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LYSOPHOSPHOLIPID RECEPTORS: Signaling and Biology

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Key Words lysophosphatidic acid, lysophosphatidylcholine, sphingosine 1-phosphate, sphingosylphosphorylcholine, G protein-coupled receptor, LPA, S1P

■ **Abstract** Lysophospholipids (LPs), such as lysophosphatidic acid and sphingosine 1-phosphate, are membrane-derived bioactive lipid mediators. LPs can affect fundamental cellular functions, which include proliferation, differentiation, survival, migration, adhesion, invasion, and morphogenesis. These functions influence many biological processes that include neurogenesis, angiogenesis, wound healing, immunity, and carcinogenesis. In recent years, identification of multiple cognate G protein-coupled receptors has provided a mechanistic framework for understanding how LPs play such diverse roles. Generation of LP receptor-null animals has allowed rigorous examination of receptor-mediated physiological functions in vivo and has identified new functions for LP receptor signaling. Efforts to develop LP receptor subtype-specific agonists/antagonists are in progress and raise expectations for a growing collection of chemical tools and potential therapeutic compounds. The rapidly expanding literature on the LP receptors is herein reviewed.

CONTENTS

INTRODUCTION 322
1. LYSOPHOSPHOLIPIDS 323
2. LYSOPHOSPHOLIPID RECEPTORS 330
2.1 Lysophosphatidic Acid Receptor 1 331
2.2 Lysophosphatidic Acid Receptor 2 332
2.3 Lysophosphatidic Acid Receptor 3 332
2.4 Lysophosphatidic Acid Receptor 4 333
2.5 Sphingosine 1-Phosphate Receptor 1 333
2.6 Sphingosine 1-Phosphate Receptor 2 334
2.7 Sphingosine 1-Phosphate Receptor 3 335

2.8 Sphingosine 1-Phosphate Receptor 4 336
2.9 Sphingosine 1-Phosphate Receptor 5 336
2.10 G2A 337
2.11 Ovarian Cancer G Protein-Coupled Receptor 1 337
2.12 G Protein-Coupled Receptor 4 338
2.13 G Protein-Coupled Receptor 12 338
2.14 Candidates for Additional LP Receptors 339
2.15 LP Receptor Agonists and Antagonists 339
3. NON-GPCR TARGETS FOR LYSOPHOSPHOLIPIDS 340
4. BIOLOGY OF LYSOPHOSPHOLIPIDS 341
4.1 Nervous System 341
4.2 Angiogenesis and Cardiovascular Development 343
4.3 Wound Healing 345
4.4 Immunity 345
4.5 Ovarian Cancer and Preservation of Female Reproduction 346
CONCLUDING REMARKS 348

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INTRODUCTION

Lysophospholipids (LPs) are quantitatively minor lipid species, compared to their major phospholipid counterparts (e.g., phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin) that structurally compose mammalian cell membranes. Prominent among the LPs are lysophosphatidic acid (LPA), sphingosine 1-phosphate (S1P), lysophosphatidylcholine (LPC), and sphingosylphosphorylcholine (SPC); all of which share rather simple chemical structures of a 3-carbon glycerol or sphingoid backbone on which is attached a single acyl chain of varied length and saturation (Figure 1). LPs were initially identified as precursors and metabolites in the de novo biosynthesis of phospholipids. They were subsequently observed to have properties resembling extracellular growth factors or signaling molecules, although the mechanisms of action for LPs remained unclear for decades after description of their bioactivities.

In recent years, LPs have been shown to act through sets of specific G protein-coupled receptors (GPCRs) in an autocrine or paracrine fashion. Since the identification of the first LP receptor, LPA_1, in 1996 (1), a growing family of GPCRs has been identified as high-affinity LP receptors in mammals (Table 1), with sequence and functional homologies that extend evolutionarily at least through Amphibia (2). The best characterized receptors have been renamed for their high-affinity ligands and constitute the LPA and S1P receptors (3), whereas other orphan receptor names have been maintained for those receptors with provisional ligand identities. For a given LP ligand, LP receptors generally share high amino acid similarities, although exceptions are also evident (Figure 2). One or more LP receptor genes and gene products are expressed in most mammalian tissues with spatially and temporally regulated expression patterns (Table 2). Many cell types express more than one LP receptor, and each receptor can couple

with multiple types of G proteins to activate a range of downstream effectors that mediate a variety of cellular responses upon LP stimulation (Table 2) [reviewed in (4)]. The identification of LP receptors has provided a major focus for understanding not only the signaling pathways activated by LPs but also their biology in vivo. This review focuses on our current knowledge about the LP receptors, with emphasis on their signaling and biological roles. We have deferred detailed discussion of the basic biochemistry and biosynthesis/metabolism of the LPs, along with in-depth specialty areas, to many excellent reviews and the papers cited therein (5–18).

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1. LYSOPHOSPHOLIPIDS

LPA (1-acyl-2-hydroxy-*sn*-glycero-3-phosphate) and S1P are in many ways prototypic examples of LPs relevant to this review. Early hints of a role for LPA as a biological effector molecule were recognized during the 1960s (19, 20), and an increasingly diverse range of physiological actions were identified in the ensuing decades, including effects on blood pressure, platelet activation, and smooth muscle contraction (21–23). A wealth of cell biological studies in the mid-1980s defined a variety of cellular effects, which include cell growth, cell rounding, neurite retraction, and actin stress fiber formation [reviewed in (24)]. These findings suggested the existence of specific receptors that could mediate the effects of this small lipid. However, biophysical properties of LPA or the possibility of second messenger activities were also proposed as competing mechanisms for LPA actions, and this mechanistic ambiguity persisted in the absence of identified receptors [reviewed in (4)]. The identification of cloned LPA receptors, combined with molecular genetic strategies to establish their functions, allowed determination of both signaling and biological effects that are dependent on receptor mechanisms. Activation of these LPA receptors demonstrated that a range of downstream signaling cascades mediate LPA signaling. These include mitogen-activated protein kinase (MAPK) activation, adenylyl cyclase (AC) inhibition/activation, phospholipase C (PLC) activation/Ca^{2+} mobilization, arachidonic acid release, Akt/PKB activation, and the activation of small GTPases, Rho, Rac, and Ras (Table 2). It is critical to note that the actual pathway and realized end point are dependent on a range of variables that include receptor usage, cell type, expression level of a receptor or signaling protein, and LPA concentration. Many discrepancies in the literature can be explained by the different experimental conditions employed.

LPA is produced from activated platelets, activated adipocytes, neuronal cells, and other cell types [reviewed in (4, 8)]. Although mechanisms of LPA synthesis in individual cell types remain to be elucidated, serum LPA is produced by multiple enzymatic pathways that involve monoacylglycerol kinase, phospholipase A_1, secretory phospholipase A_2, and lysophospholipase D (lysoPLD), including autotaxin [reviewed in (9, 26)]. Several enzymes are involved in LPA

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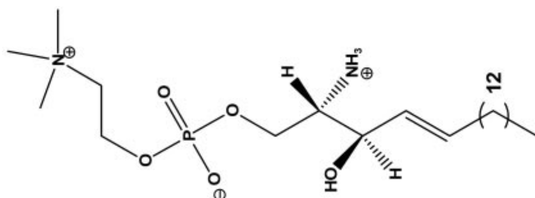

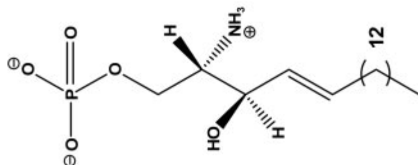

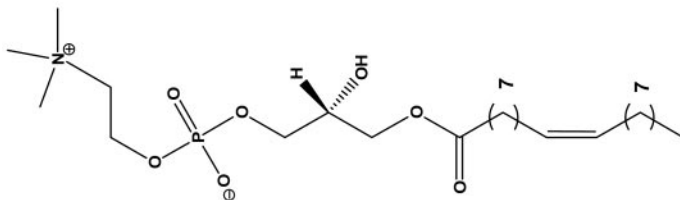

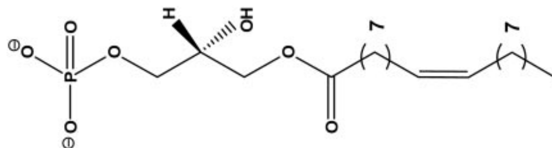

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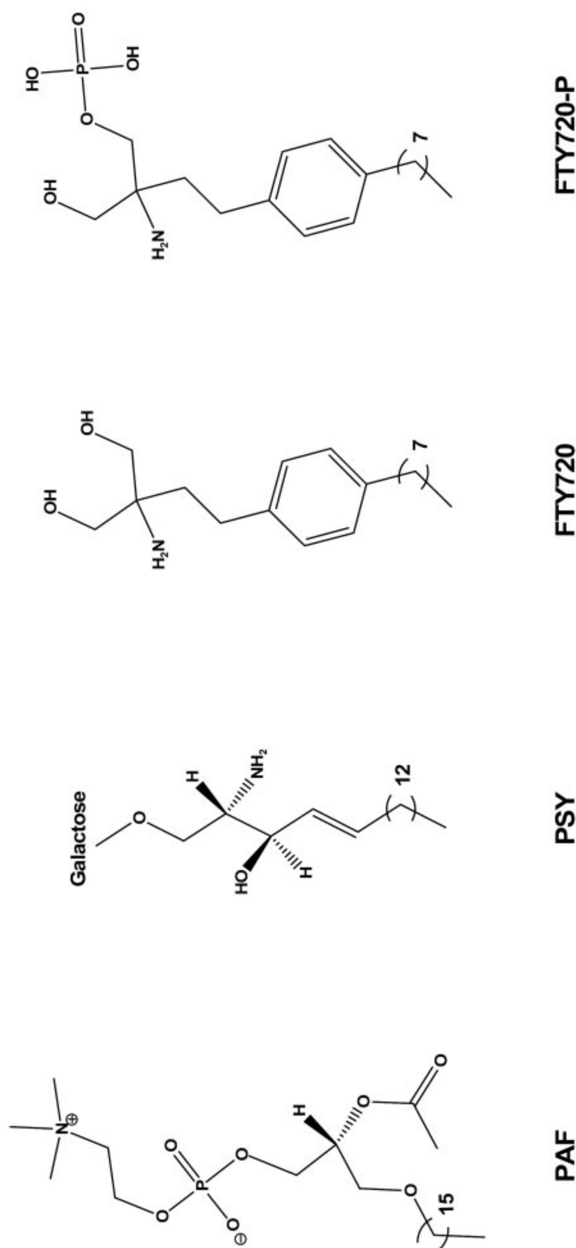

Figure 1 Chemical structures of signaling lysophospholipids and two related bioactive lipids, platelet-activating factor (PAF) and psychosine (PSY). LPA, (1-oleoyl) lysophosphatidic acid; LPC, (1-oleoyl) lysophosphatidylcholine; S1P, sphingosine 1-phosphate; SPC, sphingosylphosphorylcholine; FTY720-P, the phosphorylated form of FTY720.

