

# LYSOPHOSPHOLIPID RECEPTORS

---

Nobuyuki Fukushima, Isao Ishii, James JA Contos,  
Joshua A Weiner, Jerold Chun

Neuroscience Program, Department of Pharmacology, University of California,  
San Diego, La Jolla, California 92093-0636; e-mail: [nfukushima@ucsd.edu](mailto:nfukushima@ucsd.edu),  
[iishii@ucsd.edu](mailto:iishii@ucsd.edu), [jcontos@ucsd.edu](mailto:jcontos@ucsd.edu), [weinerj@pcg.wustl.edu](mailto:weinerj@pcg.wustl.edu), [jchun@ucsd.edu](mailto:jchun@ucsd.edu)

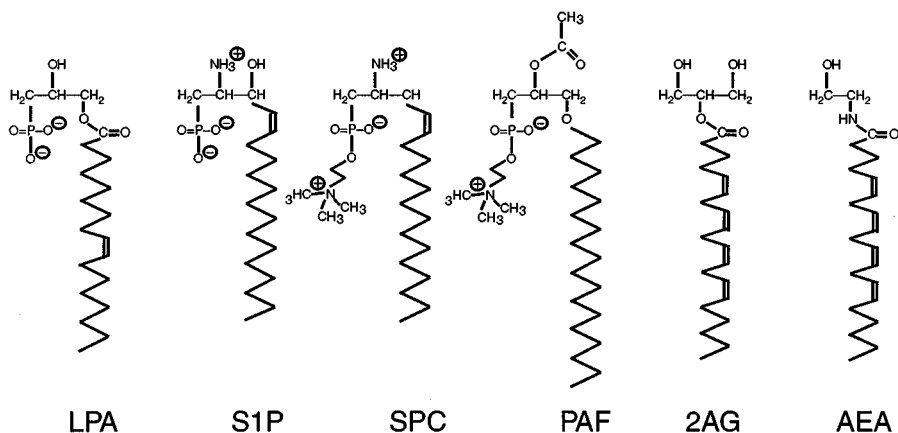
**Key Words** lysophosphatidic acid, sphingosine 1-phosphate, G protein-coupled receptor, nervous system, development

■ **Abstract** Lysophospholipids (LPs), including lysophosphatidic acid and sphingosine 1-phosphate, produce many cellular effects. However, the prolonged absence of any cloned and identified LP receptor has left open the question of how these lipids actually bring about these effects. The cloning and functional identification of the first LP receptor, *lp<sub>AI</sub>/vzg-1*, has led rapidly to the identification and classification of multiple orphan receptors/expression sequence tags known by many names (e.g. *edg*, *mrecl.3*, *gpcr26*, *H218*, *AGR16*, *nrg-1*) as members of a common cognate G protein-coupled receptor family. We review features of the LP receptor family, including molecular characteristics, genomics, signaling properties, and gene expression. A major question for which only partial answers are available concerns the biological significance of receptor-mediated LP signaling. Recent studies that demonstrate the role of receptor-mediated LP signaling in the nervous system, cardiovascular system, and other organ systems indicate the importance of this signaling in development, function, and pathophysiology and portend an exciting time ahead for this growing field.

## INTRODUCTION

Lysophospholipids (LPs) have been long considered to be metabolites in the biosynthesis of membrane phospholipids. Two prominent examples of LPs are lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) (see Figure 1 for chemical structures of LPs and related lipids). In addition to their metabolic roles, however, both of these structurally related LPs also have cell-signaling properties, producing similar types of responses in a range of cells. Some of the earliest examples of potential LP signaling were reported in the 1960s when LPA was found to induce intestinal contraction (1).

In early mechanistic studies, LPs were postulated to act primarily as intracellular second messengers, as suggested for LPA (2-5) or based upon intrinsic chemical



**Figure 1** Chemical structures of signaling lysophospholipids and related lipids. LPA, lysophosphatidic acid; S1P, sphingosine 1-phosphate; SPC, sphingosylphosphorylcholine; PAF, platelet-activating factor; 2AG, 2-arachidonyl glycerol; AEA, arachidonyl ethanolamide.

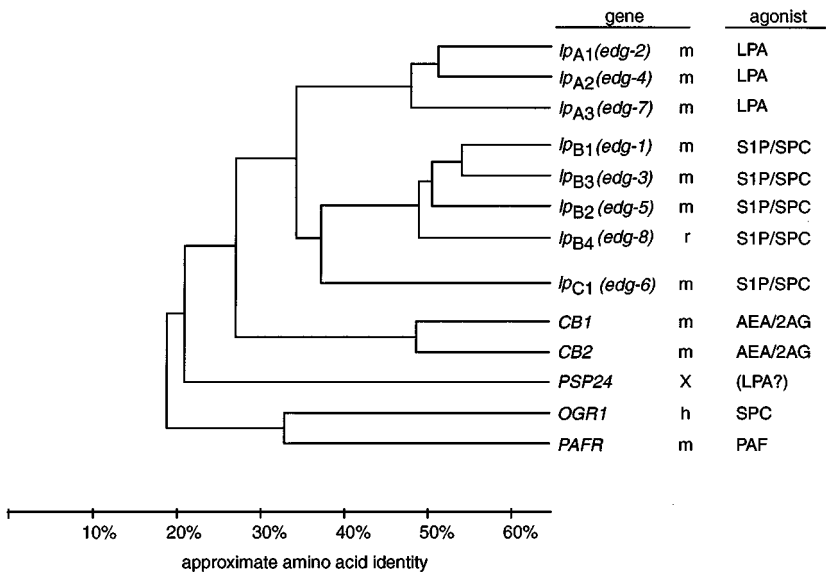
properties (e.g.  $\text{Ca}^{2+}$ -binding activity of LPA) (2, 6). Just as with LPA, a mechanism postulated to be responsible for S1P effects included second-messenger roles (5, 7).

However, a seminal study by van Corven et al demonstrated that the proliferative effects of LPA in fibroblasts were mediated by G proteins (8). This finding raised the probability that a cognate G protein-coupled receptor (GPCR) could exist, which would account for many of the diverse cellular effects induced by LPA in many types of cells (9, 10). Similarly, exogenous application of S1P also was shown to result in various cellular responses (11–15) that implicated GPCR-based mechanisms (13, 14). In addition, the similarity of the cellular responses documented for both LPA and S1P suggested that these two LPs might even share the same GPCRs (16–18).

However, despite circumstantial data supporting the existence of LPA or S1P GPCRs (17, 19, 20), cloned LP receptors defied identification. This failure was in part a reflection of the lipophilic nature of the ligands, which complicated standard approaches such as expression cloning. The absence of cloned receptors was compounded by an absence of any specific competitive antagonists to block LP-dependent responses, leaving open alternative possibilities to explain these responses.

Identification of the first LP receptor gene, *ventricular zone gene 1* (*vzg-1*), was reported in 1996 during studies on mammalian neurogenesis in which a cloned orphan receptor was isolated by virtue of its expression in the neurogenic zone of the embryonic cerebral cortex (21–23). *vzg-1* encoded a GPCR that had the properties of a high-affinity LPA receptor. Based on the nucleotide sequence of *vzg-1*, it immediately became apparent that several orphan receptor genes or expression sequence tags (ESTs) in DNA sequence databases most certainly

interacted with LPA or similar LP ligands such as S1P—particularly in view of the above-mentioned studies suggesting a common receptor for these two lipids. Following identification of the first LPA receptor, the first S1P receptor was identified independently by both Zondag et al (24) and Lee et al (25) in 1998. Subsequent studies on homologous genes by many groups (noted below) have revealed the existence of a gene family of LP receptors with a high degree of predicted amino acid similarity (22, 23). Based on sequence similarity and genomic structures, this GPCR gene family consists of at least two major subgroups (22, 23). One group consists of the LPA receptor subfamily (commonly referred to by an orphan receptor gene nomenclature: *vzg-1*/endothelial differentiation gene, *edg* (*edg-2/mrec1.3*, *edg-4*, and *edg-7*), whereas the other group (*edg-1*, *edg-3*, *H218/AGR16/edg-5*, and *nrg-1/edg-8*) represents the receptor subfamily for S1P (Figure 2, Table 1). We currently use a nomenclature based on receptor function in which LPA receptors are termed LP receptors of an “A” group consisting of three known members ( $LP_{A1}$ ,  $LP_{A2}$ , and  $LP_{A3}$ ) and a “B” group consisting of S1P receptors ( $LP_{B1}$ ,  $LP_{B2}$ ,  $LP_{B3}$ , and  $LP_{B4}$ ; Figure 2, Table 1). The subscript numbers indicate the order in which function was reported. (22, 23, 26). Another related receptor gene, *edg-6*, also has been shown to mediate S1P responses (27, 28). However, based on its



**Figure 2** Divergence of the lysophospholipid and related lipid receptor gene families. *CB*, cannabinoid receptor; *PAFR*, PAF receptor; m, mouse; r, rat; X, *Xenopus*; h, human. The *edg-4* sequences are nonidentical with *lpA2*, and sequence similarities were calculated based on *lpA2* sequences (see text for details). The closest branch point between two genes is located at the point on the horizontal axis corresponding to the approximate percentage of amino acid identity between the encoded proteins.

**TABLE 1** Characteristics of lysophospholipid receptors

Name (gene)	Synonyms	Chromosomal location	Coupled G proteins	Cellular function
LP <sub>A1</sub> ( <i>lp<sub>A1</sub></i> )	VZG-1 MREC1.3 GPCR26 EDG-2	Mouse 4, close to <i>vc</i> Human 9q31.3-32	G <sub>i/o</sub> G <sub>q/11/14</sub> G <sub>12/13</sub>	AC inhibition SRE activation DNA synthesis PLC activation Actomyosin stimulation
LP <sub>A2</sub> ( <i>lp<sub>A2</sub></i> )	Nonmutated EDG-4	Mouse 8, close to <i>kat</i> Human 19p12	G <sub>i/o</sub> G <sub>q/11/14</sub> G <sub>12/13</sub>	AC inhibition MAP kinase activation SRE activation PLC activation Actomyosin stimulation
LP <sub>A3</sub> ( <i>lp<sub>A3</sub></i> )	EDG-7 (EDG-5)	Mouse 3, close to <i>va</i> Human 1	G <sub>i/o</sub> G <sub>q/11/14</sub>	AC stimulation or inhibition MAP kinase activation PLC activation
LP <sub>B1</sub> ( <i>lp<sub>B1</sub></i> )	EDG-1	Mouse 3	G <sub>i/o</sub>	AC inhibition MAP kinase activation PLC activation
LP <sub>B2</sub> ( <i>lp<sub>B2</sub></i> )	AGR16 H218 EDG-5	Mouse 9 Rat 8, close to <i>lp<sub>B4</sub></i>	G <sub>i/o</sub> G <sub>q/11/14</sub> G <sub>12/13</sub>	AC stimulation MAP kinase activation SRE activation PLC activation Actomyosin stimulation
LP <sub>B3</sub> ( <i>lp<sub>B3</sub></i> )	EDG-3	Mouse 13 Human 9q22.1	G <sub>i/o</sub> G <sub>q/11/14</sub> G <sub>12/13</sub>	AC inhibition/ stimulation MAP kinase activation SRE activation PLC activation
LP <sub>B4</sub> ( <i>lp<sub>B4</sub></i> )	NRG-1 EDG-8	Rat 8, close to <i>lp<sub>B2</sub></i>	G <sub>i/o</sub>	AC inhibition
LP <sub>C1</sub> ( <i>lp<sub>C1</sub></i> )	EDG-6	Mouse 10 Human 19p13.3	G <sub>i/o</sub>	PLC activation MAP kinase activation

AC, adenyl cyclase; SRE, serum response element; PLC, phospholipase C.

distinct amino acid residues, this gene may warrant classification as a member of a third C group (LP<sub>C1</sub>) or as a dissimilar member of the B group. This nomenclature also avoids confusion over names shared by nonreceptor molecules [e.g. *edg-3*, (29)].

