of $S1P_1$ and $S1P_4$ receptors renders the cells unable to undergo a chemotactic response to S1P (Graeler and Goetzl 2002). FTY720, an S1P receptor agonist, also blocks T cell chemotactic responses, downregulates S1P receptor expression, and prevents lymphocytes from exiting peripheral lymphoid organs in vivo (Brinkmann et al. 2000, 2001; Graeler and Goetzl 2002; Mandala et al. 2002). Thus, $S1P_1$ and $S1P_4$ may act to recruit naïve T cells to areas necessary for an immune response, whereas activated T cells may be retained in the same areas due to a decreased chemotactic response to S1P.

$S1P_1$ and $S1P_4$ are also expressed on $CD4^+CD25^+$ regulatory T cells (Wang et al. 2004). In response to S1P treatment, these cells decrease the ability of naïve $CD4^+$ T cells to produce IL-2 and undergo proliferation (Wang et al. 2004). The inhibitory effects of S1P on regulatory T cells are also blocked by FTY720 treatment, indicating that S1P receptors are responsible for this effect (Wang et al. 2004). In addition S1P can inhibit T cell proliferation in response to PMA and ionomycin treatment, anti-CD3 plus anti-CD8 antibodies, and dendritic cell stimulation (Jin et al. 2003).

One major caveat to these experiments is that FTY720 does not appear to downregulate $S1P_4$ expression, indicating that chemotactic and immunoregulatory responses are mediated by $S1P_1$ and not $S1P_4$ (Fig. 1) (Wang et al. 2004). Furthermore, chemotaxis in response to S1P occurs in a rat hepatoma cell line when $S1P_1$ is overexpressed but not $S1P_4$ (Graeler and Goetzl 2002). The generation of $S1P_4$ null mice will help to determine whether $S1P_4$ has a role of mediating these responses in vivo.

$S1P_5$

The $S1P_5$ receptor was identified in expressed sequence tag data bases and cloned from rat cDNA (Glickman et al. 1999; Im et al. 2000a). This gene has a relatively restricted expression pattern with high RNA levels present in the spleen, skin, lung, and brain (Glickman et al. 1999; Im et al. 2000a, 2001; Ishii et al. 2001, 2002; Niedernberg et al. 2002). Northern blot analysis showed that $S1P_5$ transcripts are highly expressed in a number of brain regions (Glickman et al. 1999; Im et al. 2000a, 2001). In situ hybridization further localized $S1P_5$ expression to white matter tracts throughout the brain at every stage of oligodendrocyte differentiation (Im et al. 2000a; Jaillard et al. 2005; Terai et al. 2003; Yu et al. 2004). This expression pattern suggests that $S1P_5$ plays a role in myelination.

The $S1P_5$ receptor has been found to couple to G_i and $G_{12/13}$ proteins (Fig. 2). In several experiments, expression of $S1P_5$ in cell lines was shown to inhibit AC activity in response to S1P through a G_i regulated pathway (Im et al. 2000a, 2001; Malek et al. 2001; Niedernberg et al. 2002). In addition, S1P stimulation of the $S1P_5$ receptor repressed ERK activation while inducing c-Jun NH_2-terminal kinase (JNK) activation (Malek et al. 2001). It is not known at the present time how the activation states of ERK and JNK are regulated by $S1P_5$. Serum-stimulated proliferation of $S1P_5$ overexpressing CHO cells was inhibited with S1P treatment (Malek et al. 2001; Niedernberg et al. 2002).