TABLE 1 Mammalian lysophospholipid receptors

Receptors (Synonyms)	Species	Accession number	Amino acids	Predicted MW (kDa)	Chromosomal location
LPA_1	Human	(NM_057159)	364	41.1	9q32
(EDG-2/VZG-1)	Mouse	(NM_010336)	364	41.2	4 B3
	Rat	(NM_053936)	364	41.1	5q22
LPA_2	Human	(NM_004720)	351	39.1	19p12
(EDG-4)	Mouse	(XM_193070)	348	39.0	8 B3.3
LPA_3	Human	(NM_012152)	353	40.1	1p22.3-p31.1
(EDG-7)	Mouse	(NM_022983)	354	40.3	3 H2
	Rat	(AB051164)	354	40.3	1q55
LPA_4	Human	(NM_005296)	370	41.9	Xq13-q21.1
($p2y_9$/GPR23)	Mouse	(NM_175271)	370	41.9	X D
$S1P_1$	Human	(NM_001400)	382	42.8	1p21
(EDG-1/LP_{B1})	Mouse	(NM_007901)	382	42.6	3 G1
	Rat	(NM_017301)	383	42.7	2q41
$S1P_2$	Human	(NM_004230)	353	38.8	19p13.2
(EDG-5/AGR16/	Mouse	(XM_134731)	352	38.8	9 A3
H218/LP_{B2})	Rat	(NM_017192)	352	38.7	5q36
$S1P_3$	Human	(NM_005226)	378	42.3	9q22.1-q22.2
(EDG-3/LP_{B3})	Mouse	(NM_010101)	378	42.3	13 B1
$S1P_4$	Human	(NM_003775)	384	41.6	19p13.3
(EDG-6/LP_{C1})	Mouse	(NM_010102)	386	42.3	10 C1
$S1P_5$	Human	(NM_030760)	398	41.8	19p13.2
(EDG-8/NRG-1	Mouse	(NM_053190)	400	42.3	9 A3
LP_{B4})	Rat	(NM_021775)	400	42.4	5q36
G2A	Human	(NM_013345)	380	42.5	14q32.3
	Mouse	(NM_019925)	382	42.7	12 F2
OGR1	Human	(NM_003485)	365	41.1	14q31
(GPR68)	Mouse	(NM_175493)	365	41.2	12 E
	Rat	(XM_234483)	365	41.2	6q32
GPR4	Human	(NM_005282)	362	41.0	19q13.3
	Mouse	(NM_175668)	365	41.1	7 A1
	Rat	(NM_218415)	365	41.3	1q21
GPR12	Human	(NM_005288)	334	36.7	13q12
(GPCR01)	Mouse	(NM_008151)	334	36.6	5 G3
	Rat	(NM_030831)	334	36.7	12p11
TDAG8[a]	Human	(NM_003608)	337	39.3	14q31-q32.1
(GPCR25/GPR65)	Mouse	(NM_008152)	337	39.4	12 E
PAFR[a]	Human	(NM_000952)	342	39.2	1p35-p34.3
	Mouse	(D50872)	341	39.1	4 D2.2

[a]Not a lysophospholipid receptor

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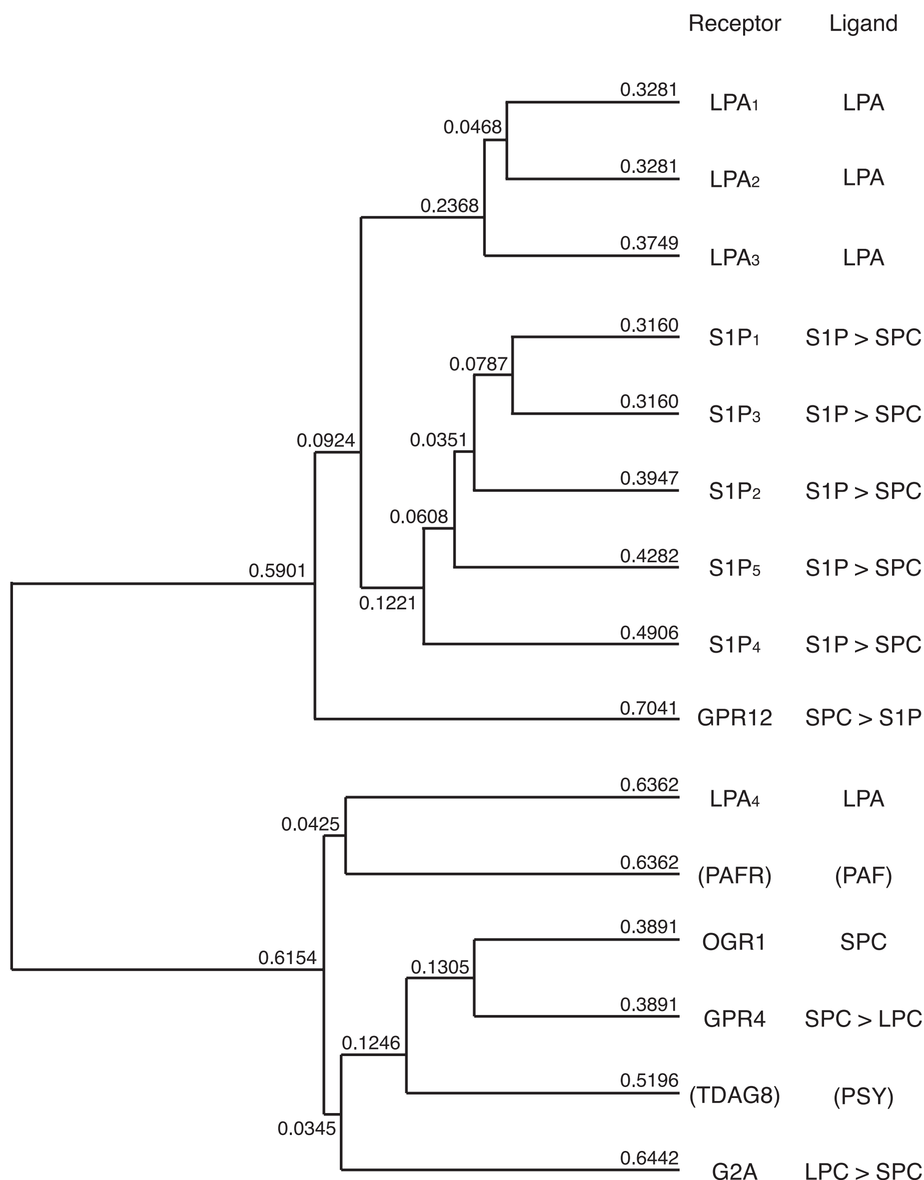

Figure 2 Phylogenic tree of the human lysophospholipid receptor family. For comparison, receptors for two related but distinct lipids are also shown in parentheses, the platelet activating factor (PAF) receptor and the putative psychosine receptor (TDAG8). The tree was derived by the neighbor joining method run on a Genetyx-Mac program (Genetyx Corp., Tokyo, Japan). The amino acid sequence divergence between any pair of sequences is equal to the sum of the lengths of the horizontal branches connecting two sequences. Preferential ligands for each receptor are aligned with the potency orders.

TABLE 2 Lysophospholipid receptor signaling and distribution in mice

Receptors	G-protein coupling	Cellular signaling	Tissue distribution in mice
LPA_1	$G_{i/o}$, G_q, $G_{12/13}$	[a]DNA ↑, SRE ↑, MAPK ↑, AC ↓, PLC/Ca ↑, Rho ↑, PI3K/Akt ↑	Ubiquitous
LPA_2	$G_{i/o}$, G_q, $G_{12/13}$	DNA ↑, SRE ↑, MAPK ↑, AC ↓, PLC/Ca ↑, Rho ↑, PI3K/Akt ↑	Ubiquitous
LPA_3	$G_{i/o}$, G_q, G_s	MAPK ↑, AC ↑ ↓, PLC/Ca ↑	Ubiquitous
LPA_4	Unknown	Ca ↑, AC ↑	(Ov, Pa, Th in human)
$S1P_1$	$G_{i/o}$	MAPK ↑, AC ↓, PLC/Ca ↑, (Rho ↑), Rac ↑, PI3K/Akt ↑	Ubiquitous
$S1P_2$	$G_{i/o}$, G_q, $G_{12/13}$, G_s	SRE ↑, MAPK ↑, AC ↑, PLC/Ca ↑, Rho ↑, Rac ↓	Ubiquitous
$S1P_3$	$G_{i/o}$, G_q, $G_{12/13}$, G_s	SRE ↑, MAPK ↑, AC ↑ ↓, PLC/Ca ↑, Rho ↑, Rac ↑, PI3K/Akt ↑	Ubiquitous
$S1P_4$	$G_{i/o}$, $G_{12/13}$, G_s	MAPK ↑, AC ↑, PLC/Ca ↑, Rho ↑	Ln, Sp, Lg, Th
$S1P_5$	$G_{i/o}$, $G_{12/13}$	DNA ↓, MAPK ↓, AC ↓, PLC/Ca ↑	Br, Sk, Sp
G2A	$G_{i/o}$, G_q, $G_{12/13}$, G_s	SRE ↑, MAPK ↑, AC ↑, PLC/Ca ↑, Rho ↑, Rac ↑, Ras ↑	Th, Sp, Bm
OGR1	$G_{i/o}$, G_q	DNA ↓, MAPK ↑, PLC/Ca ↑	(Ubiquitous in human)
GPR4	$G_{i/o}$	DNA ↑, SRE ↑, MAPK ↑, PLC/Ca ↑	(Ubiquitous in human)
GPR12	$G_{i/o}$, G_s	AC ↑, PLC/Ca ↑	Br, Ts, Li
TDAG8	G_q, G_z	AC ↓, PLC/Ca ↑	Th, Sp, Bm

[a]Abbreviations used are: DNA, DNA synthesis (proliferation); SRE, serum-responsive element; MAPK, mitogen-activated protein kinase; AC, adenylyl cyclase; PLC, phospholipase C; PI3K, phosphoinositide 3-kinase; Ov, ovary; Pa, pancreas; Th, thymus; Ln, lymph node; Sp, spleen; Lg, lung; Br, brain; Sk, skin; Ts, testis; Li, liver; and Bm, bone marrow.

degradation: lysophospholipase, lipid phosphate phosphatase, and LPA acyl transferase such as endophilin [reviewed in (9)]. LPA concentrations in human serum are estimated to be 1–5 μM (27). Serum LPA is bound to albumin, low-density lipoproteins, or other proteins, which possibly protect LPA from rapid degradation [reviewed in (9)]. LPA molecular species with different acyl chain lengths and saturation are naturally occurring, including 1-palmitoyl (16:0), 1-palmitoleoyl (16:1), 1-stearoyl (18:0), 1-oleoyl (18:1), 1-linoleoyl (18:2), and 1-arachidonyl (20:4) LPA (27) [reviewed in (8)]. Quantitatively minor alkyl LPA has biological activities similar to acyl LPA (28), and different

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LPA species activate LPA receptor subtypes with varied efficacies (29). Thus far, three LPA receptors (LPA_1-LPA_3) that share high amino acid sequence similarity have been identified and are complemented by a fourth (LPA_4) that is dissimilar.