In addition to the LP receptor family, two other receptors are notable. A reported *Xenopus* LPA receptor called PSP24 has low sequence similarity to LP

receptors (30; Figure 2). It also has low sequence similarity to another, distinct receptor that binds another phospholipid-signaling molecule, platelet-activating-factor (PAF), despite use of cloning strategies based on PAF receptor homology (30). It currently remains unclear whether PSP24 is an LPA receptor (30a), and for this reason it will not be discussed further in this review. Of more interest to this discussion of LP receptors is a receptor with some sequence similarity to the PAF receptor that recently has been identified and referred to as OGR1 (31). OGR1 appears to be a cognate GPCR for sphingosylphosphorylcholine [SPC (32; Figure 1)]. Here we review what is known about the LP receptor family. Aspects of their predicted amino acid sequence, genomic structure, expression patterns, and signaling properties are considered. Biological roles of receptor-mediated LP signaling are also discussed. Glimpses of these roles have come from more recent studies, and pertinent examples will be noted. The historical background for LPA-, S1P-, and SPC-induced cellular responses has been extensively examined in many excellent reviews (5, 9, 10, 33, 34) and will not be covered here.

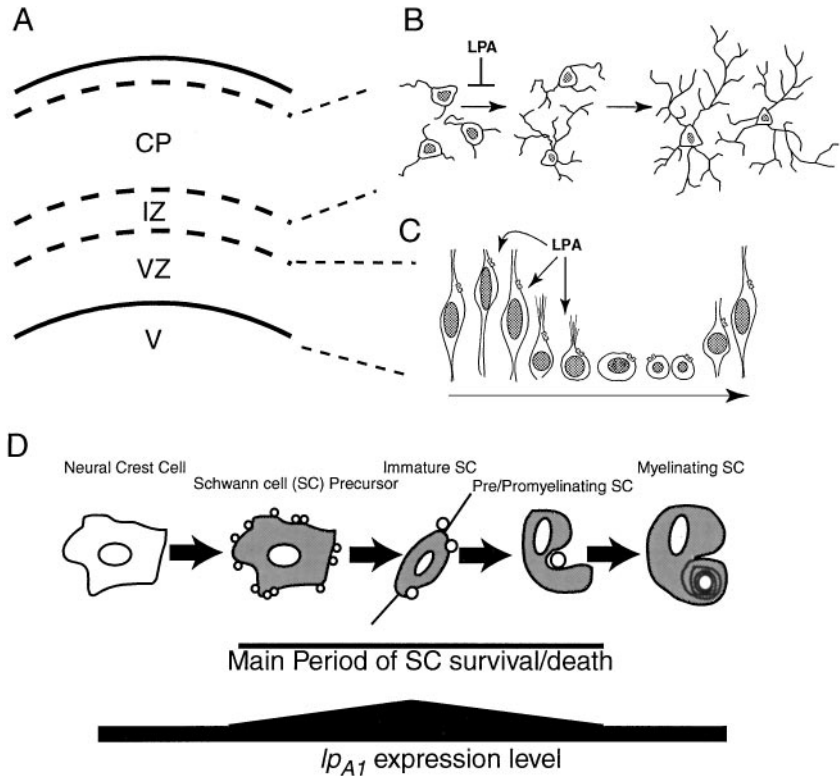
## LYSOPHOSPHATIDIC ACID RECEPTORS

### *lp<sub>A1</sub>/vzg-1/mrec1.3/edg-2*

The first LPA receptor gene was identified during developmental studies of the mouse cerebral cortex in which experiments were designed to identify novel GPCR genes associated with the production of neurons (21–23). The search for novel GPCRs associated with neurogenesis was based on the existence of G proteins found during embryonic development and the clear role of such receptors in cell proliferation and differentiation in other organisms (35, 36). GPCR gene fragments were amplified by degenerate polymerase chain reaction (PCR) of cDNAs from immortalized mouse cortical neuroblast cell lines (37). The resulting products were used for in situ hybridization screening to identify genes with enriched expression within the embryonic cortex, particularly within neurogenic regions [ventricular zone or VZ (Figure 3A)]. This analysis led to the cloning of a then novel orphan receptor gene, *vzg-1*, so named because of its enriched expression pattern within the VZ of the embryonic cerebral cortex (21; reviewed in 22, 23). This name has since been replaced by its functional nomenclature name, *lp<sub>A1</sub>*.

Analyses of the obtained mouse cDNA revealed that it encoded a 41-kDa protein consisting of 364 amino acids with seven transmembrane domains. It shared homology with two known receptors, the cannabinoid and melanocortin receptors (30% and 32% amino acid identity, respectively). The greatest similarity was shared with an orphan receptor cloned from human endothelial cells called *edg-1* [37% amino acid identity (Figure 2)] (38).

Subsequent analyses of mouse *lp<sub>A1</sub>* genomic structure revealed that the gene spans >45 kilobases (kb) and contains five exons with one intron located within the middle of transmembrane domain VI, which, to date, appears to be a feature unique to these LPA receptor genes (23, 39). Alternative use of an exon also produces



**Figure 3** Background on development of cerebral cortical neuroblasts and Schwann cells. (A) Anatomical zones in the embryonic cerebral cortex. CP, cortical plate; IZ, intermediate zone; VZ, ventricular zone; V, lateral ventricle. (B) Differentiating neurons undergoing process outgrowth in the CP. LPA may alter process formation and/or growth. (C) Neuroblasts undergoing morphological changes associated with cell-cycle progression (known as "interkinetic nuclear migration") in the VZ. LPA actions may be involved in this phenomenon. (D) Schwann cell development. As Schwann cells differentiate to form myelin contacts with axons, supernumerary cells are removed by apoptosis. Temporal expression of  $lp_{A1}$  corresponds with this period.

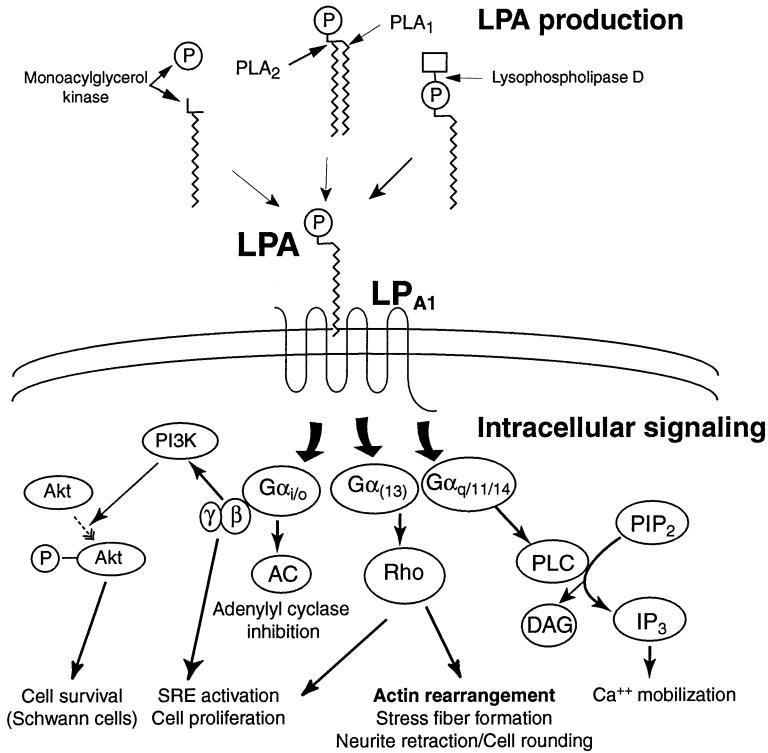
another variant of this receptor, *mrec1.3*; the name reflects its first report as an orphan GPCR gene (40). This isotype lacks 18 amino acids in the amino-terminal region. The  $lp_{A1}$  gene is located on mouse chromosome 4 for most of the mouse strains examined, with an additional, partial duplication of the locus present in *Mus spretus* on chromosome 6 (39). The location of  $lp_{A1}$  on chromosome 4 maps near to a gene called *vacillans* (*vc*). This extinct mutant mouse showed nervous system defects (41; for more about this relationship, see below).

In our initial studies using gene overexpression in cortical neuroblast cell lines, from which  $lp_{A1}$  was isolated, we concluded that  $LP_{A1}$  was a functional LPA

receptor that mediated the inhibition of cyclic AMP (cAMP) production and the stimulation of cell rounding (21). These overexpression studies included analyses of endogenous receptor protein expression and also utilized an extensive repertoire of independent analyses. Although somewhat surprising skepticism over this identity persisted (42, 43), further analyses have eliminated the few remaining doubts.

A key part of such analyses of LPA receptors was the development of a heterologous expression system based on identification of two mammalian cell lines that did not express  $lp_{A1}$  or respond to LPA (44). These cell lines were derived from two distinct cellular lineages—hepatoma (RH7777) and neuroblastoma cells (B103)—and are now in common use in this field (45, 46). Because of the lipophilic nature of LPA, which precludes detailed approaches to receptor–ligand-binding studies, several alternative strategies were developed that reliably assessed LPA-dependent receptor activation. These strategies were based on rapidly occurring, cell biological changes (induction of cell rounding in B103 and stress fiber formation in RH7777) and [ $^{35}$ S]GTP $\gamma$ S binding to plasma membranes (44, 47). The use of an epitope-tagged  $LP_{A1}$  combined with these assays for LPA responses shows that  $lp_{A1}$  expression is sufficient to produce LPA-dependent signaling, including G protein activation, Rho-mediated actin rearrangement, increased cell proliferation, and stimulation of serum response elements (SREs) in these cells (Figure 4) (44). These data have confirmed that  $LP_{A1}$  couples to  $G_{i/o}$ , which leads to cell proliferation. In addition,  $LP_{A1}$  is also coupled to the Rho pathway, leading to actin rearrangement and cell proliferation. Both  $G_i$  and Rho activation are also observed in Schwann cells, with the major cell survival pathway, rather than proliferation, apparently involving  $G_i$ -mediated activation of phosphatidylinositol 3-kinase and Akt (48) (JA Weiner, N Fukushima, J Chun, in preparation; Figure 4). Recently, our data with heterologous expression of  $lp_{A1}$  in B103 neuroblastoma cells have indicated that  $LP_{A1}$  induces phospholipase C (PLC) activation through a pertussis toxin (PTX)-insensitive pathway, implicating the interaction of  $LP_{A1}$  with  $G_q$  family members (48a). These data demonstrate that  $LP_{A1}$  acts as a multifunctional LPA receptor that couples to  $G_{i/o}$  and PTX-insensitive G proteins ( $G_{12/13}$  and  $G_{q/11/14}$ ) in distinct cell types (Figure 4). This finding is supported in part by the demonstration of coupling between  $LP_{A1}$  and  $G_{i/o}$  or  $G_{11}$  (49). It should be noted that coupling may vary depending on receptor expression levels or amounts of available G protein during the assay, which might explain the differing results that have been obtained using different cell lines or expression systems (46, 50, 51).