Although $S1P_5$ is expressed at all stages of oligodendrocyte development, mice deficient for $S1P_5$ do not display obvious myelination abnormalities, and they have no other apparent phenotypic defects (Table 1) (Jaillard et al. 2005). However, Rho kinase-dependent process retraction in response to S1P was abolished in cultured preoligodendrocytes derived from S1P-null mutant mice (Jaillard et al. 2005). At more mature stages of oligodendrocyte development, repression of $S1P_5$ expression by siRNAs negated the ability of S1P to act as a survival factor in response to differentiation signals in vitro (Jaillard et al. 2005). Interestingly, S1P treatment of mature oligodendrocytes induces phosphorylation of AKT (Jaillard et al. 2005). The ability of S1P to protect mature oligodendrocytes from cell death and to promote AKT phosphorylation is diminished upon PTX treatment (Jaillard et al. 2005). It should be noted that oligodendrocyte survival experiments were not done with oligodendroctyes cultured from $S1P_5$-deficient mice. It is possible that functional redundancies with other lysophospholipid receptors account for the absence of myelination defects in $S1P_5$-deficient mice. The generation of $S1P_5$-deficient mice crossed with mice null for other lysophospholipid receptors will help further our understanding of the role lysophospholipids play in the myelination process.

LPA receptors

There are four known LPA receptors that mediate LPA signaling in a wide variety of tissues and cell types. These receptors are designated LPA_1, LPA_2, LPA_3, and LPA_4. The numbering system reflects their initial order in the literature.

LPA_1

The LPA_1 receptor was the first lysophospholipid receptor identified. It was cloned from neural progenitor cell lines using degenerate primers designed against transmembrane do-

mains in G protein-coupled receptors (Hecht et al. 1996). In adult mouse tissues, high levels of RNA are also detected in the lung and testis (Contos et al. 2002a; Hecht et al. 1996). The LPA_1 receptor also has a biphasic expression pattern in the developing brain. LPA_1 levels are high in the developing cerebral cortex throughout development of the embryonic brain followed by a decrease in expression at or near birth, then reemergence of gene expression soon after birth (Hecht et al. 1996; Weiner et al. 1998). In situ hybridization experiments show that LPA_1 expression is localized to proliferative zones of the embryonic cerebral cortex (Hecht et al. 1996). LPA_1 expression in the postnatal brain correlates with white matter tracts (Weiner et al. 1998). In situ hybridization experiments with oligodendrocyte-specific and LPA_1 probes shows that LPA_1 is expressed in oligodendrocytes during a period that coincides with the onset of myelination (Weiner et al. 1998). LPA_1 transcripts are also present in Schwann cells (Weiner and Chun 1999).

LPA exerts diverse effects on a variety of cell types through several signal transduction pathways (Fig. 3). For example, LPA inhibits AC activation in neuronal cell lines overexpressing LPA_1 (Ishii et al. 2000). This response is inhibited by PTX treatment, indicating that LPA_1 is coupled to G_i proteins (Ishii et al. 2000). LPA_1-overexpressing cells also activate a SRE element reporter and MAPK in response to LPA (An et al. 1997b; Fukushima et al. 1998; Ishii et al. 2000). However, this response is sensitive to PTX and C3 exoenzyme, indicating that G_i proteins and the GTPase Rho mediate this effect (Fukushima et al. 1998; Ishii et al. 2000). Overexpression of LPA_1 in cell lines also leads to Rho-dependent morphological changes such as stress fiber formation, neurite retraction, and cell rounding in response to LPA (Fukushima et al. 1998; Ishii et al. 2000). In addition to G_i and Rho-mediated responses, LPA can activate PLC in LPA_1-overexpressing cells (Ishii et al. 2000). The role of LPA_1 in these signaling pathways was confirmed in MEFs derived from LPA_1-null mutant mice (Contos et al. 2002a). In addition to decreases in other responses that will be discussed below, MEFs from LPA_1-deficient mice displayed a marked reduction in PLC activation, and a minimal inhibition of forskolin-induced cAMP accumulation in response to LPA (Contos et al. 2002a).

The importance of the LPA_1 gene in mammalian development is evident by the reduced viability of LPA_1-null mutant mice (Contos et al. 2002a) (Table. 1). The loss of LPA_1 in mice results in an approximately 50% reduction of the expected number of pups homozygous for the LPA_1 mutant allele. The majority of LPA_1 null mutant mice die between birth and three weeks of age. One reason for this reduced viability is an abnormal suckling behavior that appears to be due to an olfaction defect. LPA_1-null mutant mice that survive to adulthood have craniofacial defects that include flattened faces, shortened snouts, an increased distance between the eyes and reduced body mass. Some LPA_1-deficient embryonic and neonatal mice also have frontal hematomas or exencephaly.