S1P represents the second major prototype of bioactive LPs whose biological effects were first described in the early 1990s. S1P was found to induce Ca^{2+} mobilization from internal Ca^{2+} stores (30), and it was subsequently observed to stimulate fibroblast proliferation and morphological changes (31). These phenomena were attributed to S1P acting as a second messenger in fibroblast proliferation induced by platelet-derived growth factor (PDGF) and serum (32). Like inositol triphosphate, S1P was proposed to act as a Ca^{2+}-mobilizing second messenger that is produced intracellularly from membranes upon cell activation [reviewed in (6)], and a receptor-independent mechanism remains a viable explanation for some S1P-mediated effects (33). However, following the functional identification of LPA receptors, multiple S1P receptors were also identified based on their shared sequence similarities [reviewed in (4, 6, 34)]. S1P has been shown to exert most of its previously documented effects through cell surface receptors with signaling pathways and cellular/organismal physiologies that are comparable to LPA (Table 2) [reviewed in (4, 6, 8, 35)]. Thus far, five high-affinity receptors ($S1P_1$-$S1P_5$) and a possible low-affinity receptor (GPR12) have been identified in mammals, and additional receptors are likely to exist (Figure 2).

S1P, unlike LPA, is stored in platelets at relatively high concentrations and released from platelets upon activation (36, 37) [reviewed in (10)]. S1P in serum is bound to albumin or lipoproteins, and its concentration is estimated to be 0.5–0.8 μM in human serum and 0.2–0.4 μM in human plasma [reviewed in (11)]. S1P is synthesized exclusively from sphingosine by sphingosine kinases and is degraded either by S1P lyases or by S1P phosphatases [reviewed in (10, 12)]. As a calcium-mobilizing second messenger, intracellular S1P homeostasis maintains low concentrations by appropriately balanced synthesis and degradation. Platelets are the primary source of S1P in serum because of the presence of sphingosine kinase and the absence of S1P lyase [reviewed in (10)]. Recent genetic-null studies in lower organisms indicate that deficiency of S1P lyase leads to abnormal development of *Dictyostelium discoideum*, *Caenorhabditis elegans*, or *Drosophila melanogaster* (38–40). In *Drosophila*, most of the examined 31 genes, whose mammalian homologues were shown to be involved in LPA or S1P metabolism, are expressed in spatio-temporal patterns that suggest physiological roles for LPs during *Drosophila* development (41).

In addition to LPA and S1P, two phosphorylcholine-containing LPs, SPC and LPC, have been shown to induce a variety of biological responses [reviewed in (13, 42)]. SPC induces cell proliferation, migration, and cytoskeletal rearrangement; all are actions shared by the structurally related ligand S1P. SPC may activate high-affinity S1P receptors as a lower affinity ligand (Figure 2), although it can also be converted to S1P by enzymes like autotaxin, underscoring a need for cautious interpretation of observed effects. Moreover, SPC mobilizes Ca^{2+}

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from internal stores as does S1P, suggesting common sites of action and mechanisms for SPC and S1P [reviewed in (13, 14)]. It is notable, however, that SPC has also been shown to act differentially from S1P, as observed in human platelets in which S1P initiates, but SPC inhibits, activation (43). SPC can be produced from sphingomyelin by sphingomyelin deacylase, but little is known about the synthesis/degradation of SPC in a physiological context [reviewed in (13)]. Elevated SPC levels were observed in some tissues of Niemann–Pick disease patients, who lack sphingomyelinase that degrades sphingomyelin, but the relevance of SPC to the observed pathological conditions remains unclear [reviewed in (13)].

LPC is present as a component of oxidized low-density lipoprotein (LDL); it has been proposed to be involved in atherosclerosis and inflammatory diseases [reviewed in (14, 42)]. It can be produced from phosphatidylcholine by phospholipase A_2. Compared to other signaling LPs, the physiological concentrations of LPC in body fluids including blood and ascitic fluid can be very high (5–180 μM), and LPC is capable of cell lysis at >30 μM, which suggests the operation of nonreceptor actions (that include toxicity) in addition to receptor-based activities in considering LPC actions [reviewed in (14, 42)]. Possible high-affinity receptors for both lipids have been reported; these include three high-affinity SPC receptors (OGR1, GPR4, and GPR12) and one high-affinity LPC receptor (G2A) (44–47). The identity of OGR1 and GPR4 as bona fide SPC receptors is currently unclear in view of a recent report finding them unresponsive to SPC (48), and this receptor group in general requires additional analysis. Further characterization of these receptors will clarify ambiguities, and they are reviewed while noting these caveats.

2. LYSOPHOSPHOLIPID RECEPTORS

A growing number of receptors are reported to interact with lysophospholipids, albeit with variable apparent affinities. The best characterized are those for LPA (LPA_1-LPA_4) and S1P ($S1P_1$-$S1P_5$ and possible low-affinity interactions with GPR12). In addition, provisional identifications were reported for SPC receptors (OGR1, GPR4, GPR12, $S1P_1$-$S1P_5$, and G2A) and LPC receptors (G2A and GPR4). The receptors and relative ligand interactions are listed in Figure 2. Historical details on identification of the first LP receptors were previously covered (4, 25). Many of the LP receptor genes have been referred to by different names, such as *EDGs* (*Endothelial Differentiation Genes*). However, this review adopts the recommended IUPHAR nomenclature that is based on the optimal biological ligand for a given receptor (Table 1) (3). The LP receptors generally share high amino acid similarity within a ligand group and also share some similarity with other GPCRs for structurally related bioactive lipids, such as platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphorylcholine), psychosine (Figure 1), and endogenous cannabinoids [reviewed in (4)].

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Here we review the basic characteristics of each LP receptor, which include gene structures, signaling properties, tissue distribution, cellular functions, and in vivo roles, particularly as revealed by genetic-null studies.

2.1 Lysophosphatidic Acid Receptor 1

LPA_1 (previously called VZG-1/EDG-2/mrec1.3) was the first identified, high-affinity receptor for LPA (1) [reviewed in (4)]. The mammalian (human, mouse, and rat) lpa_1 genes encode 41-kDa proteins consisting of 364 amino acids with 7 putative transmembrane domains (Table 1). The mouse receptor is encoded by two of five exons with a characteristic conserved intron in transmembrane domain 6 that is shared with lpa_2 and lpa_3, and amino terminus isoforms generated by alternative exon usage exist (49). Functional analyses with mammalian heterologous receptor expression systems reveal the multi-functionality of this receptor [reviewed in (4, 25)]. LPA_1 couples with three types of G proteins, $G_{i/o}$, G_q, and $G_{12/13}$ (50, 51). Through activation of these G proteins, LPA induces a range of cellular responses through LPA_1: cell proliferation, serum-response element (SRE) activation, MAPK activation, AC inhibition, PLC activation, Ca^{2+} mobilization, Akt activation, and Rho activation (Table 2) [reviewed in (4, 25)].

Wide expression of lpa_1 is observed in adult mice, with clear presence in testis, brain, heart, lung, small intestine, stomach, spleen, thymus, and skeletal muscle (25). Similarly, human tissues also express lpa_1; it is present in brain, heart, placenta, colon, small intestine, prostate, testis, ovary, pancreas, spleen, kidney, skeletal muscle, and thymus (52). In situ hybridization studies reveal varied patterns of expression within a single tissue (1, 53, 54).

The nervous system is a major locus for lpa_1 expression; there it is spatially and temporally regulated throughout brain development [reviewed in (4, 25)]. Its embryonic central nervous system (CNS) expression is restricted to the neocortical neurogenic region called ventricular zone, which disappears at the end of cortical neurogenesis, just before birth (53). During postnatal life, lpa_1 expression is apparent in and around developing white matter tracts, and its expression coincides with the process of myelination (53). In situ hybridization and immunohistochemistry show that oligodendrocytes, the myelinating cells in the CNS, express lpa_1 in mammals (53, 55, 56). In addition, Schwann cells, the myelinating cells of the peripheral nervous system, also express lpa_1 (57), which is involved in regulating Schwann cell survival and morphology (57, 58). These observations identify important functions for receptor-mediated LPA signaling in neurogenesis, cell survival, and myelination.

The targeted disruption of lpa_1 in mice revealed unanticipated in vivo functions of this receptor (59). The $lpa_1^{(-/-)}$ mice show ~ 50% lethality in the perinatal period in a mixed genetic background. Survivors have reduced body size, craniofacial dysmorphism with flattened facies, and increased apoptosis in sciatic nerve Schwann cells (58, 59). Defective suckling, attributable to olfactory defects, likely accounts for neonatal lethality. Small fractions of $lpa_1^{(-/-)}$ embryos have exencephaly (~5%) or frontal cephalic hemorrhage (~2.5%). Loss of LPA responsivity in embryonic

neuroblasts and fibroblasts demonstrates nonredundant functions and roles for lpa_1 in vivo (59, 60).