$lp_{A1}$  in situ hybridization and Northern blot analyses first detected  $lp_{A1}$  expression within the embryonic nervous system (21). During embryonic life,  $lp_{A1}$  is expressed prominently in the VZ of the developing cerebral cortex in the mouse (Figure 3A). This locus of expression developmentally follows neurogenesis within the cerebral cortex. The expression gradually diminishes with disappearance of the VZ by the end of cortical neurogenesis, which occurs just before birth (21, 52). During this embryonic period, a second locus of  $lp_{A1}$  expression is present near the pial (outer) surface of the embryonic cerebral cortex, although the precise cell type responsible for this expression has not been determined.



**Figure 4** LP<sub>A1</sub>-mediated signaling. LPA production, receptor binding, and resultant activation of diverse cellular responses.

During postnatal life, *lp<sub>A1</sub>* expression reemerges in a different cellular locus in the central nervous system (43, 52). The *lp<sub>A1</sub>* expression first reappears in the hindbrain around postnatal day 3 and subsequently expands from caudal to rostral regions with peak expression around postnatal day 18. The expression is concentrated in and around white matter tracts in which oligodendrocytes, the myelinating cells in the central nervous system, are prevalent. Studies using double-label in situ hybridization with *lp<sub>A1</sub>*, combined with either the proteolipid protein gene (an oligodendrocyte marker) or glial fibrillary acidic protein gene (an astrocyte marker), confirmed the *lp<sub>A1</sub>*-expressing cells as oligodendrocytes but not astrocytes. In addition, Schwann cells, the myelinating cells in the peripheral nervous system, also express *lp<sub>A1</sub>*, with the highest levels of expression being observed in the first postnatal week (48). Thus, it is likely that *lp<sub>A1</sub>* expression correlates both temporally and spatially with the development of oligodendrocytes and Schwann cells, implicating LPA signaling as a regulator of some aspect of the biology of these myelinating cells (see below for details).

The expression of *lp<sub>A1</sub>* is widespread outside the nervous system (21). Northern blot analyses revealed that *lp<sub>A1</sub>* was expressed prominently in testis and

intestine and modestly in heart, lung, kidney, spleen, thymus, muscle, and stomach (21, 40, 52a). However, detailed cellular loci remain to be determined, and no immunohistochemical data for endogenous receptor expression are currently available. In addition to this expression in normal organs,  $lp_{A1}$  is also expressed in several cancers (53), which suggests pathological roles for receptor-mediated LPA signaling (see below).

### $lp_{A2}/edg-4$ (Nonmutant Form)

GenBank searches revealed a novel human genomic sequence that encoded a GPCR with ~60% amino acid similarity to  $lp_{A1}$  (Figure 2, Table 1) (22, 39). Our characterization of an isolated mouse homolog (cDNA and genomic DNA) (54) for this gene,  $lp_{A2}$ , demonstrated a predicted amino acid sequence of 348 amino residues, yielding a calculated molecular mass of 39 kDa. Analyses reveal that the  $lp_{A2}$  gene structure consists of three exons with the previously noted intron inserted within putative transmembrane domain VI, as observed for  $lp_{A1}$ , indicating that  $lp_{A1}$  and  $lp_{A2}$  were derived from a common ancestral gene. The  $lp_{A2}$  gene is located on chromosome 8, near loci that account for myodystrophy (*myd*) and "kidney-anemia-testis" (*kat*), although it is likely that neither phenotype is caused by the  $lp_{A2}$  mutation. Another intriguing feature of the  $lp_{A2}$  gene is the existence of several human variants observed in cancer cells. This feature was most obvious in a large number of nucleotide variations in 3' untranslated regions (mostly by single nucleotide replacement). The prevalence of mutations within this gene likely accounts for the inadvertent use of a carboxyl-terminal mutant, which was used in a study designed to analyze human  $lp_{A2}$  (named *edg-4*) (51, 55). This aberrant clone was obtained from an ovarian tumor cell line, and subsequent analyses demonstrated that the clone contained a G deletion that produced a frame-shift mutation. This mutation results in a predicted mutant protein with 4 replaced and 31 additional amino acids (382 amino acids as compared with native  $lp_{A2}$  which has 351 amino acids) (54). Because C-terminal regions of GPCRs are generally essential for appropriate coupling to G proteins, this mutated  $LP_{A2}$  may produce different cellular responses compared with wild-type  $LP_{A2}$ , such as those associated with cellular transformation.

Despite initial examination of a mutant  $LP_{A2}$ , the properties of both mutant and wild-type  $LP_{A2}$  support its identity as a second LPA receptor (46, 48a, 51, 55).  $LP_{A2}$  couples to  $G_i$  and  $G_q$ , which mediates LPA-induced PLC activation and leads to intracellular  $Ca^{2+}$  increases and inositol phosphate production (48a, 50, 51). The estimated 50% effective concentrations to achieve a variety of responses are also, as with  $LP_{A1}$ , in the low nanomolar range. These properties, taken together, demonstrate that  $LP_{A2}$  is a multifunctional LPA receptor and thus functionally is similar to  $LP_{A1}$  (Table 1). As with  $LP_{A1}$ , the coupling of  $LP_{A2}$  to specific G proteins also appears to depend on the assay system used.

Northern blot analyses showed that embryonic and neonatal mouse brains expressed two  $lp_{A2}$  transcripts of distinct size (2.8 kb and 7 kb); however, neither transcript was detectable in adult brain tissue (52a). This temporal expression profile was also confirmed by our in situ hybridization studies, which suggest

expression of  $lp_{A2}$  in postmitotic neuronal regions (e.g. developing cortical plate, Figure 3A) as well as the VZ of embryonic mice (C McGiffert & J Chun, in preparation). These findings suggest a role for  $lp_{A2}$  in brain development. Two species of transcripts were also observed in human lymphoid cell lines, although these transcripts differed in size (1.8 kb and 8 kb) from those found in the mouse (55). The generation of these two species may be caused by distinct polyadenylation sites (54). Outside the nervous system, the smaller transcript was abundant in testis, kidney, and lung in adult mice (52a).

### $lp_{A3}/edg-7(edg-5)$

In the course of studies on  $lp_{A1}$  and  $lp_{A2}$ , we identified a third related gene,  $lp_{A3}$ , (filed 12/1997 as *edg-5* in U.S. patent 6057126).  $lp_{A3}$  encodes a 40-kDa GPCR of 354 amino acids with ~60% amino acid similarity to mouse  $lp_{A1}$  and  $lp_{A2}$  (Figure 2). Independent isolation by reverse transcriptase-polymerase chain reaction, with degenerate primers and Jurkat T-cell RNA, also identified the same human gene (called *edg-7*) (51). Mouse  $lp_{A3}$  is present on chromosome 3 near the varint waddler (*va*) locus and consists of three exons along with an identically placed intron located within transmembrane domain VI, just as is found for  $lp_{A1}$  and  $lp_{A2}$  (52a). Based on the similarity in the conserved intron insertion and predicted amino acid sequence, this gene is clearly a third member of the same  $LP_A$  family of receptors.

One anticipated function of  $LP_{A3}$ , LPA-dependent increases in intracellular  $Ca^{2+}$  concentration, was confirmed by studies of mammalian (RH7777 cell line) and insect (Sf9 cell line) cells overexpressing  $lp_{A3}$  (46, 51). This  $LP_{A3}$ -mediated  $Ca^{2+}$  increase was not blocked by pretreatment with PTX. Our studies also confirmed that  $LP_{A3}$  activated PLC in a PTX-independent manner (48a). These results suggest coupling of  $LP_{A3}$  to a member of the  $G_q$  family. Less certain is the possible  $LP_{A3}$  coupling to  $G_i$ , where studies have yielded contradictory or equivocal results.  $LP_{A3}$  activation leads to stimulatory pathways for adenylyl cyclase in insect cells (51); whereas in mammalian cells, there is no (46) or significant coupling (48a). Thus, the involvement of  $G_{i/o}$ , as well as  $G_{12/13}$ , has not yet been fully determined, although it would be somewhat surprising if these pathways were not activated, and leaves open the question of actual signaling properties for this particular receptor.

$lp_{A3}$  expression was most abundant in human testis, prostate, heart, and frontal regions of cerebral cortex (46, 51), although it was detected prominently in mouse lung, kidney, and testis (52a).

## BIOLOGICAL FUNCTIONS OF LYSOPHOSPHATIDIC ACID

Essential questions about receptor-mediated LPA signaling have moved from the identification of receptors to understanding the *in vivo* roles of receptor-mediated signals.

## Nervous System

A major locus of expression for LPA receptors is the nervous system. Analyses of  $lp_{A1}$  expression by in situ hybridization identified three major cellular loci in the nervous system: cortical neuroblasts of the neuroproliferative VZ, oligodendrocytes, and Schwann cells (21, 43, 48, 52). Preliminary studies suggest that  $lp_{A2}$  may be expressed in both overlapping and complementary populations of cells, such as postmitotic or differentiating neurons during development (C McGiffert & J Chun, in preparation). We have used the nervous system as a model for exploring the function of receptor-based LPA signaling, and we will now discuss the possible roles for LPA signaling in the nervous system.

**Cortical Neuroblasts** In mammals, most cerebral cortical neurons are generated by neuroblasts that proliferate in the VZ, a pseudostratified zone overlying the lateral ventricle (56) (Figure 3A). As the cell cycle proceeds during neurogenesis, neuroblast nuclei show a to-and-fro movement along the radial (apico-basal) axis. This movement has been termed "interkinetic nuclear migration" (57) (Figure 3C). During the S phase of the cell cycle, neuroblasts are fusiform with nuclei positioned at the superficial border of the VZ. During the G2 phase, the nucleus descends to the ventricular surface, the pial process is retracted, and the cell rounds up. The cell then divides at the M phase, after which daughter cells either migrate away as postmitotic neurons, undergo programmed cell death (58, 59), or reextend their bipolar processes to enter the cell cycle again (57, 60). Several peptidergic growth factors or neurotransmitters have been shown to influence neuroblast proliferation by activating receptor tyrosine kinase pathways or modulating ionic conductance (61). However, the molecular mechanisms underlying neuroblast morphological changes and cell cycle progression remain unclear.

To determine whether exogenous LPA signaling could affect cerebral cortical neuroblasts from the VZ on a short time scale, LPA-dependent ionic conductance changes were examined by using whole-cell recordings on acutely dissociated VZ neuroblasts (62). Exogenous application of LPA induces two clear types of ionic changes within the majority of VZ neuroblasts, changes that occur within 30 to 60 s. These changes consist of increases in either chloride or nonselective cation conductances and result in depolarization of the cells, based on the resting potential. It is interesting that the ionic responses developmentally precede or coexist in a neuroblast population with the previously identified  $\gamma$ -aminobutyric acid and/or L-glutamate ionotropic conductance changes (62, 63). The LPA-dependent ionic changes might modulate DNA synthesis or cell proliferation, which is consistent with recent studies showing proliferative effects of LPA in VZ neuroblasts (63a).

In addition to inducing electrophysiological changes, LPA induces morphological changes (cellular and nuclear migration, cell rounding, and the formation of fine retraction fibers) of VZ neuroblast in cluster cultures and whole brain explant cultures (63b). The intracellular signaling pathways (Rho and actomyosin pathways) activated in response to LPA are similar to those mediated by  $LP_{A1}$  in neuroblastoma

cell lines (44). The LPA-induced morphological changes in neuroblasts are similar to those observed during interkinetic nuclear migration (60), suggesting the involvement of LPA signaling in this classical feature of VZ neuroblasts (Figure 3A).