In agreement with the finding that LPA is a Schwann cell survival factor, an increased number of apoptotic Schwann cells are present in the sciatic nerve of LPA_1-deficient mice (Contos et al. 2002a; Weiner and Chun 1999). In contrast to Schwann cells derived from wild-type mice, Schwann cells from LPA_1-deficient mice fail to undergo normal actin rearrangements in response to LPA treatment (Weiner et al. 2001). LPA_1 was originally identified as a gene present in the ventricular zone of the cerebral cortex (Hecht et al. 1996). LPA treatment induces morphological changes and enhances the proliferation of neuroblasts derived from this region (Contos et al. 2000; Fukushima et al. 2000). In response to LPA, cortical neuroblast cultures derived from LPA_1-deficient mice fail to undergo LPA-dependent cell rounding and compaction and show slightly reduced proliferation compared to cultures derived from wild-type mice (Contos et al. 2000). Elegant experiments conducted using ex vivo cultured mouse embryonic cortices showed that LPA induces dramatic folding

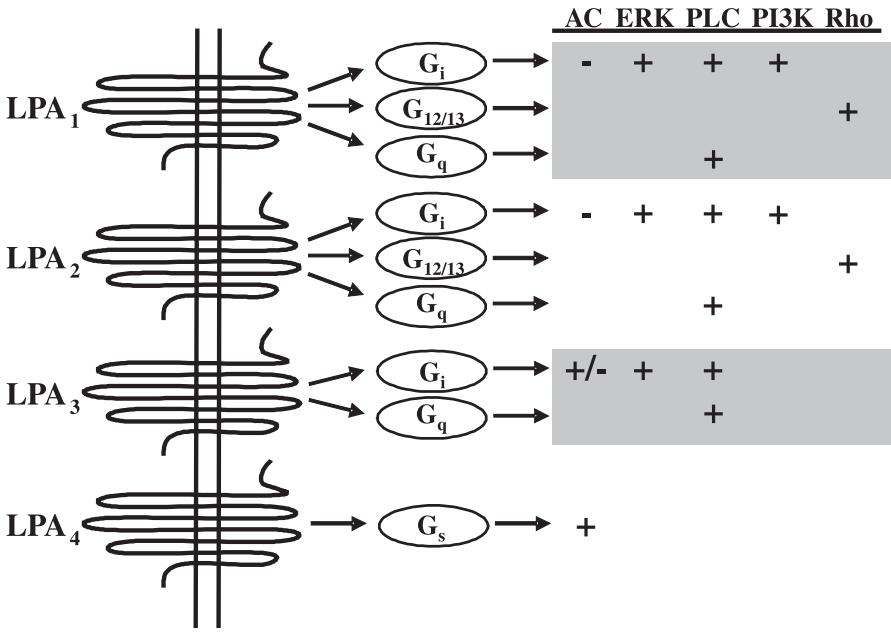

Fig. 3 Signaling pathways regulated by the LPA family of G-protein coupled receptors. Shown are the four known LPA receptors and the G proteins coupled to them. Cell signaling pathways downstream of receptor signaling are indicated. *AC* adenylyl cyclase, *ERK* mitogen-activated protein kinase, *PLC* phospholipase C, *PI3K* phosphoinositol 3-kinase

and thickening of the developing cerebral cortex (Kingsbury et al. 2003). This remarkable morphological change is due to an LPA-dependent decrease in cell death and increased terminal mitosis of neural progenitor cells (Kingsbury et al. 2003). These changes are not apparent in LPA treated cortices of mice deficient for both LPA_1 and LPA_2 (Kingsbury et al. 2003), demonstrating the necessity for these two receptors in this response.