2.2 Lysophosphatidic Acid Receptor 2

LPA_2 (EDG-4 nonmutant form) was identified from GenBank homology searches of orphan GPCR genes [reviewed in (4)]. A related carboxyl-terminus mutant termed EDG-4 that was isolated from a neoplasm (52) is not present in wild-type genomes, and it should not be confused with wild-type LPA_2. The mouse LPA_2 gene contains three exons with the coding region in exons two and three. As with LPA_1, LPA_2 couples with three types of G proteins, $G_{i/o}$, G_q, and $G_{12/13}$, to mediate LPA-induced cellular signaling (Table 2) [reviewed in (4, 25)]. Expression of lpa_2 is observed in the testis, kidney, lung, thymus, spleen, and stomach of adult mice (25) and in the human testis, pancreas, prostate, thymus, spleen, and peripheral blood leukocytes (52). Expression of lpa_2 is upregulated in various cancer cell lines, and several human lpa_2 transcriptional variants with mutations in the 3′-untranslated region have been observed (see Section 4.5).

Targeted deletion of lpa_2 in mice (60) does not result in any obvious phenotypic abnormalities. However, significant loss of normal LPA signaling (e.g., PLC activation, Ca^{2+} mobilization, and stress fiber formation) is observed in primary cultures of mouse embryonic fibroblasts (MEFs) (60). Creation of $lpa_1^{(-/-)}lpa_2^{(-/-)}$ double-null mice (60) does not reveal obvious additional phenotypic abnormalities beyond those attributable to $lpa_1^{(-/-)}$ except for a higher incidence of frontal cephalic hemorrhage (26% versus 2.5% in $lpa_1^{(-/-)}$ mice); however, more subtle phenotypes may be present (59, 60), along with effects observed under gain-of-function conditions (see Section 4.1). Importantly, many LPA-induced responses, which include cell proliferation, AC inhibition, PLC activation, Ca^{2+} mobilization, JNK and Akt activation, and stress fiber formation, are absent or severely reduced in double-null MEFs. All these responses, except for AC inhibition (AC inhibition is nearly abolished in $lpa_1^{(-/-)}$ MEFs), are only partially affected in either $lpa_1^{(-/-)}$ or $lpa_2^{(-/-)}$ MEFs (60). These results indicate that LPA_2 contributes to normal LPA-mediated signaling responses in at least some cell types.

2.3 Lysophosphatidic Acid Receptor 3

LPA_3 (EDG-7) was isolated as an orphan GPCR gene by degenerate polymerase chain reaction (PCR)-based cloning and homology searches: As with the three exon structure of the LPA_2 gene, LPA_3 is also encoded by exons two and three (61, 62) [reviewed in (4)]. The LPA_3 receptor is distinct from LPA_1 and LPA_2 in its ability to couple with $G_{i/o}$ and G_q but not $G_{12/13}$ (51) and is much less responsive to LPA species with saturated acyl chains (61, 62). Nonetheless, LPA_3 can mediate pleiotropic LPA-induced signaling that includes PLC activation, Ca^{2+} mobilization, AC inhibition/activation, and MAPK activation (51, 61, 62). LPA_3 has variable effects on AC that likely depend on cell type and expression levels.

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This could explain the elevated intracellular cAMP levels seen in Sf9 insect cells compared to slightly decreased levels in neuronal cells or no change in cAMP levels in hepatoma cells (51, 61, 62). Additionally, LPA_3 does not couple to actomyosin machinery that produces cell rounding in neuronal cells in which $G_{12/13}$ and Rho are involved (51). Overexpression of LPA_3 in neuroblastoma cells leads, surprisingly, to neurite elongation, whereas that of LPA_1 or LPA_2 results in neurite retraction and cell rounding when stimulated with LPA (51). Null receptor mutations for LPA_3 have not yet been reported. Expression of lpa_3 is observed in adult mouse testis, kidney, lung, small intestine, heart, thymus, and brain (25). In humans, it is found in the heart, pancreas, prostate, testis, lung, ovary, and brain (frontal cortex, hippocampus, and amygdala) (61, 62).

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2.4 Lysophosphatidic Acid Receptor 4

LPA_4 ($p2y_9$/GPR23) was identified from orphan GPCR gene libraries within another evolutionary branch of the LP receptor superfamily (Figure 2). Unlike the other three LPA receptors, LPA_4 is encoded by a single exon. The orphan receptor, $p2y_9$/GPR23, is a functional high-affinity LPA receptor (K_d = 45 nM) that is now classified as LPA_4 (63). It is of divergent sequence compared to LPA_1-LPA_3 with closer similarity to the PAF receptor. LPA_4 mediates LPA-induced Ca^{2+} mobilization and cAMP accumulation, and functional coupling to G_s for AC activation is probable, although coupling preferences to other G proteins are currently unknown. Among 16 human tissues tested with quantitative real-time PCR, the lpa_4 gene is expressed at very high levels in the ovary and, to a much lesser extent, in the pancreas, thymus, and human kidney and skeletal muscle (63). Its physiological roles are currently unknown.

2.5 Sphingosine 1-Phosphate Receptor 1

$S1P_1$ (EDG-1/LP_{B1}) was the first identified S1P receptor that was initially isolated as an orphan GPCR in human endothelial cells, and it was later shown to encode a high-affinity (K_d = 8 nM) S1P receptor. The $S1P_1$ gene contains two exons with the coding region entirely on exon two [reviewed in (4)]. The $S1P_1$ receptor primarily couples with PTX-sensitive $G_{i/o}$ proteins and mediates S1P-induced MAPK activation, AC inhibition, PLC activation, Ca^{2+} mobilization, cell aggregation, Rac (and often Rho) activation, and cell migration [reviewed in (4, 15–17)]. Akt-mediated phosphorylation of $S1P_1$ is required for Rac activation, cortical actin assembly, and cell migration, but not for $G_{i/o}$-dependent signaling in endothelial cells (64). $S1P_1$ mutants lacking an intracellular Akt phosphorylation site (Thr^{236} in human, mouse, and rat) act as dominant negatives and inhibit S1P-induced chemotaxis and angiogenesis (64). Computational modeling suggests that basic amino acids, Arg^{120} and Arg^{292}, may form an ion-pair with the phosphate group of S1P, whereas the acidic Glu^{121} residue forms an ion pair with the ammonium moiety of S1P (65).

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Several lines of evidence suggest that $S1P_1$ signaling is involved in PDGF-induced cellular responses. First, PDGF-induced Src activation, focal adhesion kinase activation, and cell migration are defective in $s1p_1^{(-/-)}$ MEFs, yet neither PDGF receptor autophosphorylation nor DNA synthesis are altered in this setting (66, 67). Second, PDGF activates sphingosine kinase, inducing its membrane translocation (66), which may increase S1P levels in local areas where the $S1P_1$ receptor is present (68, 69). Third, immunoprecipitation experiments suggest possible protein interactions between the PDGF receptor and $S1P_1$ (68, 69), although this finding may not be universal and requires further examination (70).

Expression of $s1p_1$ is pervasive, including spleen, brain, heart, lung, adipose tissues, liver, thymus, kidney, and skeletal muscle (71, 72). The deletion of $s1p_1$ in mice results in embryonic lethality (73). The $s1p_1^{(-/-)}$ embryos appear to be normal by E11.5, but they are identifiable at E12.5 by their edematous yolk sac with less blood. All $s1p_1^{(-/-)}$ embryos show hemorrhage at E12.5 to E14.5 and fail to survive beyond E14.5. Vasculogenesis and angiogenesis are normal, contrasting with vascular maturation that is incomplete because of defects in surrounding vascular smooth muscle cells (VSMCs)/pericytes in $s1p_1^{(-/-)}$ embryos (73). S1P-induced Rac activation and cell migration are both defective in $s1p_1^{(-/-)}$ MEFs, suggesting that defects in VSMC/pericyte migration result in vascular immaturity that leads to embryonic death (73). Recent studies utilizing cell type-specific $s1p_1$ deletion in mice via a *Cre/loxP* system show that $S1P_1$ receptors in vascular endothelial cells (VECs), rather than those in VSMCs, are responsible for the constitutive deletion phenotype (74) (see Section 4.2).

2.6 Sphingosine 1-Phosphate Receptor 2

$S1P_2$ (EDG-5/AGR16/H218/LP_{B2}) was first isolated as an orphan GPCR gene from rat cardiovascular and nervous systems. $S1P_2$ was later identified by many groups as a high-affinity (K_d = 20–27 nM) S1P receptor and low-affinity SPC receptor, and it is also encoded on a single exon [reviewed in (4)]. It couples with $G_{i/o}$, G_q, $G_{12/13}$, and possibly G_s, and it can mediate S1P-induced cell proliferation, cell survival, cell rounding, SRE activation, MAPK activation, AC activation, PLC activation, Ca^{2+} mobilization, and Rho activation [reviewed in (4, 15–17)]. $S1P_2$ inhibits Rac activity and prevents cell migration (75), contrasting with $S1P_1$ [reviewed in (17)], although it appears that $S1P_2$ can also produce counteracting signals (G_i versus $G_{12/13}$-Rho pathways) to influence cell migration (76).

Expression of $s1p_2$ is widespread; it is present in heart, lung, thymus, brain, liver, kidney, spleen, adipose tissues, and all other tissues tested in adult mouse (71, 72) and in lung, heart, stomach, intestine, and adrenal glands in rat (77). During early stages of rat CNS development, $s1p_2$ may be expressed in young, differentiating neuronal cell bodies and axons (78), although no significant expression is observed during embryonic mouse development (134).

Vertebrate $s1p_2$ genetic models include a zebra fish (*Danio rerio*) mutant (see Section 4.2) as well as $s1p_2$-null mice generated by two independent groups (79,

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80). One report indicates that $s1p_2^{(-/-)}$ mice do not show anatomical/histological defects but have spontaneous and sporadic seizures, which were occasionally lethal (79). Electroencephalographic abnormalities are also observed both during and between seizures, and whole-cell patch-clamp recording revealed a significant increase in the excitability of neocortical pyramidal neurons (79). Independently generated $s1p_2^{(-/-)}$ mice also do not show gross phenotypic abnormalities nor evidence of seizure activity. However, a slight but statistically significant decrease in litter size is observed (80). The $s1p_2^{(-/-)}$ mice are born at the expected Mendelian ratios without sexual bias, and they are fertile and healthy. However, a significant loss of S1P-induced intracellular signaling is observed in $s1p_2^{(-/-)}$ MEFs. Wild-type MEFs express $s1p_1$, $s1p_2$, and $s1p_3$ but neither $s1p_4$ nor $s1p_5$. The observation that S1P-induced Rho activation is significantly impaired while PLC activation/Ca^{2+} mobilization remains intact in $s1p_2^{(-/-)}$ MEFs indicates that $S1P_2$ is critical for S1P-induced Rho activation but not for PLC activation/Ca^{2+} mobilization (80). The discrepancy in phenotypes, observed between two groups of $s1p_2^{(-/-)}$ mice, may derive from differences in mouse genetic backgrounds used for analyses. All these results indicate that $S1P_2$ has identifiable signaling properties in MEFs, and it plays a minor yet discernible role in normal mouse development.