Collectively, these multiple functions for LPA in proliferative cortical neuroblasts implicate receptor-mediated LPA signaling as a biologically relevant mechanism during cortical neurogenesis. Many peptide growth factors or transmitters, as well as their receptors, are also present during the same embryonic period (61), suggesting that these components are likely to function harmoniously with one another. Indeed, it has been documented that LPA-induced activation of intracellular signaling pathways can cross-talk with those pathways activated by other stimuli via receptor tyrosine kinases [e.g. epidermal growth factor receptor (64)]. The cooperative interaction between LPA and other neurogenic signals is an area for future investigation.

**Differentiating Neurons** Cerebral cortical neuroblasts exit the cell cycle to become postmitotic neurons and leave the VZ by migrating toward the subplate, intermediate zone, and cortical plate. As these cells reach their final destination in the cortical plate, they continue differentiation that includes axonal elongation and dendritic branching (Figure 3B). Both cell migration and neurite formation require cytoskeletal rearrangement. Extracellular signaling factors, such as chemorepellants or chemoattractants, have been demonstrated to play guidance roles in migration and neurite formation through cytoskeletal rearrangement (65, 66). LPA may have pertinent signaling roles based on several observations. First, LPA induces cytoskeletal rearrangement in neuroblastoma cells (67, 68) or primary neurons (69, 70). Second, LPA has regulatory effects on cell migration in some cell lines (17, 71, 72). Third,  $lp_{A2}$  expression appears to occur in these differentiating neurons. Preliminary analyses in support of this relationship suggest that LPA signaling influences neurite formation of differentiating neurons (N Fukushima & J Chun, in preparation).

**Myelinating Cells: Oligodendrocytes and Schwann Cells**  $lp_{A1}$  is expressed in myelinating cells of both the peripheral and central nervous systems (48, 52). This finding implicates LPA signaling in the biology of these cells. In addition, two observations relate to possible  $lp_{A1}$  expression in previously described mutant mice that show dysfunction of myelinating cells. In the *jimpy* mouse, increased oligodendrocyte apoptosis is associated with decreased expression of  $lp_{A1}$  (52). As mentioned above, another mutant, *vacillans*, mapped to the same proximal region of chromosome 4 as  $lp_{A1}$  (39), and this extinct mutant mouse exhibited signs of peripheral neuropathy consistent with a defect in nerve function that can accompany demyelination (41). These data suggest that  $LP_{A1}$ -mediated signaling could influence the basic neurobiology of myelinating cells, including their ability to survive.

To address this possibility in an experimentally tractable culture system, Schwann cells were examined because of their ease of growth in culture compared

with oligodendrocytes. Primary cultures of rat Schwann cells are usually grown in the presence of serum but can be induced into apoptosis via serum withdrawal (73, 74). Part of this apoptotic response may reflect the loss of LPA signaling because LPA is present in serum at micromolar concentrations (75). To assess potential survival roles, the effects of LPA were examined in a well-defined culture model of Schwann cell survival. Exogenously added LPA in serum-free medium protects these cells from apoptosis following serum withdrawal without affecting cell proliferation (48). The potency is equivalent to that of neuregulins, which are peptidergic survival factors for Schwann cells that signal through cognate receptor tyrosine kinases (73). Of particular note is that LPA-induced survival effects are mediated by  $LP_{A1}$ -activated phosphatidylinositol 3 kinase/Akt pathways (Figure 4) that are also activated by neuregulins. These results indicate that divergent extracellular signaling can produce convergent signaling, which, in theory, might also compensate should disruption of one signal occur. It is interesting that S1P showed no effect on Schwann cell survival, in spite of the relatively abundant expression of its cognate GPCR,  $LP_{B3}$ , which indicates a nonredundant role for LP receptors in this system.

As Schwann cells differentiate and myelinate axons during postnatal development, Schwann cell survival is regulated via apoptotic mechanisms (Figure 3D) (48, 73). During this period,  $lp_{A1}$  expression is upregulated (48), which suggests a physiological role for  $LP_{A1}$ -mediated LPA signaling in peripheral nerve development. Because similar intracellular signaling pathways (e.g. phosphatidylinositol 3 kinase/Akt) are involved in oligodendrocyte survival (76),  $LP_{A1}$ -mediated signaling may play a similar role in the survival of central myelinating cells.

**Other Nervous System Loci** Little is known about the role for LP receptor signaling in neurotransmission. However, a recent report has demonstrated that local injection in vivo of antisense oligonucleotides against  $lp_{A1}$  partially attenuates LPA-induced nociception (77). This study suggests the involvement of  $LP_{A1}$  in pain transmission in the peripheral nervous system.

## Tumor Cells

LPA has been shown to influence cell motility in cancer cells by means of cytoskeletal rearrangement via Rho and/or Rac signaling pathways, although other types of signaling [e.g. PLC, protein kinase C, or  $Ca^{2+}$  signaling] are also important (17, 70, 78–80). These changes lead to the stimulation or inhibition of cell migration or invasion of cancer cells, depending on cell types. Given the presence of LPA in ascites from ovarian cancer patients (81, 82), the effects of LPA on cancer cells are an important issue that needs to be addressed. Although it is still unclear how individual LPA receptors are involved in LPA-dependent invasion of cancer cells, accumulating evidence suggests roles for certain types of LPA receptors in tumor biology. For example, in ovarian cancer cells,  $lp_{A1}$  has been suggested to be a negative regulator for cell growth, although this effect is independent of

LPA (53).  $lp_{A2}$  has also been suggested to be involved in the LPA-dependent cell proliferation (83).

## Recent Studies on Genetic Nulls

A major impediment to assessing the roles for any LP in a biological setting is the absence of specific competitive antagonists for any receptor. An alternative strategy that is being pursued by many groups is the creation of genetic nulls for these receptors. Our analyses of  $lp_{A1}$ -null mice have revealed that many of these mice have impaired suckling behavior, and all have reduced total body mass and craniofacial defects (63a). These findings implicate a nonredundant role for single LP receptors, and further demonstrate the requirement for members of this gene family in mediating LP effects in vivo.

## PRODUCTION AND DEGRADATION OF EXTRACELLULAR LYSOPHOSPHATIDIC ACID

To better understand the biology of signaling LPA, the cells of origin and metabolic and biosynthetic pathways need to be determined. Major cellular sources of LPA include platelets and adipocytes (75, 84). Both release LPA when stimulated by thrombin or alpha-receptor agonists, respectively. Platelet-derived LPA is present in serum at micromolar levels and is bound to albumin and/or low-density lipoprotein, which provides stabilization against degradation by phospholipases (85, 86). This LPA has been considered to play an important role in pathological conditions, such as wound healing. Adipocyte-derived LPA appears to mediate the proliferation of preadipocytes, suggesting paracrine mechanisms for the growth of adipose tissue. In addition to these LPA sources, LPA has also been detected in ascites from ovarian cancer patients (81, 82). Recently we have demonstrated that postmitotic cortical neurons are capable of producing LPA (63b). Secreted LPA may be a signaling component regulating cortical neurogenic events. This list is surely a partial one considering the wide distribution of LPA receptors. The major difficulty in addressing the site of synthesis for signaling LPA is that the biochemical pathways of production are incompletely defined (87), especially within primary cells from specific anatomical regions such as the many cell types within the central nervous system. Secreted  $PLA_2$  is thought to be involved in LPA production (88). However, there are other types of phospholipases or lipid kinases that may be involved in LPA production, including lysophospholipase D,  $PLA_1$ , and monoacylglycerol kinase; the latter two produce arachidonyl-LPA (Figure 4) (87, 89).

Several enzymes that are potentially involved in the degradation of LPA have been documented, including lysophospholipases, lipid phosphate phosphohydrolases, and endophilin (90–92). However, lysophospholipases are considered to be intracellular enzymes that can also cleave other nonsignaling LPs, such as lysophosphatidylcholine (91). Whether these enzymes physiologically function in

the degradation of extracellular LPA remains to be determined. Lipid phosphate phosphohydrolases are hydrophobic enzymes that have four potential membrane-spanning regions (90). Lipid phosphate phosphohydrolases can degrade both LPA and S1P. Recently, another role for LPA in synaptic vesicle recycling has been demonstrated in studies of endophilin, which has acyl-CoA transferase activity that produces phosphatidic acid from LPA that is present within synaptic vesicle membranes (92). Its relationship to extracellular signaling, if it exists, remains to be determined.

## SPHINGOSINE 1-PHOSPHATE RECEPTORS

With the identification of  $LP_{A1}$  as the first LP receptor (21), comparative analyses of ESTs and orphan receptor genes indicated the existence of a closely related group, the  $lp_B$  family. Although the identity of the high-affinity ligand for  $LP_B$  receptors was not known at the time, the high degree of sequence similarity made it most likely that the ligand would have similarities to LPA, particularly in view of prior functional studies that postulated a shared receptor for LPA and S1P (16–18). Two groups independently reported  $LP_{B1}$  as the first S1P receptor in 1998 (24, 25), and a wealth of subsequent studies from many groups has supported the identification of other  $LP_B$  members as high-affinity S1P receptors (10, 22, 23).

### $lp_{B1}/edg-1$

$lp_{B1}$  was originally isolated by Hla and Maciag as an immediate early gene induced by phorbol ester in human endothelial cells, and its orphan receptor name, *edg-1*, reflects a postulated role for the gene in endothelial differentiation (38). Analyses of the human  $lp_{B1}$  gene revealed that it encoded a GPCR consisting of 381 amino acids with a molecular mass of 43 kDa. Mouse  $lp_{B1}$  consists of two exons; the second exon includes the entire open reading frame and is localized to chromosome 3 (93; J Contos & J Chun, unpublished data). This genomic organization is common to all  $lp_B$  family members but is distinct from  $lp_A$  family members (see above).

Formal mammalian heterologous expression studies for  $LP_{B1}$ , unlike  $LP_{A1-3}$ , have not been possible because of a continued low level of expression of  $lp_{B1}$  or related receptor genes in all examined cell lines. Nevertheless, based on the concordance of data obtained for  $LP_A$  receptors using both overexpression and heterologous expression, it is very likely that essentially similar data will be obtained using heterologous expression approaches (see below). On the basis of  $LP_{B1}$  overexpression studies, two independent groups identified S1P as a high-affinity ligand for  $LP_{B1}$  ( $K_d = 8$  nM) (24, 25). Studies from these and other groups also demonstrate that  $LP_{B1}$  activates multiple cellular responses, all mediated by a PTX-sensitive pathway (Table 1). These responses include PLC activation, intracellular  $Ca^{2+}$  mobilization, MAP kinase activation, and inhibition of forskolin-stimulated cAMP production (24, 25, 94–96). Consistent with these findings, coupling of  $LP_{B1}$

to other types of G proteins—including  $G_q$ ,  $G_{12}$ ,  $G_{13}$ , and  $G_s$ —is not detectable in an overexpression system using insect cells (97), and thus far,  $LP_{B1}$  has been shown to couple only with  $G_{i/o}$ . However, this finding is somewhat surprising in view of LP receptors that couple with multiple G proteins; indeed, morphogenetic differentiation in  $LP_{B1}$ -overexpressing cells is not blocked by PTX but is inhibited by C3 exoenzyme (25), indicating activation of PTX-insensitive signaling mechanisms such as those present for  $LP_A$  receptors. The determination of which G proteins are involved in this process, assuming that these are not secondary effects, remains for future studies.