MEFs isolated from LPA_1-null mutant mice also display abnormal responses to LPA signaling (Contos et al. 2002a). When LPA_1 is overexpressed in cell lines, LPA can activate PLC, inhibit AC activity, and induce morphological changes such as cell rounding (Fukushima et al. 1998; Hecht et al. 1996; Ishii et al. 2000). LPA_1-deficient MEFs show a substantial but incomplete loss of PLC activation, and a near complete loss of AC inhibition when treated with LPA (Contos et al. 2002a). These data argue that other LPA receptors contribute to LPA-dependent activation of PLC and that LPA_1 is the only LPA receptor involved in AC inhibition in MEFs. Stress fiber formation in response to LPA treatment is also reduced in MEFs derived from the meninges of LPA_1 knockout mice as compared to wild-type controls (Contos et al. 2002a). LPA_1 mutant mice have been extremely useful in demonstrating the importance of this receptor in mammalian development and cell function (Table 1). In addition, these mice have indicated that other lysophospholipid receptors are likely important for Schwann cell development, cortical neuroblast development, and cellular responses to LPA signaling.

LPA_2

The LPA_2 receptor was discovered in a search for genes similar to LPA_1, although the first published sequence contained a non-wild-type mutation in the carboxy terminus (An et al. 1998). In contrast to LPA_1, LPA_2 has a more restricted pattern of expression. LPA_2 RNA levels are high in adult human peripheral blood lymphocytes, thymus, spleen, and testis (An et al. 1998). In mice, LPA_2 RNA transcripts are abundant in testis, kidney, $CD4^+$ and $CD8^+$ T cells, and B cells (Contos et al. 2002a; Goetzl et al. 2004).

Similar to LPA_1, overexpression of LPA_2 in cell lines leads to an inhibition of AC activity in response to LPA treatment (Ishii et al. 2000). Like LPA_1, AC activation is sensitive to PTX treatment, indicating that this response occurs through a G_i-mediated pathway (Ishii et al. 2000) (Fig. 3). Also, similar to LPA_1 activation, LPA appears to induce activation of the SRE in cell lines overexpressing LPA_2, and this response is partially inhibited by both PTX and C3 exoenzyme, suggesting mediation of the response by both G_i- and Rho-dependent pathways (An et al. 1998) (Fig. 3). In neuroblastoma cell lines infected with LPA_2 expressing virus, LPA causes Rho-dependent cell rounding and processes retraction (Ishii et al. 2000). LPA signaling through LPA_2 also activates PLC (Ishii et al. 2000) (Fig. 3).

Since MEFs express LPA_1 and LPA_2 but not LPA_3, MEFs deficient for LPA_2 were useful in helping to determine the extent that LPA signaling through LPA_2 receptor contributes to these responses in vivo (Contos et al. 2002a). For example, LPA_2-deficient MEFs have a greater reduction in PLC activation than MEFs deficient for LPA_1 (Contos et al. 2002a). However, PLC activation in response to LPA signaling is nearly abolished in LPA_1/LPA_2 double knockout MEFs (Contos et al. 2002a). This result shows that both LPA_1 and LPA_2 receptors play a role in PLC activation but that PLC is activated by LPA_2 to a greater extent in MEFs. While overexpression of LPA_2 in cell lines can result in an inhibition of AC, the loss of LPA_2 in MEFs does not have a major effect on AC activity (Contos et al. 2002a; Ishii et al. 2000). AC activity was not further inhibited in double knockout MEFs, nor was it activated in double knockout MEFs infected with LPA_2-expressing retrovirus (Contos et al. 2002a). These data show that in MEFs, the LPA receptor responsible for LPA-mediated inhibition of AC is LPA_1. LPA_1 and LPA_2 were found to have redundant roles in LPA-induced stress fiber formation, as well as activation of JNK, AKT, and MAPK (Contos et al. 2002a).

Mice deficient for LPA_2 have no obvious phenotype (Contos et al. 2002a) (Table 1), and mice deficient for both LPA_1 and LPA_2 display the same abnormalities as LPA_1 deficient mice except for an increase in the incidence of frontal hematomas and a slight decrease in survival rate (Contos et al. 2002a).