2.7 Sphingosine 1-Phosphate Receptor 3

$S1P_3$ (EDG-3/LP_{B3}) was isolated as an orphan GPCR gene by degenerate PCR-based cloning from a human genomic DNA library (81). Like $S1P_2$, $S1P_3$ is a high-affinity (K_d = 23–26 nM) S1P receptor and low-affinity SPC receptor and is encoded on a single exon [reviewed in (4)]. $S1P_3$ is evolutionarily more related to $S1P_1$ than to $S1P_2$ (Figure 2), but the intracellular signaling mediated by $S1P_3$ appears to resemble that of $S1P_2$, except for regulation of Rac (75) [reviewed in (17)]. The $S1P_3$ receptor couples with $G_{i/o}$, G_q, $G_{12/13}$, and possibly G_s, and it induces cell proliferation, cell survival, cell rounding, MAPK activation, SRE activation, AC activation/inhibition, PLC activation, Ca^{2+} mobilization, Rho activation, Rac activation, and cell migration [reviewed in (4, 15–17)].

Expression of $s1p_3$ is widespread; it is present in the spleen, heart, lung, thymus, kidney, testis, brain, and skeletal muscle in adult mice (71, 72) and, in humans, in the heart, placenta, kidney, liver, pancreas, skeletal muscle, lung, and brain (81). Targeted disruption of $s1p_3$ in mice results in no obvious abnormality (72). The $s1p_3^{(-/-)}$ mice are born at the expected Mendelian ratios without sexual bias, are fertile, and appear healthy. The litter size from $s1p_3^{(-/-)}$ crosses is modestly smaller (5.6 pups per litter) than that from $s1p_3^{(+/-)} \times$ wild-type crosses (7.5 pups per litter), but the reason for this is unknown (72). However, significant loss of S1P signaling is observed in $s1p_3^{(-/-)}$ MEFs that normally express only three of the five $s1p$ genes ($s1p_{1,2,3}$) (80). S1P-induced PLC activation and Ca^{2+} mobilization are abolished, but Rho activation and AC inhibition remain intact in $s1p_3^{(-/-)}$ MEFs (72). In vitro functional analyses reveal that $S1P_3$ and $S1P_2$ can mediate many of the same S1P responses except for Rac regulation [reviewed in

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(17)]. However, the results from analyses of MEFs demonstrate that $S1P_3$ is indispensable for PLC activation/Ca^{2+} mobilization. Nevertheless, the *s1p*$_3$ and *s1p*$_2$ genes are coexpressed in many mouse tissues (72), and functional redundancy might exist in vivo in other cell types. Production of $s1p_2^{(-/-)}s1p_3^{(-/-)}$ double-null mice (80) results in a clear phenotype of reduced litter sizes compared to single-null crosses, and most of the $s1p_2^{(-/-)}s1p_3^{(-/-)}$ pups do not survive beyond the first three postnatal weeks (only 1.2 pups per litter survived beyond three weeks) (80). Surprisingly, the double-null survivors display no obvious phenotype and are fertile. Although *s1p*$_3$ deletion alone does not significantly affect S1P-induced Rho activation, *s1p*$_2$ deletion partially impairs it, and deletion of both receptors eliminates it in MEFs (80). These data demonstrate that $S1P_2$ and $S1P_3$ can function redundantly in vivo, whereas elimination of both receptors in mice results in marked perinatal lethality and S1P signaling defects. The reasons for the lethality remain to be elucidated.

2.8 Sphingosine 1-Phosphate Receptor 4

$S1P_4$ (EDG-6/LP_{C1}) was isolated from in vitro differentiated human and murine dendritic cells (82) as an orphan GPCR gene. It was found to encode a high-affinity (K_d = 13–63 nM) receptor for S1P and a low-affinity receptor for SPC (83, 84) and is also encoded on a single exon [reviewed in (4)]. Its comparatively low amino acid sequence similarity compared to the other high-affinity S1P receptors (Figure 2) suggested that this receptor could have a distinct, preferred ligand (4, 85), and indeed, phytosphingosine 1-phosphate (4D-hydroxysphinganine 1-phosphate) has a 50-fold higher affinity for $S1P_4$ (at 1.6 nM) compared to S1P itself in at least one assay system (86). Virtually all other analyses on $S1P_4$ were conducted using S1P as the activating ligand, which could have relevance to reported signaling properties of $S1P_4$ in some biological settings. $S1P_4$ couples with $G_{i/o}$, $G_{12/13,}$ and possibly G_s, and it mediates S1P-induced MAPK activation, PLC activation, Ca^{2+} mobilization, AC activation, Rho activation, cytoskeletal rearrangement (stress fiber formation and cell rounding), and cell motility (72, 83, 84, 87).

Unlike *s1p*$_1$– *s1p*$_3$ receptors, *s1p*$_4$ expression is restricted in human and mouse to lymph node, spleen, lung, and thymus (72, 82). This expression pattern suggests potential roles of $S1P_4$ in the immune system. In vivo roles and functions of $S1P_4$ are still unknown.

2.9 Sphingosine 1-Phosphate Receptor 5

$S1P_5$ (EDG-8/LP_{B4}) was isolated as an orphan GPCR gene from rat pheochromocytoma 12 (PC12) cells (88), and it was later found to encode a high-affinity S1P receptor (K_d = 2–10 nM) and low-affinity SPC receptor (89–91) that also has a single exon coding region [reviewed in (4)]. Expression of *s1p*$_5$ is restricted to specific tissues: brain, spleen, and peripheral blood leukocytes in human and brain, skin, and spleen in rat and mouse (72, 88, 89). In rat brain, *s1p*$_5$ is

predominantly expressed in white matter tracts and cells of oligodendrocyte lineage (89, 92), suggesting its potential roles in maturation and myelination of oligodendrocytes. $S1P_5$ can couple with $G_{i/o}$ and $G_{12/13}$, and it mediates S1P-induced AC inhibition and Ca^{2+} mobilization like the other S1P receptors. However, unlike the other S1P receptors, it mediates inhibition of MAPK activation/cell proliferation (72, 89, 90, 93). Physiological roles for $S1P_5$ have not been reported in the published literature.

2.10 G2A

The *G2A* gene was isolated as an orphan GPCR gene from mouse bone marrow cells during a search for genes that were induced by BCL-ABL tyrosine kinase oncogene (94). It was named because ectopic *G2A* expression resulted in accumulation of NIH3T3 cells at G2/M cell cycle boundary, which blocked further progression to mitosis (94). G2A has properties of a high-affinity LPC receptor (K_d = 65 nM) and low-affinity SPC receptor (K_d = 230 nM) (46).

G2A appears to couple with $G_{12/13}$ and mediates the activation of small GTPases (Rho, Rac, and Ras), stress fiber formation that requires G_{13} and Rho, and SRE activation (95, 96). It also mediates LPC-induced Ca^{2+} mobilization and MAPK activation in a pertussis toxin (PTX)-sensitive manner, suggesting coupling to $G_{i/o}$. G2A can also mediate LPC-induced cell migration and apoptosis (46, 97). A recent report indicates that G2A expression also produces ligand-independent, PTX-insensitive activation of PLC and AC (probably via G_q and G_s, respectively) and that G2A couples with G_q and G_{13} for NF-κB activation (97). This observation has theoretical implications for identifying with certainty the specificity of LPC as the biological, high-affinity ligand for G2A. The current availability of a genetic-null mouse for G2A should help to clarify this issue (98).

G2A expression is restricted to lymphoid tissues, such as thymus, spleen, and bone marrow in mice (95). Overexpression of G2A may antagonize fibroblast transformation by the BCL-ABL (94) or induce it (95). Disruption of *G2A* in mice leads to immunological disorders (98). The *G2A*$^{(-/-)}$ mice develop enlarged spleens and lymph nodes with abnormal expansion of both T- and B-lymphocytes (98). Older *G2A*$^{(-/-)}$ mice (>1 year) develop a late-onset autoimmune syndrome, indicating that G2A also plays a role in the control of lymphocyte homeostasis (98).

2.11 Ovarian Cancer G Protein-Coupled Receptor 1

OGR1 was originally isolated as an orphan GPCR gene in HEY human ovarian cancer cells (99) and was later reported to encode a high-affinity (K_d = 33 nM) receptor for SPC (44). The mammalian *OGR1* gene encodes a 41-kDa protein consisting of 365 amino acids with relatively low similarity to any of the *lpa* or *s1p*-type receptors (Figure 2). It is notable that $S1P_1$-$S1P_5$ receptors can be activated by SPC with much lower affinities than by S1P. In contrast, OGR1 is

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only activated by SPC, not S1P (44). A recent report raises questions about the identity of OGR1 based on the reported inability of SPC to activate it (48).

OGR1 appears to couple with both $G_{i/o}$ and G_q, mediating SPC-induced Ca^{2+} mobilization via $G_{i/o}$ and MAPK activation via G_q. It also mediates SPC inhibition of cell proliferation (44). The *OGR1* gene is expressed in various human tissues: lung, placenta, brain, heart, spleen, testis, small intestine, and peripheral blood lymphocytes (99). Current information on functions and physiological roles of OGR1 is still limited [reviewed in (13, 14)]. Comparatively little independent confirmation of OGR1 as a bona fide SPC receptor exists, and based on the current literature, its identity as an SPC receptor should be considered provisional.

2.12 G Protein-Coupled Receptor 4

The GPR4 gene was originally isolated as an orphan human GPCR (100, 101) and was later reported to encode a high-affinity receptor for SPC (K_d = 36 nM) and low-affinity receptor for LPC (K_d = 159 nM) (45). As with OGR1, the identity of GPR4 is currently unclear in view of its unresponsiveness to SPC in recently reported assays (48).

The GPR4 protein shows the highest homology with OGR1 (~50% amino acid identity) (Figure 2) and appears to couple (at least) with $G_{i/o}$ to mediate cell proliferation, SRE activation, MAPK activation, Ca^{2+} mobilization, and cell motility (45) [reviewed in (13, 14)]. It is widely expressed in human tissues: ovary, liver, lung, kidney, heart, and lymph node (45, 101), and its physiological function remains to be determined. As with OGR1, its identity as a high-affinity SPC receptor should be considered provisional.