Northern blot analyses have shown wide expression of  $lp_{B1}$  in adult mice (38, 47). In addition to vascular endothelial cells, from which  $lp_{B1}$  was isolated, the major cellular loci identified are murine cerebellar Purkinje cells and hippocampal granule cells (93).

### *lp<sub>B2</sub>/AGR16/H218/edg-5*

$lp_{B2}$  was independently cloned by two groups and given the orphan GPCR gene names of *AGR16*, for a rat gene isolated from cardiovascular system (98), or *H218*, for a rat gene from the nervous system (Table 1) (99). Our independent isolation of mouse  $lp_{B2}$  gene revealed that it encodes a 39-kDa protein of 352 amino acids with ~58% amino acid similarity to  $lp_{B1}$  (47). It is localized on chromosome 9 (G Zhang, J Contos & J Chun, in preparation) and has a genomic organization similar to that found in other members of the  $lp_B$  family.

Functional analyses of this receptor from many laboratories, including ours, confirmed that  $LP_{B2}$  also acts as a high-affinity S1P receptor ( $K_d = 20\text{--}27$  nM) (47, 94, 97, 100–102).  $LP_{B2}$ -mediated activation of signaling pathways appears to be more divergent than those mediated by  $LP_{B1}$  (Table 1). Following S1P exposure to  $LP_{B2}$ -expressing cells, the activation of multiple effector pathways occurs, including the activation of PTX-sensitive and PTX-insensitive G proteins, which leads to PLC stimulation,  $Ca^{2+}$  mobilization, MAP and SAPK kinase activation, and actin rearrangement. However, unlike  $LP_{B1}$ , which mediates a decrease in cAMP accumulation stimulated by forskolin, the stimulation of  $LP_{B2}$  results in an increase in basal (no stimulation by forskolin) cAMP formation (94, 102).

$lp_{B2}$  expression is abundant in the lung and heart, but less so in the brain in the adult mouse and rat (47, 98). However, it is prominent in the brain during embryonic development (99). Immunohistochemical studies revealed that  $LP_{B2}$  is possibly localized in neuronal cell bodies during differentiation and in the axon during the period of neurite outgrowth (103), which suggests a role for  $LP_{B2}$ -mediated signaling in neuronal development. This finding may be related to S1P-induced neurite retraction mediated by  $LP_{B2}$  in a neuronal cell line (100).

### *lp<sub>B3</sub>/edg-3*

$lp_{B3}$  is synonymous with a third gene, *edg-3*, isolated from human genomic DNA (104). Human and mouse  $lp_{B3}$  were mapped on chromosome 9q22.1 and

chromosome 13, respectively (104, G Zhang, J Contos & J Chun, in preparation). Mouse  $lp_{B3}$  encodes a 42-kDa protein that consists of 378 amino acids with ~60% amino acid similarity to  $lp_{B1}$  (47). The binding affinity for S1P to  $LP_{B3}$  was comparable with that for other  $LP_B$  members ( $K_d = 23\text{--}26$  nM) (94, 100). The intracellular signaling pathways activated through  $LP_{B3}$  appear similar to those activated by  $LP_{B2}$ , although they may have comparative differences in efficacy and potency in S1P stimulation between these two receptors (Table 1) (94, 97, 105).

The expression of human  $lp_{B3}$  is detected in heart and kidney tissues and at lower levels in lung tissue (104), whereas mouse  $lp_{B3}$  was highly expressed in lung, heart, and kidney tissues (47). In addition to expression in these tissues,  $lp_{B3}$  is prominently expressed in rat Schwann cells (48) and mouse embryonic brain tissue (J Contos & J Chun, unpublished data).

### $lp_{B4}/nrg-1/edg-8$

The fourth member of the  $lp_B$  family was identified as a result of a screening for genes associated with differentiation of rat pheochromocytoma 12 (PC12) cells (106) and as an EST homologous to other  $lp_B$  genes. This gene was called *nrg-1* (nerve growth factor-regulated gene-1), and the gene encodes a protein consisting of 400 amino acids. Rat  $lp_{B4}$  gene maps to chromosome 8, near the locus of  $lp_{B2}$  (106).  $LP_{B4}$  has features of a high-affinity S1P receptor ( $K_d = 2$  nM) (45), which couples to  $G_{i/o}$  pathways (Table 1). Whether  $LP_{B4}$  stimulates other pathways has not yet been determined.

In PC12 cells, treatment with nerve growth factor or other differentiation-inducing reagents resulted in the repressed expression of  $lp_{B4}$ , which suggests an inverse relationship to differentiation (106). Rat  $lp_{B4}$  expression is abundant in brain, particularly in the midbrain, hindbrain, and spinal cord. Although the precise cellular loci in the nervous system remains uncertain, its apparent expression in white matter implicates oligodendrocytes as a locus (45), as was previously observed for other  $LP$  genes (52).

### $lp_{C1}/edg-6$

This gene encodes a protein consisting of 384 (human) or 386 (mouse) amino acids (107). Comparison of the amino acid sequence to  $lp_A$  and  $lp_B$  family members revealed 30%–34% identity and 40%–44% identity, respectively, which is significantly less than the percentage of identity observed within  $lp_A$  or  $lp_B$  family members (48%–52%). This finding may warrant categorization of this gene as the first member of a distinct subfamily (Figure 2), although its genomic organization is similar to other  $lp_B$  family members (J Contos, V Sah & J Chun, submitted). Human  $lp_{C1}$  is located on chromosome 19p13.3, whereas the mouse gene maps to chromosome 10 (J Contos, V Sah & J Chun, submitted).

In spite of the relatively distal position of  $lp_{C1}$  from the  $lp_B$  family in the dendrogram (Figure 2),  $LP_{C1}$  has many attributes of a high-affinity S1P receptor ( $K_d = 13\text{--}63$  nM) (27, 28). It mediates S1P-induced PLC activation, intracellular

Ca<sup>2+</sup> mobilization, and MAP kinase activation, all of which are blocked by PTX treatment.

*lp<sub>Cl</sub>* is expressed in lymphoid and hematopoietic tissues as well as the lung. This expression pattern is more restricted when compared with other *lp<sub>B</sub>* family members.

## SPHINGOSYLPHOSPHORYLCHOLINE RECEPTOR

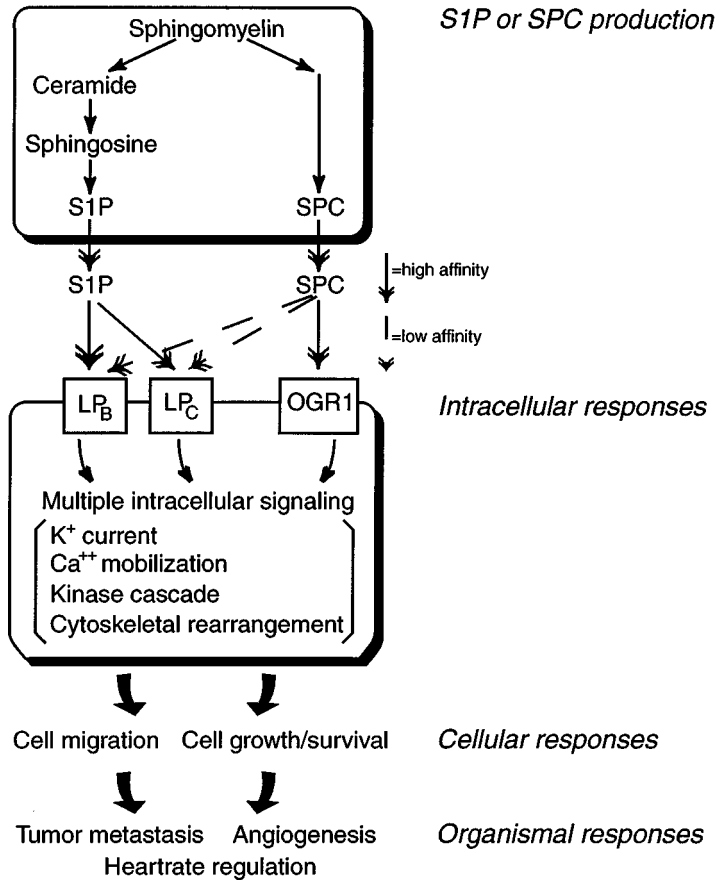
*OGR1* was originally identified as an orphan GPCR from a human ovarian cancer cell line (31). It shares high homology with another orphan GPCR, GPR4, but it also has modest sequence homology (28% amino acid identity and 46% similarity) with a PAF receptor (31). It has low similarity to any LP receptor family member (e.g. 25% identity to *lp<sub>B1</sub>* in a small stretch-containing transmembrane domain VII; Figure 2). It maps to human chromosome 14q31. Other information on genomic structure is not yet available. OGR1 binds SPC with high affinity ( $K_d = 33$  nM), resulting in intracellular Ca<sup>2+</sup> mobilization through a PTX-sensitive pathway and MAP kinase activation through a PTX-insensitive pathway, which suggests some similarity to LP receptor signaling (32). Neither S1P nor PAF activate OGR1. It is notable that all LP<sub>B</sub>/LP<sub>Cl</sub> family members also can be activated by SPC, albeit at lower apparent affinities (~10- to 100-fold less efficiently than S1P) (27, 45, 95, 102, 105, 108). This finding may reflect different configurations and/or binding sites. *OGR1* is expressed in the placenta, lung, liver, spleen, testis, and brain but not in the thymus, ovary, or kidney, which suggests a pathological origin for its expression within ovarian cell lines (31). The dissimilar molecular appearance of this receptor may reflect a basic difference in how choline-containing ligands appear, based upon the dissimilarity of the PAF receptor and OGR1 to the LP receptor noted here. Alternatively, there may be a need to expand the LP receptor family to include less similar receptors. This need is not without precedence, in view of the comparatively low similarity observed among prostanoid receptors (109).

## BIOLOGICAL FUNCTIONS OF SPHINGOSINE 1-PHOSPHATE AND SPHINGOSYLPHOSPHORYLCHOLINE

### Cardiovascular System

S1P- and SPC-induced responses in atrial myocytes suggest biological actions for these lipids in the cardiovascular system (13, 110, 111). Both S1P and SPC potently activate G<sub>i</sub>-regulated, inwardly rectifying potassium channels, which suggest physiological roles for these lipids in heart-rate regulation (Figure 5). A recent study has demonstrated that LP<sub>B3</sub> is involved in these actions (111a).

Biological relevance of receptor-mediated S1P signaling in the vascular system is likely, based on several findings. First, treatment of vascular endothelial cells



**Figure 5** Sphingolipid signaling. Sphingosine 1-phosphate (S1P) or sphingosylphosphorylcholine (SPC) generated from sphingomyelin is released and can bind to LP<sub>B</sub>, LP<sub>C</sub>, or OGR1 receptors to elicit diverse cellular responses.

with phorbol esters results in morphological differentiation, accompanied by the acute expression of *lp<sub>B1</sub>* (38). Second, application of S1P to these cells or vascular smooth-muscle cells increases intracellular Ca<sup>2+</sup> concentrations (112, 113). Third, LP<sub>B1</sub> overexpressed in fibroblasts mediates biological responses induced by S1P (25, 94, 95). Finally, endothelial cells also express *lp<sub>B3</sub>*, whose receptor protein activates pathways distinct from those of *lp<sub>B1</sub>* (114).