LPA_3

The gene for LPA_3 was cloned using two different PCR approaches. Reverse transcription was used to amplify a cDNA sequence with similarities to LPA_1 and LPA_2 in one approach, while PCR with degenerate primers to LPA_1 and LPA_2 transmembrane domains was used in the other (Bandoh et al. 1999; Im et al. 2000b).

High LPA_3 RNA levels are found in the heart, pancreas, prostate, testis, and brain in humans (Bandoh et al. 1999; Im et al. 2000b). An overlapping expression pattern is observed in rodent tissues (Contos and Chun 2001; Im et al. 2000b). In the mouse brain, postnatal expression of LPA_3 is higher than that observed in embryonic development (Contos and Chun 2001).

LPA-mediated stimulation of LPA_3 activates signal transduction pathways that both overlap and are distinct from those activated by LPA_1 and LPA_2 (Table 1). While LPA_1 and LPA_2 mediate LPA-induced cell rounding and processes retraction, LPA_3 inhibits cell rounding and causes neurites to elongate in the B103 neuroblastoma cell line (Ishii et al. 2000). Like LPA_1 and LPA_2, LPA_3 activation causes an upregulation of IP_3 production, an inhibition of AC activity, and an activation of MAP kinase when overexpressed in B103 cells (Ishii et al. 2000). AC inhibition, and MAP kinase activation are both sensitive to PTX treatment (Ishii et al. 2000). Previous reports demonstrated that overexpression of LPA_3 in cell lines or *Xenopus* oocytes activates a calcium response in response to LPA treatment (Bandoh et al. 1999; Im et al. 2000b). This is consistent with LPA_3 coupling to G_q and activating PLC and IP_3 accumulation (Fig. 3). The PTX sensitivity of AC inhibition and MAP kinase activation indicates that LPA_3 couples to G_i proteins to mediate these effects (Ishii et al. 2000). It should be noted that forskolin-induced cAMP accumulation is not affected in Sf9 insect cells or RH7777 cells overexpressing LPA_3 (Bandoh et al. 1999). This difference may reflect different downstream signaling components or signaling properties in these cells.

Mice deficient for LPA_3 have recently been generated and show a surprising phenotype (Ye et al. 2005) (Table1). LPA_3-null mutant females have reduced litter sizes compared to wild-type and LPA_3 heterozygous females. When the uteri of pregnant LPA_3 deficient mice were analyzed, it was discovered that the number of implantation sites was reduced and there were multiple embryos per placenta. The implantation sites that were present were predominantly located proximal to the cervix. On average, the embryos recovered from these implantation sites were smaller in size relative to embryos isolated from wild-type female mice. Pregnancy was slightly prolonged in LPA_3-deficient mice, and although the number of newborn pups was reduced, they were slightly larger than wild-type pups of the same age.

LPA_3 expression is upregulated in the luminal endometrial epithelium during a period critical for implantation and the data suggest that maternal LPA_3 signaling is crucial for proper embryo implantation and spacing (Ye et al. 2005). These abnormalities are similar to those observed in mice deficient for phospholipase $A_{2\alpha}$, cyclooxygenase 2 (COX2), and mice treated with indomethacin, an inhibitor of cyclooxygenases (COXs) (Frenkian et al. 2001; Kennedy 1977; Kinoshita et al. 1985; Lim et al. 1997; Reese et al. 1999; Song et al. 2002). Cytoplasmic phospholipase $A_{2\alpha}$ ($cPLA_{2\alpha}$) is involved in arachidonic acid synthesis, while COX proteins are involved in the conversion of arachidonic acid to prostaglandins. Thus, LPA signaling through LPA_3 appears to regulate prostaglandin synthesis (Fig. 4). In support of this model, COX2 expression, as well as prostaglandin synthesis, is reduced in the uteri of LPA_3-deficient mice (Ye et al. 2005). Administration of exogenous prostaglandins partially rescues implantation abnormalities in mice lacking LPA_3 (Ye et al. 2005). In support of a role for LPA signaling in the regulation of COX2, it was recently reported that LPA induces COX2 expression in ovarian cancer cell lines (Symowicz et al. 2005).