2.13 G Protein-Coupled Receptor 12

The GPR12 (GPCR01) gene was isolated from mouse cDNA by degenerate PCR during homology searches for orphan GPCR genes, and it was originally called *GPCR01* (102). GPR12 has been reported to be a moderate-affinity S1P receptor, unresponsive to SPC (103), and as a high-affinity SPC receptor (47). This discrepancy requires clarification.

There is little information regarding GPR12 signaling properties. SPC induces a G protein-gated, inwardly rectifying K^+ channel in *Xenopus* oocytes expressing GPR12 via PTX-sensitive pathways (47). S1P was reported to activate AC via PTX-insensitive pathways and Ca^{2+} mobilization via PTX-sensitive pathways, suggesting coupling with both G_s and $G_{i/o}$ (103). The *GPR12* gene is expressed in mouse brain, testis, and liver (102). In situ hybridization reveals *GPR12* expression in all areas of the developing mouse CNS from E14.5; this occurs especially in regions of neuroblast differentiation but not in areas of neuroblast proliferation. SPC stimulates cell proliferation and clustering in hippocampal HT22 cell lines, and it increases amounts of synaptophysin (a neuronal differentiation marker) in primary rat cortical cells that could be mediated by *GPR12*

(47), suggesting roles for SPC–GPR12 signaling in neuronal differentiation as observed in LPA-LPA receptor signaling (54, 104). However, the identity of this receptor must still be clarified beyond provisional classification as a receptor for S1P and/or SPC.

2.14 Candidates for Additional LP Receptors

A human orphan receptor GPR63 (accession no. NM_030784) was reported to act as a low-affinity receptor for S1P, dihydro-S1P, and dioleoylphosphatidic acid (105). The *GPR63* gene is highly expressed in human brain (especially in thalamus and caudatus), thymus, stomach, and small intestine, and a lower transcript abundance is present in kidney, spleen, pancreas, and heart (105). GPR63 overexpressed in CHO cells mediates S1P-induced Ca^{2+} mobilization and cell proliferation via PTX-insensitive pathways (105). In addition, GPR3 and GPR6, which are similar to GPR12, may mediate S1P-induced Ca^{2+} mobilization and AC activation (103), and further characterization is again necessary for their identification as functional S1P receptors. The orphan GPCRs continue to be an ample source for the discovery of new LP receptor members.

2.15 LP Receptor Agonists and Antagonists

As with many other GPCRs, LP receptors should be amenable to the development of highly specific and potent agonists or antagonists that have favorable pharmacokinetic, bioavailability, and metabolic characteristics. The anthelmintic drug suramin (106), despite its use in many earlier studies as a possible receptor inhibitor, should be considered to have poor specificity for LP receptors. Contrary to earlier speculations, receptor-mediated actions of LPs are stereoselective (107). Currently available compounds represent a promising start to the development of useful chemical tools, although none can be considered definitive in determining receptor selectivity or biological functions, especially for studies in vivo. With these caveats in mind, a partial list of compounds with reported LP receptor selectivity includes an LPA_1 antagonist, 3-(4-[4-([1-(2-chlorophenyl)-ethoxy]carbonylamino)-3-methyl-5-isoxazolyl] benzylsulfanyl) propanoicacid (Ki16425) (108); an LPA_1 antagonist that is an ethanolamide derivative (109, 110); LPA_2 agonists that are decyl and dodecyl fatty alcohol phosphates (FAP-10 and FAP-12) (111); an LPA_3 agonist that is a phosphothionate analog of LPA (112); an LPA_3 agonist that is a monofluorinated analog of LPA (107); an LPA_3 antagonist DGPP 8:0 that is a diacylglycerol pyrophosphate (110, 113); and an $S1P_2$ antagonist, JTE-013, pyrazolopyridine (114). Perhaps most encouraging are studies on a nonselective S1P receptor agonist prodrug that is an analog of myriocin, called FTY720, and becomes active following phosphorylation by sphingosine kinase (Figure 1), because these studies mark the entry of major pharmaceutical companies into the LP receptor field (108, 115, 116). Appropriately validated compounds are essential for in vivo studies, particularly in view of potential off-target effects. Combining chemical compounds with genetic

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mutants for receptors and/or related signaling components offers an attractive strategy for validating compounds and revealing new biological functions.

3. NON-GPCR TARGETS FOR LYSOPHOSPHOLIPIDS

Recently, the nuclear hormone receptor peroxisome proliferator-activated receptor γ (PPARγ) was proposed as an intracellular receptor for LPA based on the ability of LPA to displace a synthetic PPARγ agonist (rosiglitazone) (117). The physiological significance of this observation for mechanistically explaining the effects of LPA signaling is currently unclear on the basis of several criteria. Specificity of LPA binding to PPARγ is absent, and numerous biological ligands including eicosanoids, anionic fatty acids, and components of oxidized LDL can also bind PPARγ (118). Similarly, LPA itself clearly acts at other loci beyond PPARγ. Independent molecular genetic studies, in which PPARγ deletion was coupled with lacZ expression (119) or analyzed as chimeras (120), demonstrated restricted expression and effects of PPARγ deletion, primarily in adipose tissues. By contrast, LPA effects are well known in many tissue types that are unaffected by PPARγ elimination or that do not express PPARγ. Morever, the loss of LPA signaling associated with LPA_1 and LPA_2 receptor deletion, along with the observed null phenotypes, are clearly not rescued by the concomitant expression of PPARγ (59, 60). These data do not detract from the observation that LPA can interact with PPARγ, and future studies should clarify its physiological significance.

It remains possible that other intracellular LPA receptors exist. The LPA_1 receptor can apparently be expressed in nuclear membranes to mediate LPA-induced signaling, which leads to proinflammatory gene expression (121). This finding suggests that the GPCR-type LPA receptors could theoretically serve as intracellular receptors. Further, the lipid bilayer of membranes can be the direct target of LPA action. Endophilin I mediates synaptic vesicle formation by its LPA acyl transferase activity that produces phosphatidic acid (PA) from LPA (122). The local balance between PA and LPA concentrations could affect membrane curvature, leading to membrane invagination and synaptic vesicle uncoating (123). Because endophilins are essential for clathrin-mediated endocytosis and form complexes with various signaling molecules, which include cell surface receptors, metalloprotease disintegrins, and germinal center kinase-like kinase, local accumulation of LPA could result in altered cellular signaling [reviewed in (124)].

In comparison to LPA, the second messenger-like actions of S1P were first documented in the early 1990s. Molecular identification of S1P intracellular targets remains to be elucidated. S1P is rapidly produced intracellularly from sphingosine by sphingosine kinase upon cell activation with mitogens, such as PDGF and serum, and mobilizes Ca^{2+} from internal stores via inositol triphosphate (IP_3)-independent pathways (32, 125). SPC has been shown to mobilize

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Ca^{2+} from internal stores by activating the IP_3 receptor and/or ryanodine receptor. In addition, an SPC-gated Ca^{2+} channel called sphingolipid Ca^{2+} release-mediating protein of endoplasmic reticulum (SCaMPER) (30, 126–128) has also been implicated, although some disagreement exists over the functional distribution of SCaMPER in endoplasmic reticulum (129) [reviewed in (130)]. In cardiomyocytes, however, SCaMPER is localized to the sarcotubular junction on the plasma membrane where connections between the transverse tubules and sarcoplasmic reticulum function to regulate cell calcium levels and mediate SPC-induced Ca^{2+} release (131). S1P and SPC seem to act differentially as possible second messengers. They mobilize Ca^{2+} from different internal pools that could be distinguished by sensitivity to thapsigargin pretreatment (132). S1P has also been proposed as a "calcium influx factor," which links internal calcium store depletion to downstream store-operated calcium entry (133). Superimposed on these studies is the action of both known and perhaps unknown GPCR-type S1P receptors, some of which might function intracellularly; future studies should provide mechanistic clarification.

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4. BIOLOGY OF LYSOPHOSPHOLIPIDS

4.1 Nervous System

The nervous system is one of the major loci for LP receptor expression (25, 53, 72, 134). The expression profiles are correlated with neuronal development processes, such as neurogenesis, neuronal migration, neuritogenesis, and myelination [reviewed in (4)]. Exogenous application of LPA or S1P to neural cells induces responses that are relevant to both the development and function of the nervous system. Furthermore, both lipids exist in the brain at relatively high concentrations (28, 135). LPs affect most neural cell types, such as neural cell lines, neural progenitors, primary neurons, oligodendrocytes, Schwann cells, astrocytes, and microglia [reviewed in (136)]. Receptor-mediated LPA signaling induces a variety of cellular responses in all these cell types, and additional functions are being identified, such as roles in some forms of pain (137). By comparison, S1P-induced responses have thus far been demonstrated in only neural cell lines and glia, although a role for S1P signaling in other cells of neural origin is likely because several S1P receptors ($s1p_1$– $s1p_3$, $s1p_5$) are expressed in the developing and mature nervous system (72, 80, 134). Here we briefly review cellular effects of LPA on neural cell lines, neuroblasts, and neurons and discuss their biological relevance. Actions of LPA on other neural cell types, particularly Schwann cells, have been described elsewhere [reviewed in (4, 136)].

Historically, studies using peripheral nervous system cell lines hinted at roles for LPA in the nervous system (138, 139). Exposure of those cells to LPA produced a rapid retraction of their processes resulting in cell rounding, which was, in part, mediated by polymerization of the actin cytoskeleton. Combined

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with the fact that LPA was present in serum at high concentrations, it has been proposed that LPA may cause neuronal degeneration under pathological conditions when the blood-brain barrier is damaged and serum components leak into the brain (140). LPA receptor gene expression is not clearly detectable in adult neurons (53), and therefore, direct influences of LPA on adult CNS neurons remain unclear, although up-regulation of LPA receptor expression following insults remains a possibility. Immortalized CNS neuroblast cell lines from the cerebral cortex also display retraction responses to LPA exposure through Rho activation and actomyosin interactions (1, 51, 141, 142). Neurite retraction occurs in minutes following LPA exposure; however, an even earlier response has been identified in LPA-responsive cells that involves loss of membrane ruffling associated with actin depolymerization (142). This phenomenon is independent of Rho activation and requires interactions between Ca^{2+} and α-actinin, an actin-cross-linking protein (142). These cellular phenomena may reflect collapse and retraction of basal neuroblast processes that are perhaps involved in the proper reorganization of the actin cytoskeleton following LPA exposure.