Support for such a role comes from studies that use *lp<sub>B1</sub>* or *lp<sub>B3</sub>* antisense oligonucleotide treatment of endothelial cells (114). This treatment results in the loss of S1P-induced adherens junction assembly and cortical actin fiber formation and affects cell survival, which results in inhibition of S1P-induced effects. S1P treatment enhances fibroblast growth factor-induced angiogenesis in vivo, and cotreatment of both antisense oligonucleotides blocks these effects. Lee and

colleagues conclude that both  $LP_{B1}$  and  $LP_{B3}$  cooperate in S1P-induced endothelial cell morphogenesis through activation of divergent signaling pathways and that both are required for S1P-induced angiogenesis (Figure 5). These findings implicate receptor-mediated S1P signaling in the formation of the endothelium. A recent report on generating null mice for  $lp_{B1}$  has shown the involvement of this gene in vascular maturation, but not angiogenesis and vasculogenesis (114a).

## Tumor Cells

Like LPA, S1P has been shown to be capable of either promoting or inhibiting tumor cell migration, depending on cell type (17, 78). A common feature in S1P signaling in these cells is the involvement of the Rho pathway. Other pathways, including  $G_i$  or  $G_q$  signaling, have also been reported to mediate S1P actions on cell motility (78, 94), and  $G_i$  pathways activated by S1P also may contribute to tumor cell proliferation (115). Recent  $lp_B$  studies have supported the expression of multiple  $LP_B$  members in several types of cancer cells.  $lp_{B2}$  and  $lp_{B3}$  are expressed in breast cancer cells, where S1P stimulates SRE activation and cell proliferation (115). Human bladder carcinoma cells, whose motility is regulated by S1P, express  $lp_{B1}$  and  $lp_{B3}$  (116). On the other hand, SPC appears to inhibit cell growth of ovarian cancer cell lines that express *OGR1* (32). The actual roles for receptor-mediated S1P or SPC signaling in initiation, propagation or therapy of cancers remain open areas of research.

## PRODUCTION AND DEGRADATION OF EXTRACELLULAR SPHINGOSINE 1-PHOSPHATE AND SPHINGOSYLPHOSPHORYLCHOLINE

Activated platelets have been demonstrated to be a major source of extracellular S1P (16, 117). When activated by thrombin or phorbol esters, platelets can release S1P into the bloodstream where it is present as an albumin- and/or lipoprotein-bound form at physiologically relevant concentrations ( $\sim 500$  nM) (118, 119). In platelets, S1P is thought to be produced exclusively through the phosphorylation of sphingosine by sphingosine kinase (117). An accumulation of generated S1P occurs through a lack of the major hydrolysing enzyme, S1P lyase, which is usually present in other types of cells (16). As with LPA, the wide distribution of  $LP_B$  members in both embryonic and adult tissues implicates other sources of extracellular S1P.

The hydrolysis of sphingomyelin by sphingolipid *N*-deacylase is the only known pathway for SPC generation (5, 34). Little is known about whether this pathway produces signaling SPC. An accumulation of SPC has been demonstrated in several types of diseases. For example, ascites fluid from ovarian cancer patients contains significant concentrations (50–190 nM) of SPC, which suggests a role for SPC signaling in ovarian cancer (32).

The degradation of extracellular S1P or SPC could theoretically regulate the concentration of these lipids relevant to receptor-mediated signaling. One potential enzyme in such regulation is lipid phosphate phosphohydrolases, as mentioned in the LPA section (90). No doubt further insights into degradative pathways relevant to the biology and pathology of these sphingolipids will emerge in the near future.

## CONCLUSIONS AND FUTURE PROSPECTS

With the identification of the LP receptors, our understanding of extracellular LP signaling has moved rapidly from debate over the existence of receptors, to a clear understanding that many of the effects documented for LPs are caused by their activation of multiple, cognate GPCRs. The existence of numerous LP receptors raises the major question of the biological role for each receptor. Are these receptors simply redundant means of signaling the presence of extracellular LPs, or are there more specific roles for individual receptors? Given the current lack of receptor-subtype-specific agonists and antagonists, traditional pharmacological analyses of LPs, both in vitro and in vivo, are not feasible. On the other hand, molecular genetic strategies, particularly when used in vivo, can assess receptor function by misexpressing, overexpressing, or mutating receptors [e.g. the production of receptor-null mice (63a, 114a)]. This approach will provide clear opportunities to understanding new and important features of receptor-mediated LP signaling in the near future. Improved knowledge of these features, combined with further understanding of how extracellular LPs are synthesized, released, degraded, and regulated, should make this decade an exciting period for unraveling the complexities and intricacies for the receptor-mediated effects of these simple phospholipids. This new information will provide a foundation for understanding the biological roles for LPs as well as realizing their medicinal potential.

## ACKNOWLEDGMENTS

We thank Casey Cox for editing the manuscript. This work was supported by the National Institute of Mental Health (JJAC, JAW, JC), the Uehara Memorial Foundation (NF, II), and a sponsored research agreement with Allelix Biopharmaceuticals.

**Visit the Annual Reviews home page at [www.AnnualReviews.org](http://www.AnnualReviews.org)**

## LITERATURE CITED

1. Vogt W. 1963. Pharmacologically active acidic phospholipids and glycolipids. *Biochem. Pharmacol.* 12:415–20
2. Gerrard J, Robinson P. 1984. Lysophosphatidic acid can activate platelets without increasing  $^{32}\text{P}$ -labelling of phosphatidic acid. *Biochim. Biophys. Acta* 795:487–92
3. Fukami K, Takenawa T. 1992. Phosphatidic

- acid that accumulates in platelet-derived growth factor-stimulated Balb/c 3T3 cells is a potential mitogenic signal. *J. Biol. Chem.* 267:10988–93
4. McCrea JM, Robinson P, Gerrard JM. 1985. Mepacrine (quinacrine) inhibition of thrombin-induced platelet responses can be overcome by lysophosphatidic acid. *Biochim. Biophys. Acta* 842:189–94
  5. Spiegel S, Milstien S. 1995. Sphingolipid metabolites: members of a new class of lipid second messengers. *J. Membr. Biol.* 146:225–37
  6. Simon MF, Chap H, Douste-Blazy L. 1984. Platelet aggregating activity of lysophosphatidic acids is not related to their calcium ionophore properties. *FEBS Lett.* 166:115–19
  7. Olivera A, Spiegel S. 1993. Sphingosine-1-phosphate as second messenger in cell proliferation induced by PDGF and FCS mitogens. *Nature* 365:557–60
  8. van Corven EJ, Groenink A, Jalink K, Eichholtz T, Moolenaar WH. 1989. Lysophosphatidate-induced cell proliferation: identification and dissection of signaling pathways mediated by G proteins. *Cell* 59:45–54
  9. Moolenaar WH, Kranenburg O, Postma FR, Zondag GC. 1997. Lysophosphatidic acid: G-protein signalling and cellular responses. *Curr. Opin. Cell Biol.* 9:168–73
  10. Moolenaar WH. 1999. Bioactive lysophospholipids and their G protein-coupled receptors. *Exp. Cell Res.* 253:230–38
  11. Desai NN, Zhang H, Olivera A, Mattie ME, Spiegel S. 1992. Sphingosine-1-phosphate, a metabolite of sphingosine, increases phosphatidic acid levels by phospholipase D activation. *J. Biol. Chem.* 267:23122–28
  12. Zhang H, Desai NN, Olivera A, Seki T, Brooker G, et al. 1991. Sphingosine-1-phosphate, a novel lipid, involved in cellular proliferation. *J. Cell. Biol.* 114:155–67
  13. Bünemann M, Liliom K, Brandts BK, Pott L, Tseng JL, et al. 1996. A novel membrane receptor with high affinity for lysophosphomyelin and sphingosine 1-phosphate in atrial myocytes. *EMBO J.* 15:5527–34
  14. van Koppen CJ, Meyer zu Heringdorf D, Zhang C, Laser KT, Jakobs KH. 1996. A distinct G(i) protein-coupled receptor for sphingosylphosphorylcholine in human leukemia HL-60 cells and human neutrophils. *Mol. Pharmacol.* 49:956–61
  15. Postma FR, Jalink K, Hengeveld T, Mooleenaar WH. 1996. Sphingosine-1-phosphate rapidly induces Rho-dependent neurite retraction: action through a specific cell surface receptor. *EMBO J.* 15:2388–92
  16. Yatomi Y, Yamamura S, Ruan F, Igarashi Y. 1997. Sphingosine 1-phosphate induces platelet activation through an extracellular action and shares a platelet surface receptor with lysophosphatidic acid. *J. Biol. Chem.* 272:5291–97
  17. Yamamura S, Yatomi Y, Ruan F, Sweeney EA, Hakomori S, et al. 1997. Sphingosine 1-phosphate regulates melanoma cell motility through a receptor-coupled extracellular action and in a pertussis toxin-insensitive manner. *Biochemistry* 36:10751–59
  18. Okajima F, Tomura H, Sho K, Kimura T, Sato K, et al. 1997. Sphingosine 1-phosphate stimulates hydrogen peroxide generation through activation of phospholipase C-Ca<sup>2+</sup> system in FRTL-5 thyroid cells: possible involvement of guanosine triphosphate-binding proteins in the lipid signaling. *Endocrinology* 138:220–29
  19. van der Bend RL, Brunner J, Jalink K, van Corven EJ, Moolenaar WH, et al. 1992. Identification of a putative membrane receptor for the bioactive phospholipid, lysophosphatidic acid. *EMBO J.* 11:2495–501
  20. Thomson FJ, Perkins L, Ahern D, Clark M. 1994. Identification and characterization of a lysophosphatidic acid receptor. *Mol. Pharmacol.* 45:718–23
  21. Hecht JH, Weiner JA, Post SR, Chun J.