LPA_4

A novel LPA receptor has recently been identified in a project to de-orphan known G protein-coupled receptors (Noguchi et al. 2003). This receptor previously known as p2y/GPR23, is the most divergent member of the LPA receptor family and shares only 20–24% amino acid identity with all known LPA receptors (Noguchi et al. 2003). LPA_4 is expressed predominantly in the ovary and moderately in the thymus (Noguchi et al. 2003). CHO cells stably expressing LPA_4 accumulate cAMP in response to LPA treatment either in the presence or absence of forskolin (Noguchi et al. 2003). PTX treatment does not attenuate the cAMP re-

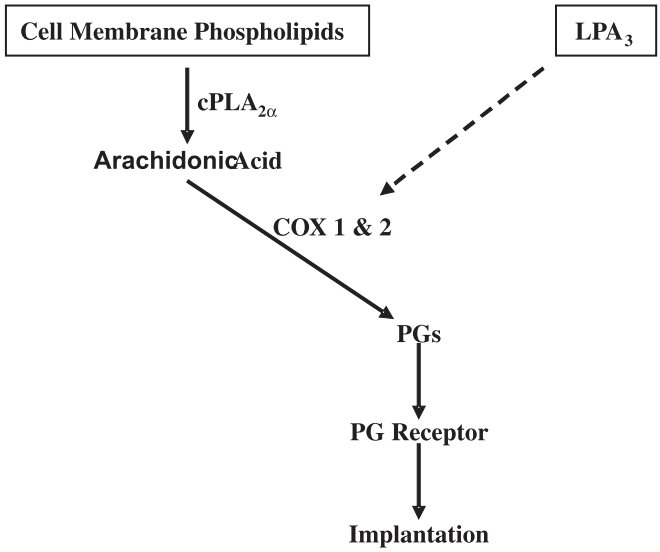

Fig. 4 Model for LPA_3 in the regulation of prostaglandin synthesis. LPA_3 signaling upregulates COX expression, leading to the formation of prostaglandins (PGs) from arachidonic acid. PGs bind to receptors in the epithelium and provide signals for implantation

sponse, indicating that this pathway is mediated by LPA_4 coupling to G_s proteins, although it is likely that other G proteins are activated as well (Noguchi et al. 2003) (Fig. 3). LPA also induces a dose-dependent increase in intracellular calcium levels in CHO cells stably expressing LPA_4.

A more extensive analysis of this receptor and signal transduction pathways downstream of this receptor is needed to provide further insight into the function of this receptor in vivo.

Conclusions and future prospects

The identification and characterization of lysophospholipid receptors has provided new insights into our understanding of how lysophospholipids exert their biological effects. In addition, mice deficient for specific lysophospholipid receptors have provided important clues about the function and significance of lysophospholipid signaling in vivo. However, there are still many questions left to answer. For example, functional redundancies between receptors likely exist and the generation of mice deficient for multiple lysophospholipid receptors will help determine if this is indeed the case. Also, given the overlapping expression pattern of many lysophospholipid receptors, combinatorial deletions could elucidate how these receptors might cooperate in physiological and pathophysiological conditions. Furthermore, receptor-specific agonists and antagonists that are currently being developed will be useful in determining the functional properties of lysophospholipid receptors in vitro and in vivo, particularly when combined with defined receptor null animals. The coming years should see an increasing depth of basic and medicinal biology related to the signaling of this novel group of simple lipids.

Acknowledgements. We thank Brigitte Anliker and Christine Paczkowski for critical reading of the manuscript. This work was supported by NIH grants MH51699, MH01723, and NS048478 to J.C. from the National Institute of Mental Health and the National Institute of Neurological Disorders and Stroke.

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