Growth cone collapse is a well-known cellular response triggered by extracellular stimuli, occurring during axonal pathfinding or migration of differentiating neurons. LPA signaling might influence neurite formation and migration of differentiating neurons. For example, low concentrations of LPA induce repulsive turning of extending growth cones for *Xenopus* spinal cord neurons (143). Ubiquitin-dependent proteolysis via proteosomes is required for LPA-induced growth cone collapse in retinal neurons in which the apoptotic pathway involving p38 kinase and caspase-3 plays an important role (144, 145). In addition to a classical view of cytoskeletal rearrangement in growth cone motility, these observations raise the intriguing possibility that regulation of protein degradation is involved in not only collapse but also in turning of growth cones that involve LPA signaling.

Neuroprogenitor cells that express lpa_1 appear to include precursor cells of neurons and glia. As observed in neural cell lines, LPA induces cellular and nuclear rounding and migration, accompanied by the formation of fine retraction fibers (54). These morphological changes resemble the well known rounding-up phase of "to-and-fro" nuclear movement present in the cerebral cortical ventricular zone called "interkinetic nuclear migration" (146). LPA receptor-mediated cell proliferation is only modestly increased by LPA and is distinct from the more prominent proliferative responses produced by basic fibroblast growth factor (bFGF) (59). LPA also stimulates depolarizing ionic conductances in cortical neuroblasts (147), consisting of increases in both chloride and nonselective cation conductances.

LPA can be produced by postmitotic cortical neurons (54), and the interaction between neuroblasts and neurons by means of released LPA is likely involved in cortical neurogenic processes. Expression of lpa_2 in cortical plate or differentiating neurons (104, 134) combined with the effects of LPA on their cytoskeleton suggest a role of LPA in neuronal migration and/or neurite outgrowth. In young

differentiating cortical neurons, LPA induces retraction of neurites or lamellar structures, which could be related to growing axons, dendrites, or leading processes (104). The overall effects of LPA signaling in the embryonic cerebral cortex have been recently addressed in a culture settting that maintains the normal organization and growth characteristics observed in utero (148). Exogenous LPA exposure produces increases in cell number, width, and cortical folds resembling sulci and gyri. Each of these phenomena is absent in embryonic cerebral cortices from mice that are null for lpa_1 and lpa_2, demonstrating the LPA receptor dependence of this marked, growth phenomenon. Collectively, these data demonstrate multiple functions for LPA signaling during embryonic brain development, and it is probable that an equivalent biology exists for S1P (134) and perhaps related ligands.

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4.2 Angiogenesis and Cardiovascular Development

Angiogenesis is the formation of new capillary networks from preexisting vasculature by sprouting and/or splitting of capillaries; it involves coordinated proliferation, migration, adhesion, differentiation, and assembly of both VECs and their surrounding VSMCs. This process is also implicated in physiological processes, which include wound healing and myocardial angiogenesis after ischemic injury, and is precisely controlled by (both angiogenic and antiangiogenic) protein growth factors, such as vascular endothelial growth factor (VEGF), bFGF, and PDGF. It is also influenced by the lysophospholipids, LPA and S1P. Dysregulation of angiogenesis can lead to pathological conditions such as atherosclerosis, hypertension, solid tumor growth, rheumatoid arthritis, and diabetic retinopathy [reviewed in (5, 149)].

Several lines of evidence suggest that S1P receptor-mediated signaling plays a major regulatory role in angiogenesis. First, S1P induces both proliferation and migration of VECs (36, 64, 114, 150–152) while inducing proliferation but inhibiting migration of VSMCs (114, 151, 153, 154). VEC migration is inhibited by antisense oligonucleotides against $s1p_1$ or $s1p_3$ (150) or by $s1p_2$ overexpression (151). Using an $S1P_2$-specific antagonist (JTE-013), S1P-induced migration of VECs is enhanced, and inhibition of VSMCs migration is reversed (114). Overexpression of $s1p_1$ in VSMCs enhances both mitogenic and migration responses to S1P (151, 153). Furthermore, S1P protects VECs from serum-deprived apoptosis by nitric oxide production through both $S1P_1$ and $S1P_3$ receptors (155). These results implicate S1P receptor signaling in VEC/VSMC proliferation and migration. Second, S1P stimulates the formation and maintenance of VECs assembly/integrity by activating both $S1P_1$ and $S1P_3$. S1P-induced VEC adherens junction assembly and cell barrier integrity are blocked by antisense oligonucleotides against $s1p_1$ or $s1p_3$ (156, 157). Expression of a dominant negative $S1P_1$ mutant inhibits S1P-induced VEC assembly and migration (64). Although $s1p_2$ is not expressed or is expressed at only low levels in VECs (114, 150, 151, 156), overexpression of $s1p_2$ augments cell barrier

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integrity of VECs (157). Third, *slp*$_1$– *s1p*$_3$ transcripts are found in embryonic brain blood vessels (134).

Different S1P responses can be explained by altered S1P receptor expression patterns in VECs and VSMCs and by differential regulation of the two small GTPases, Rho and Rac (Table 2). In general, VEC expresses both *s1p*$_1$ and *s1p*$_3$ but not *s1p*$_2$ (36, 150–152, 154, 156), whereas VSMCs express all three receptors with high *s1p*$_2$ expression levels (114, 151, 153, 154). Functional analyses revealed that these receptors differentially regulate Rho and Rac; $S1P_1$ mediates the activation of Rac [and often Rho (150)], $S1P_2$ mediates Rho activation and Rac inhibition, and $S1P_3$ mediates the activation of both Rho and Rac (Table 2). Rac activation (via $S1P_1$) is required for migration of both VEC and VSMC (64), whereas both Rac-mediated cortical actin assembly (via $S1P_1$) and Rho-mediated stress-fiber formation (via $S1P_3$) are essential for VEC adherens junction assembly (64, 156, 157). $S1P_1$ plays a primary role in angiogenesis by its potent activation of Rac (64, 153), potentially through the intimate interplay with PDGF (as mentioned in Section 2.5). Negative regulation of Rac by $S1P_2$ in VSMCs underlies the S1P inhibitory response in migration (114, 151).

Although LPA regulates VSMC functions (158, 159), the roles of LPA signaling in angiogenesis appear to be in pathological conditions, such as wound healing (see next section) and atherosclerosis [reviewed in (160, 161)], rather than in a normal or basal conditions. However, frontal cephalic hemorrhages are observed in a significant percentage of *lpa*$_1^{(-/-)}$ or *lpa*$_1^{(-/-)}$*lpa*$_2^{(-/-)}$ embryos (59, 60) (see Sections 2.1 and 2.2), suggesting potential roles for LPA receptor signaling in some aspect of normal angiogenesis/vascular maturation.

The cardiovascular system is another major locus for LP receptor expression; at least five LP receptor genes (*lpa*$_1$, *lpa*$_3$, *s1p*$_1$– *s1p*$_3$, along with *OGR1* and *GPR4*) are expressed in mammalian heart (25, 45, 72, 99). Vasoregulatory actions of LPA were described as early as 1978 in which intravenous LPA application produced hypertension in rats/guinea pigs but hypotension in cats/rabbits (21). Later, S1P was also shown to regulate the cardiovascular system; intravenous administration of S1P decreased heart rates, ventricular contraction, and blood pressure in rats (162). The effects of LPA and S1P are predominantly receptor mediated. Direct evidence for S1P receptor signaling in angiogenesis and cardiovascular development comes from the phenotype of two genetic-null studies in mice and zebra fish (73, 163) [reviewed in (5, 6, 35)]. The *s1p*$_1$-null embryos die in utero because of defective vascular maturation in which VSMCs/pericytes do not migrate to surround the vessels (see Section 2.5). In zebra fish, the homolog of mammalian *s1p*$_2$ (the *mil* gene) was mutated (163), resulting in a cardiac phenotype. Normally cardiac muscle progenitor cells migrate from bilateral positions toward the dorsal midline and fuse to form a single heart tube. However, Mil mutant progenitors do not migrate to the midline, leading to lethality for lack of proper blood circulation. Transplanted mutant progenitors migrate normally in wild-type embryos, whereas transplanted wild-type progenitors do not migrate in the Mil mutant, suggesting defects in the guidance of

progenitor cell migration by surrounding paraxial cells (163). By contrast, deletion of $s1p_2$ in mice does not produce discernible cardiovascular defects, and $s1p_2^{(-/-)}$ mice are alive and grossly normal (see Section 2.6) (79, 80). Deletion of both $s1p_2$ and $s1p_3$ in mice leads to marked perinatal lethality, despite absence of gross anatomical and histological defects in the rare surviving double-null mice (see Section 2.7) (80). Deletion of lpa_1, lpa_2, or both in mice does not reveal obvious cardiac defects (59, 60).

4.3 Wound Healing

When wounded, damaged blood vessels activate platelets. The activated platelets play pivotal roles in subsequent repair processes by releasing bioactive mediators to induce cell proliferation, cell migration, blood coagulation, and angiogenesis. LPA and S1P are likely to be such mediators because both are released from activated platelets (36, 37) [reviewed in (10)]; this induces platelet aggregation along with mitogenic/migration effects on the surrounding cells, such as endothelial cells, smooth muscle cells, fibroblasts, and keratinocytes (164). Indeed, topical LPA application to cutaneous wounds in mice promotes repair processes (wound closure and increased neoepithelial thickness) by increasing cell proliferation/migration without affecting secondary inflammation (165, 166). However, normal wound closure is observed in $lpa_1^{(-/-)}lpa_2^{(-/-)}$ mice (60), suggesting the potential involvement of other LPA receptors and/or nonreceptor-mediated mechanisms in this process. S1P has not been reported in wound healing in vivo. S1P may have paradoxical effects on cutaneous wound healing, because S1P induces fibroblast proliferation and keratinocyte migration while inhibiting keratinocyte proliferation, a critical step for reepithelialization of the wound (167). All five high-affinity S1P receptor genes, $s1p_1$– $s1p_5$, are expressed in keratinocytes, and S1P inhibition of keratinocyte proliferation is partially inhibited by PTX pretreatment. In addition, microinjection of S1P inhibits keratinocyte proliferation. These results suggest that both S1P receptor signaling and perhaps intracellular actions may mediate this effect (167). S1P could also modulate actions of other mediators released from platelets (168). In contrast, SPC inhibits platelet activation (not via specific LP receptors) (43), and its effect on wound healing is unknown.