1996. *Ventricular zone gene-1 (vzg-1)* encodes a lysophosphatidic acid receptor expressed in neurogenic regions of the developing cerebral cortex. *J. Cell Biol.* 135:1071–83
22. Chun J, Contos JJA, Munroe D. 1999. A growing family of receptor genes for lysophosphatidic acid (LPA) and other lyso-phospholipids (LPs). *Cell Biochem. Biophys.* 30:213–42
23. Chun J. 1999. Lysophospholipid receptors: implications for neural signaling. *Crit. Rev. Neurobiol.* 13:151–68
24. Zondag GC, Postma FR, Etten IV, Verlaan I, Moolenaar WH. 1998. Sphingosine 1-phosphate signalling through the G-protein-coupled receptor Edg-1. *Biochem. J.* 330:605–9
25. Lee MJ, Van Brocklyn JR, Thangada S, Liu CH, Hand AR, et al. 1998. Sphingosine-1-phosphate as a ligand for the G protein-coupled receptor EDG-1. *Science* 279:1552–55
26. Chun J, Weiner JA, Fukushima N, Contos JJA, Zhang G, et al. 2000. Neurobiology of receptor-mediated lysophospholipid signaling, from the first lysophospholipid receptor to roles in the nervous system function and development. *Ann. NY Acad. Sci.* 905:110–17
27. Yamazaki Y, Kon J, Sato K, Tomura H, Sato M, et al. 2000. Edg-6 as a putative sphingosine 1-phosphate receptor coupling to  $Ca^{2+}$  signaling pathway. *Biochem. Biophys. Res. Commun.* 268:583–89
28. van Brocklyn JR, Grealer MH, Bernhardt G, Hobson JP, Lipp M, et al. 2000. Sphingosine-1-phosphate is a ligand for the G protein-coupled receptor EDG-6. *Blood* 95:2624–29
29. Hla T, Zimrin AB, Evans M, Ballas K, Maciag T. 1997. The immediate-early gene product MAD-3/EDG-3/IkappaB alpha is an endogenous modulator of fibroblast growth factor-1 (FGF-1) dependent human endothelial cell growth. *FEBS Lett.* 414:419–24
30. Guo Z, Liliom K, Fischer DJ, Bathurst IC, Tomei LD, et al. 1996. Molecular cloning of a high-affinity receptor for the growth factor-like lipid mediator lysophosphatidic acid from *Xenopus oocytes*. *Proc. Natl. Acad. Sci. USA* 93:14367–72
- 30a. Kawasawa Y, Kume K, Izumi T, Shimizu T. 2000. Mammalian PSP24s ( $\alpha$  and  $\beta$  isoforms) are not responsive to lysophosphatidic acid in mammalian expression systems. *Biochem. Biophys. Res. Commun.* 276:957–64
31. Xu Y, Casey G. 1996. Identification of human OGR1, a novel G protein-coupled receptor that maps to chromosome 14. *Genomics* 35:397–402
32. Xu Y, Zhu K, Hong G, Wu W, Baudhuin LM, et al. 2000. Sphingosylphosphorylcholine is a ligand for ovarian cancer G-protein-coupled receptor 1. *Nature Cell Biol.* 2:261–67
33. Hla T, Lee MJ, Ancellin N, Liu CH, Thangada S, et al. 1999. Sphingosine-1-phosphate: extracellular mediator or intracellular second messenger? *Biochem. Pharmacol.* 58:201–7
34. Meyer zu Heringdorf D, van Koppen CJ, Jakobs KH. 1997. Molecular diversity of sphingolipid signalling. *FEBS Lett.* 410:34–38
35. Crawford JA, Mutchler KJ, Sullivan BE, Lanigan TM, Clark MS, et al. 1993. Neural expression of a novel alternatively spliced and polyadenylated Gs alpha transcript. *J. Biol. Chem.* 268:9879–85
36. Brann MR. 1992. *Molecular Biology of G-Protein-Coupled Receptors*, Boston, MA: Birkhauser
37. Chun J, Jaenisch R. 1996. Clonal cell lines produced by infection of neocortical neuroblasts using multiple oncogenes transduced by retroviruses. *Mol. Cell. Neurosci.* 7:304–21
38. Hla T, Maciag T. 1990. An abundant transcript induced in differentiating human endothelial cells encodes a polypeptide with structural similarities to

- G-protein-coupled receptors. *J. Biol. Chem.* 265:9308–13
39. Contos JJA, Chun J. 1998. Complete cDNA sequence, genomic structure, and chromosomal localization of the LPA receptor gene, *lp<sub>A1</sub>/vzg-1/Gpcr26*. *Genomics* 51:364–78
40. Macrae AD, Premont RT, Jaber M, Peterson AS, Lefkowitz RJ. 1996. Cloning, characterization, and chromosomal localization of rec1.3, a member of the G-protein-coupled receptor family highly expressed in brain. *Brain Res. Mol. Brain Res.* 42:245–54
41. Sirlin JL. 1956. Vacillans, a neurological mutant in the house mouse linked to brown. *J. Genet.* 54:42–48
42. Hooks SB, Ragan SP, Hopper DW, Heonemann CW, Durieux ME, et al. 1998. Characterization of a receptor subtype-selective lysophosphatidic acid mimetic. *Mol. Pharmacol.* 53:188–94
43. Allard J, Barraon S, Diaz J, Lubetzki C, Zalc B, et al. 1998. A rat G protein-coupled receptor selectively expressed in myelin-forming cells. *Eur. J. Neurosci.* 10:1045–53
44. Fukushima N, Kimura Y, Chun J. 1998. A single receptor encoded by *vzg-1/lp<sub>A1</sub>/edg-2* couples to G proteins and mediates multiple cellular responses to lysophosphatidic acid. *Proc. Natl. Acad. Sci. USA* 95:6151–56
45. Im DS, Heise CE, Ancellin N, O'Dowd BF, Shei GJ, et al. 2000. Characterization of a novel sphingosine 1-phosphate receptor, Edg-8. *J. Biol. Chem.* 275:14281–86
46. Im DS, Heise CE, Harding MA, George SR, O'Dowd BF, et al. 2000. Molecular cloning and characterization of a lysophosphatidic acid receptor, Edg-7, expressed in prostate. *Mol. Pharmacol.* 57:753–59
47. Zhang G, Contos JJA, Weiner JA, Fukushima N, Chun J. 1999. Comparative analysis of three murine G-protein coupled receptors activated by sphingosine-1-phosphate. *Gene* 227:89–99
48. Weiner JA, Chun J. 1999. Schwann cell survival mediated by the signaling phospholipid lysophosphatidic acid. *Proc. Natl. Acad. Sci. USA* 96:5233–38
- 48a. Ishii I, Contos JJA, Fukushima N, Chun J. 2000. Functional comparisons of the lysophosphatidic acid receptors. LP<sub>A1</sub>/VZG-1/EDG-2, LP<sub>A2</sub>/EDG-4, and LP<sub>A3</sub>/EDG-7 in neuronal cell lines using a retrovirus expression system. *Mol. Pharmacol.* 58:895–902
49. Yoshida A, Ueda H. 1999. Activation of Gi1 by lysophosphatidic acid receptor without ligand in the baculovirus expression system. *Biochem. Biophys. Res. Commun.* 259:78–84
50. An S, Bleu T, Zheng Y, Goetzl EJ. 1998. Recombinant human G protein-coupled lysophosphatidic acid receptors mediate intracellular calcium mobilization. *Mol. Pharmacol.* 54:881–88
51. Bandoh K, Aoki J, Hosono H, Kobayashi S, Kobayashi T, et al. 1999. Molecular cloning and characterization of a novel human G-protein-coupled receptor, EDG7, for lysophosphatidic acid. *J. Biol. Chem.* 274:27776–85
52. Weiner JA, Hecht JH, Chun J. 1998. Lysophosphatidic acid receptor gene *vzg-1/lp<sub>A1</sub>/edg-2* is expressed by mature oligodendrocytes during myelination in the postnatal murine brain. *J. Comp. Neurol.* 398:587–98
- 52a. Contos JJA, Ishii I, Chun J. 2000. Lysophosphatidic acid receptors. *Mol. Pharmacol.* 58:1188–96
53. Furui T, LaPushin R, Mao M, Khan H, Watt SR, et al. 1999. Overexpression of *edg-2/vzg-1* induces apoptosis and anoikis in ovarian cancer cells in a lysophosphatidic acid-independent manner. *Clin. Cancer Res.* 5:4308–18
54. Contos JJA, Chun J. 2000. Genomic characterization of the lysophosphatidic acid receptor gene, *lp<sub>A2</sub>/Edg4*, and identification of a frameshift mutation in a

- previously characterized cDNA. *Genomics* 64:155–69
55. An S, Bleu T, Hallmark OG, Goetzl EJ. 1998. Characterization of a novel subtype of human G protein-coupled receptor for lysophosphatidic acid. *J. Biol. Chem.* 273:7906–10
56. Boulder Committee. 1970. Embryonic vertebrate central nervous system: revised terminology. *Anat. Rec.* 166:257–61
57. Sauer FC. 1935. Mitosis in the neural tube. *J. Comp. Neurol.* 62:377–405
58. Blaschke AJ, Staley K, Chun J. 1996. Widespread programmed cell death in proliferative and postmitotic regions of the fetal cerebral cortex. *Development* 122:1165–74
59. Blaschke AJ, Weiner JA, Chun J. 1998. Programmed cell death is a universal feature of embryonic and postnatal neuroproliferative regions throughout the central nervous system. *J. Comp. Neurol.* 396:39–50
60. Seymour RM, Berry M. 1975. Scanning and transmission electron microscope studies of interkinetic nuclear migration in the cerebral vesicles of the rat. *J. Comp. Neurol.* 160:105–25
61. Cameron HA, Hazel TG, McKay RDG. 1998. Regulation of neurogenesis by growth factors and neurotransmitters. *J. Neurobiol.* 36:287–306
62. Dubin A, Brunton T, Weiner JA, Fukushima N, Chun J. 1999. Lysophosphatidic acid (LPA) stimulates neurotransmitter-like conductance changes that precede GABA and L-glutamate in early, presumptive cortical neuroblasts. *J. Neurosci.* 19:1371–81
63. LoTurco JJ, Owens DF, Heath MJ, Davis MB, Kriegstein AR. 1995. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. *Neuron* 15:1287–98
- 63a. Contos JJA, Fukushima N, Weiner JA, Kaushal D, Chun J. 2000. Requirement for the *lp<sub>AI</sub>* lysophosphatidic acid receptor gene in normal suckling behavior. *Proc. Natl. Acad. Sci. USA* 97:13384–89
- 63b. Fukushima N, Weiner JA, Chun J. 2000. Lysophosphatidic acid (LPA) is a novel extracellular regulator of cortical neuroblast morphology. *Dev. Biol.* 228:6–18
64. Daub H, Weiss FU, Wallasch C, Ullrich A. 1996. Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. *Nature* 379:557–60
65. Brose K, Tessier-Lavigne M. 2000. Slit proteins: key regulators of axon guidance, axonal branching, and cell migration. *Curr. Opin. Neurobiol.* 10:95–102
66. Culotti JG, Merz DC. 1998. DCC and netrins. *Curr. Opin. Cell Biol.* 10:609–13
67. Jalink K, van Corven EJ, Hengeveld T, Morii N, Narumiya S, et al. 1994. Inhibition of lysophosphatidate- and thrombin-induced neurite retraction and neuronal cell rounding by ADP ribosylation of the small GTP-binding protein Rho. *J. Cell Biol.* 126:801–10
68. Tigyi G, Fischer DJ, Sebök A, Yang C, Dyer DL, et al. 1996. Lysophosphatidic acid-induced neurite retraction in PC12 cells: control by phosphoinositide- $\text{Ca}^{2+}$  signaling and Rho. *J. Neurochem.* 66:537–48
69. Saito S. 1997. Effects of lysophosphatidic acid on primary cultured chick neurons. *Neurosci. Lett.* 229:73–76
70. Jin Z, Strittmatter SM. 1997. Rac1 mediates collapsin-1-induced growth cone collapse. *J. Neurosci.* 17:6256–63
71. Sakai T, de la Pena JM, Mosher DF. 1999. Synergism among lysophosphatidic acid,  $\beta_1$  integrins, and epidermal growth factor or platelet-derived growth factor in mediation of cell migration. *J. Biol. Chem.* 274:15480–86
72. O'Connor KL, Shaw LM, Mercurio AM. 1998. Release of cAMP gating by the  $\alpha_6\beta_4$  integrin stimulates lamellae formation and the chemotactic migration of invasive carcinoma cells. *J. Cell. Biol.* 143:1749–60

73. Syroid DE, Maycox PR, Burrola PG, Liu N, Wen D, et al. 1996. Cell death in the Schwann cell lineage and its regulation by neuregulin. *Proc. Natl. Acad. Sci. USA* 93:9229–34
74. Bologna L, Cole R, Chiappelli F, Saneto RP, de Vellis J. 1988. Serum contains inducers and repressors of oligodendrocyte differentiation. *J. Neurosci. Res.* 20:182–88
75. Eichholtz T, Jalink K, Fahrenfort I, Mooleenaar WH. 1993. The bioactive phospholipid lysophosphatidic acid is released from activated platelets. *Biochem. J.* 291:677–80
76. Vemuri GS, McMorris FA. 1996. Oligodendrocytes and their precursors require phosphatidylinositol 3-kinase signaling for survival. *Development* 122:2529–37
77. Renbeack K, Inoue M, Yoshida A, Nyberg F, Ueda H. 2000. Vzg-1/lysophosphatidic acid-receptor involved in peripheral pain transmission. *Brain Res. Mol. Brain Res.* 75:350–54
78. Stam JC, Michiels F, van der Kammen RA, Moolenaar WH, Collard JG. 1998. Invasion of T-lymphoma cells: cooperation between Rho family GTPases and lysophospholipid receptor signaling. *EMBO J.* 17:4066–74
79. Imamura F, Mukai M, Ayaki M, Takemura K, Horai T, et al. 1999. Involvement of small GTPases Rho and Rac in the invasion of rat ascites hepatoma cells. *Clin. Exp. Metastasis* 17:141–48
80. Imamura F, Horai T, Mukai M, Shinkai K, Sawada M, et al. 1993. Induction of in vitro tumor cell invasion of cellular monolayers by lysophosphatidic acid or phospholipase D. *Biochem. Biophys. Res. Commun.* 193:497–503
81. Westermann AM, Havik E, Postma FR, Beijnen JH, Dalesio O, et al. 1998. Malignant effusions contain lysophosphatidic acid (LPA)-like activity. *Ann. Oncol.* 9:437–42
82. Xu Y, Shen Z, Wiper DW, Wu M, Morton RE, et al. 1998. Lysophosphatidic acid as a potential biomarker for ovarian and other gynecologic cancers. *J. Am. Med. Assoc.* 280:719–23
83. Goetzel EJ, Dolezalova H, Kong Y, Hu YL, Jaffe RB, et al. 1999. Distinctive expression and functions of the type 4 endothelial differentiation gene-encoded G protein-coupled receptor for lysophosphatidic acid in ovarian cancer. *Cancer Res.* 59:5370–75
84. Valet P, Pages C, Jeanneton O, Daviaud D, Barbe P, et al. 1998. Alpha2-adrenergic receptor-mediated release of lysophosphatidic acid by adipocytes: a paracrine signal for preadipocyte growth. *J. Clin. Invest.* 101:1431–38
85. Tigyi G, Milei R. 1992. Lysophosphatidates bound to serum albumin activate membrane currents. In *Xenopus oocytes* and neurite retraction in PC12 pheochromocytoma cells. *J. Biol. Chem.* 267:21360–67
86. Siess W, Zangl KJ, Essler M, Bauer M, Brandl R, et al. 1999. Lysophosphatidic acid mediates the rapid activation of platelets and endothelial cells by mildly oxidized low density lipoprotein and accumulates in human atherosclerotic lesions. *Proc. Natl. Acad. Sci. USA* 96:6931–36
87. Gaits F, Fourcade O, Le Balle F, Gueguen G, Gaig B, et al. 1997. Lysophosphatidic acid as a phospholipid mediator: pathways of synthesis. *FEBS Lett.* 410:54–58
88. Fourcade O, Simon MF, Viode C, Rugani N, Leballe F, et al. 1995. Secretory phospholipase A2 generates the novel lipid mediator lysophosphatidic acid in membrane microvesicles shed from activated cells. *Cell* 80:919–27
89. Tokumura A, Miyake M, Yoshimoto O, Shimizu M, Fukuzawa K. 1998. Metal ion stimulation and inhibition of lysophospholipase D which generates bioactive lysophosphatidic acid in rat plasma. *Lipids* 33:1009–15
90. Brindley DN, Waggoner DW. 1998. Mammalian lipid phosphate phosphohydrolases. *J. Biol. Chem.* 273:24281–84
91. Wang A, Dennis EA. 1999. Mammalian

- lysphospholipases. *Biochim. Biophys. Acta* 1439:1–16
92. Schmidt A, Wolde M, Thiele C, Fest W, Kratzin H, et al. 1999. Endophilin I mediates synaptic vesicle formation by transfer of arachidonate to lysophosphatidic acid. *Nature* 401:133–41
93. Liu CH, Hla T. 1997. The mouse gene for the inducible G-protein-coupled receptor edg-1. *Genomics* 43:15–24
94. Kon J, Sato K, Watanabe T, Tomura H, Kuwabara A, et al. 1999. Comparison of intrinsic activities of the putative sphingosine 1-phosphate receptor subtypes to regulate several signaling pathways in their cDNA-transfected Chinese hamster ovary cells. *J. Biol. Chem.* 274:23940–47
95. Okamoto H, Takuwa N, Gonda K, Okazaki H, Chang K, et al. 1998. EDG1 is a functional sphingosine-1-phosphate receptor that is linked via a Gi/o to multiple signaling pathways, including phospholipase C activation, Ca<sup>2+</sup> mobilization, Ras-mitogen-activated protein kinase activation, and adenylate cyclase inhibition. *J. Biol. Chem.* 273:27104–10
96. van Brocklyn JR, Lee MJ, Menzeleev R, Olivera A, Edsall L, et al. 1998. Dual actions of sphingosine-1-phosphate: extracellular through the Gi-coupled receptor Edg-1 and intracellular to regulate proliferation and survival. *J. Cell Biol.* 142:229–40
97. Windh RT, Lee MJ, Hla T, An S, Barr AJ, et al. 1999. Differential coupling of the sphingosine 1-phosphate receptors Edg-1, Edg-3, and H218/Edg-5 to the G<sub>i</sub>, G<sub>q</sub>, and G<sub>12</sub> families of heterotrimeric G proteins. *J. Biol. Chem.* 274:27351–58
98. Okazaki H, Ishizaka N, Sakurai T, Kurokawa K, Goto K, et al. 1993. Molecular cloning of a novel putative G protein-coupled receptor expressed in the cardiovascular system. *Biochem. Biophys. Res. Commun.* 190:1104–9
99. MacLennan AJ, Browe CS, Gaskin AA, Lado DC, Shaw G. 1994. Cloning and characterization of a putative G-protein coupled receptor potentially involved in development. *Mol. Cell. Neurosci.* 5:201–9
100. van Brocklyn JR, Tu Z, Edsall LC, Schmidt RR, Spiegel S. 1999. Sphingosine 1-phosphate-induced cell rounding and neurite retraction are mediated by the G protein-coupled receptor H218. *J. Biol. Chem.* 274:4626–32
101. An S, Bleu T, Zheng Y. 1999. Transduction of intracellular calcium signals through G protein-mediated activation of phospholipase C by recombinant sphingosine 1-phosphate receptors. *Mol. Pharmacol.* 55:787–94
102. Gonda K, Okamoto H, Takuwa N, Yatomi Y, Okazaki H, et al. 1999. The novel sphingosine 1-phosphate receptor AGR16 is coupled via pertussis toxin-sensitive and -insensitive G-proteins to multiple signalling pathways. *Biochem. J.* 337:67–75
103. MacLennan AJ, Marks L, Gaskin AA, Lee N. 1997. Embryonic expression pattern of H218, a G-protein coupled receptor homolog, suggests roles in early mammalian nervous system development. *Neuroscience* 79:217–24
104. Yamaguchi F, Tokuda M, Hatase O, Brenner S. 1996. Molecular cloning of the novel human G protein-coupled receptor (GPCR) gene mapped on chromosome 9. *Biochem. Biophys. Res. Commun.* 227:608–14
105. Okamoto H, Takuwa N, Yatomi Y, Gonda K, Shigematsu H, et al. 1999. EDG3 is a functional receptor specific for sphingosine 1-phosphate and sphingosylphosphorylcholine with signaling characteristics distinct from EDG1 and AGR16. *Biochem. Biophys. Res. Commun.* 260:203–8
106. Glickman M, Malek RL, Kwitek-Black AE, Jacob HJ, Lee NH. 1999. Molecular cloning, tissue-specific expression, and chromosomal localization of a novel nerve growth factor-regulated G-protein-coupled receptor, nrg-1. *Mol. Cell. Neurosci.* 14:141–52

107. Grealer MH, Bernhardt G, Lipp M. 1998. EDG6, a novel G-protein-coupled receptor related to receptors for bioactive lysophospholipids, is specifically expressed in lymphoid tissue. *Genomics* 53:164–69
108. Ancellin N, Hla T. 1999. Differential pharmacological properties and signal transduction of the sphingosine 1-phosphate receptors EDG-1, EDG-3, and EDG-5. *J. Biol. Chem.* 274:18997–9002
109. Narumiya S, Sugimoto Y, Ushikubi F. 1999. Prostanoid receptors: structures, properties, and functions. *Physiol. Rev.* 79:1193–226
110. van Koppen C, Meyer zu Heringdorf M, Laser KT, Zhang C, Jakobs KH, et al. 1996. Activation of a high affinity Gi protein-coupled plasma membrane receptor by sphingosine-1-phosphate. *J. Biol. Chem.* 271:2082–87
111. Bünemann M, Brandts B, zu Heringdorf DM, van Koppen CJ, Jakobs KH, et al. 1995. Activation of muscarinic K<sup>+</sup> current in guinea-pig atrial myocytes by sphingosine-1-phosphate. *J. Physiol.* 489:701–77
- 111a. Himmel HM, Meyer zu Heringdorf D, Graf E, Dobrev D, et al. 2000. Evidence for edg-3 receptor-mediated activation of I (K, Ach) by sphingosine-1-phosphate in human atrial cardiomyocytes. *Mol. Pharmacol.* 58:449–54
112. Meyer zu Heringdorf D, van Koppen CJ, Windorfer B, Himmel HM, Jakobs KH. 1996. Calcium signalling by G protein-coupled sphingolipid receptors in bovine aortic endothelial cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 354:397–403
113. Bornfeldt KE, Graves LM, Raines EW, Igarashi Y, Wayman G, et al. 1995. Sphingosine-1-phosphate inhibits PDGF-induced chemotaxis of human arterial smooth muscle cells: spatial and temporal modulation of PDGF chemotactic signal transduction. *J. Cell Biol.* 130:193–206
114. Lee MJ, Thangada S, Claffey KP, Ancellin N, Liu CH, et al. 1999. Vascular endothelial cell adherens junction assembly and morphogenesis induced by sphingosine-1-phosphate. *Cell* 99:301–12
- 114a. Liu Y, Wada R, Yamashita T, Mi Y, Deng C-X, et al. 2000. Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation. *J. Clin. Invest.* 106:951–61
115. Goetzl EJ, Dolezalova H, Kong Y, Zeng L. 1999. Dual mechanisms for lysophospholipid induction of proliferation of human breast carcinoma cells. *Cancer Res.* 59:4732–37
116. Reumenapp U, Leummen G, Virchow S, Hanske J, Meyer zu Heringdorf D, et al. 2000. Sphingolipid receptor signaling and function in human bladder carcinoma cells: inhibition of LPA- but enhancement of thrombin-stimulated cell motility. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 361:1–11
117. Yatomi Y, Ruan F, Hakomori S, Igarashi Y. 1995. Sphingosine-1-phosphate: a platelet-activating sphingolipid released from agonist-stimulated human platelets. *Blood* 86:193–202
118. Yatomi Y, Igarashi Y, Yang L, Hisano N, Qi R, et al. 1997. Sphingosine 1-phosphate, a bioactive sphingolipid abundantly stored in platelets, is a normal constituent of human plasma and serum. *J. Biochem.* 121:969–73
119. Sachinidis A, Kettenhofen R, Seewald S, Gouni-Berthold I, Schmitz U, et al. 1999. Evidence that lipoproteins are carriers of bioactive factors. *Arterioscler. Thromb. Vasc. Biol.* 19:2412–21