4.4 Immunity

Consistent with their roles as pleiotropic lipid mediators, LPA and S1P have been shown to regulate immunological responses by modulating activities/functions of immune cells such as T-/B-lymphocytes and macrophages [reviewed in (169–172)]. These immune cells and/or other cells involved with their normal function express several LP receptors, and their activities are regulated differentially by the expressed LP receptor subtypes. Furthermore, expression patterns of LP receptors can be altered by cell activation [reviewed in (169–171)]. Through LP receptors, T-cell migration and immune responses can be influenced with high

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receptor sensitivity (173). LPA and S1P might also protect T cells from apoptosis through LPA_1 in combination with LPA_2, and $S1P_2$ in combination with $S1P_3$, respectively (174). LPA induces migration of and inhibits interleukin-2 (IL-2) production from unstimulated T cells that predominantly express lpa_2. Mitogen activation of T cells leads to down-regulation of lpa_2 as well as up-regulation of lpa_1 expression. Therefore, in activated T cells, LPA inhibits cell migration but activates IL-2 production/cell proliferation through LPA_1 (175, 176). S1P has been reported to stimulate migration of T cells that express $s1p_1$ and $s1p_4$ under some conditions. T cell receptor-mediated activation of T cells suppresses expression of both $s1p_1$ and $s1p_4$, and it has been reported to eliminate their migration responses to S1P (177).

Immunomodulatory actions of S1P on lymphocytes represent a particularly active area of investigation [reviewed in (172, 178)]. The phosphorylated metabolite of FTY720 (Figure 1), a novel immunomodulator that causes lymphopenia, has been shown to act through S1P receptors (115, 116). FTY720 is being evaluated in human transplant studies in which it induces lymphocyte sequestration. Assayed models of autoimmunity and transplantation indicate that sequestered lymphocytes may be prevented from reacting/ migrating to inflammatory chemokines at graft sites (172, 179–181). Unlike current immunosuppressive drugs, such as cyclosporine, FTY720 neither inhibits T-cell activation/proliferation nor impairs general immunological responses (181, 182).

FTY720 is phosphorylated by sphingosine kinase in vivo and in vitro, and the phosphorylated form of FTY720 (FTY720-P in Figure 1) acts as a S1P receptor agonist (115, 116). FTY720-P can bind to each of four S1P receptors ($S1P_1$, $S1P_3$–$S1P_5$) and activates them with varied potency and efficacy compared to S1P. Because S1P concentrations are exquisitely regulated both by synthesis and degradation, S1P homeostasis may contribute to the normal status of lymphocyte homing. S1P receptors as well as enzymes involved in S1P synthesis thus represent attractive immunoregulatory targets.

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4.5 Ovarian Cancer and Preservation of Female Reproduction

Several lines of evidence suggest that abnormal LPA metabolism/signaling may contribute to the initiation and progression of ovarian cancers. First, LPA is present at significant concentrations (2–80 μM) in the ascitic fluid of ovarian cancer patients (183, 184). Ovarian cancer cells (OCCs) constitutively produce increased amounts of LPA as compared to normal ovarian surface epithelial cells (OSEs), the precursor of ovarian epithelial cancer (185). Elevated LPA levels are also detected in plasma from patients with early-stage ovarian cancers compared with controls, and therefore, the plasma LPA level might represent a potential biomarker for ovarian cancer (186). Second, LPA stimulates proliferation/ survival of OCCs (187–189). In OCCs but not OSEs, LPA induces cell proliferation (189) and activates secretion of urokinase plasminogen activator, a

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critical component of the metastasis cascade (190). Overexpression of LPA-hydrolyzing lipid phosphate phosphohydrolase-3 in OCC lines decreases colony forming activity and tumor growth in vitro and in vivo (191). Third, the expression of lpa_2 is markedly increased in OCCs as compared with OSEs, whereas lpa_1 expression is not consistently different between OCCs and OSEs (189, 190). Overexpression of lpa_1 in OCCs results in apoptosis and anoikis (192). Markedly increased lpa_3 expression is observed in OCCs as compared with OSEs (185, 190, 193), and LPA_3 is required for LPA-induced OCC migration (194). Notably, the newly identified lpa_4 has the highest expression level in ovary among human tissues examined (63). These results indicate that LPA is involved in ovarian carcinogenesis in which LPA_2 and LPA_3 (and possibly LPA_4) could mediate LPA-induced OCC proliferation and possibly metastasis (193–195). It should be noted that the first reported human lpa_2 clone was derived from an ovarian tumor library (52) and contained a frame-shift mutation that produced 31 extra amino acids at its intracellular carboxyl terminal end, which could produce a gain of function mutant (171). Several 3′-untranslated region (or coding region) variants of the lpa_2 transcripts have been found in multiple tumors, suggesting oncogenic potential by altered LPA_2 stability/signaling (196).

The source of LPA in ascites is unclear but may include macrophages, lymphocytes, mesothelial cells, or OCCs themselves (184, 185). Recent studies indicate that human plasma lysoPLD, one of the LPA-producing enzymes, is identical to autotaxin, a cell motility-stimulating ectophosphodiesterase implicated in tumor progression (197, 198) [reviewed in (26)]. Several cancer cell lines express autotaxin and release significant amounts of LPC, a substrate of autotaxin, thus producing LPA in culture media (197). LysoPLD activity in human serum is increased in normal pregnant women at the third trimester of pregnancy and to a higher extent in patients at risk for preterm delivery (198, 199). LPA can also be found in the follicular fluid of healthy individuals (200) and induces MAP kinase activation in ovarian theca cells that express lpa_1 (201). These results suggest that, in addition to its potential roles in ovarian cancer progression, LPA may have physiological functions in normal ovarian as well as in normal reproductive processes such as pregnancy and parturition.

Stimulatory roles of LPA in cancer progression were also described in other cancers. LPA is produced from and induces proliferation of prostate cancer cell lines (202, 203). LPA induces human colon carcinoma DLD1 cell proliferation, migration, adhesion, and secretion of angiogenic factors, possibly through LPA_1 (204). In other human colon carcinoma cells lines (HT29 and WiDR), LPA enhances cell proliferation and secretion of angiogenic factors, possibly through LPA_2 but not cell migration and adhesion (204). The genetic or pharmacological manipulation of LPA metabolism, specific blockade of receptor signaling, and inhibition of downstream signal transduction, represent possible approaches for cancer therapies [reviewed in (18, 205)].

In contrast with tumor-promoting effects of LPA, S1P could protect female germ cells from cancer therapy. Chemotherapy of cancers in young female patients induces oocyte apoptosis that leads to early ovarian failure and premature onset of menopause. Total body irradiation for leukemia or lymphoma before bone marrow transplantation may cause complete oocyte depletion [reviewed in (206–208)]. S1P inhibits chemotherapy-induced oocyte apoptosis (209) and suppresses radiation-induced oocyte loss in vivo without propagating genomic damage in offspring (210), raising the possibility for lipid-based therapy in clinical oocyte preservation.

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CONCLUDING REMARKS

The identification of LP receptors over the last decade has provided a mechanistic foundation from which the pleiotropic effects attributed to LPs can be understood. Continued "deorphaning" of GPCRs—and potentially, non-GPCRs—will surely result in new additions to the receptors discussed in this review. How receptor mechanisms interface with LP metabolism, particularly synthesis and degradation, will become increasingly clear with the continued identification of relevant enzymes involved in these processes. Similarly, new aspects of receptor-activated intracellular signaling will also be determined. A major current challenge is understanding the physiological and pathophysiological roles played by single LP receptor subtypes, as well as combinations of receptors, which include those from different ligand classes. This challenge will be aided by the continued generation of receptor mutants, combined with LP receptor-specific agonists and antagonists that have favorable properties allowing their use in vivo. This new information will be important for understanding the fundamental biology of LP receptors and for the development of human therapies based on targeting LP receptors and components of their signaling pathways.

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Annual Review of Biochemistry
Volume 73, 2004

CONTENTS

THE EXCITEMENT OF DISCOVERY, *Alexander Rich*	1
MOLECULAR MECHANISMS OF MAMMALIAN DNA REPAIR AND THE DNA DAMAGE CHECKPOINTS, *Aziz Sancar, Laura A. Lindsey-Boltz, Keziban Ünsal-Kaçmaz, Stuart Linn*	39
CYTOCHROME C -MEDIATED APOPTOSIS, *Xuejun Jiang, Xiaodong Wang*	87
NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF HIGH-MOLECULAR-WEIGHT PROTEINS, *Vitali Tugarinov, Peter M. Hwang, Lewis E. Kay*	107
INCORPORATION OF NONNATURAL AMINO ACIDS INTO PROTEINS, *Tamara L. Hendrickson, Valérie de Crécy-Lagard, Paul Schimmel*	147
REGULATION OF TELOMERASE BY TELOMERIC PROTEINS, *Agata Smogorzewska, Titia de Lange*	177
CRAWLING TOWARD A UNIFIED MODEL OF CELL MOBILITY: Spatial and Temporal Regulation of Actin Dynamics, *Susanne M. Rafelski, Julie A. Theriot*	209
ATP-BINDING CASSETTE TRANSPORTERS IN BACTERIA, *Amy L. Davidson, Jue Chen*	241
STRUCTURAL BASIS OF ION PUMPING BY CA-ATPASE OF THE SARCOPLASMIC RETICULUM, *Chikashi Toyoshima, Giuseppe Inesi*	269
DNA POLYMERASE , THE MITOCHONDRIAL REPLICASE, *Laurie S. Kaguni*	293
LYSOPHOSPHOLIPID RECEPTORS: Signaling and Biology, *Isao Ishii, Nobuyuki Fukushima, Xiaoqin Ye, Jerold Chun*	321
PROTEIN MODIFICATION BY SUMO, *Erica S. Johnson*	355
PYRIDOXAL PHOSPHATE ENZYMES: Mechanistic, Structural, and Evolutionary Considerations, *Andrew C. Eliot, Jack F. Kirsch*	383
THE SIR2 FAMILY OF PROTEIN DEACETYLASES, *Gil Blander, Leonard Guarente*	417
INOSITOL 1,4,5-TRISPHOSPHATE RECEPTORS AS SIGNAL INTEGRATORS, *Randen L. Patterson, Darren Boehning, Solomon H. Snyder*	437
STRUCTURE AND FUNCTION OF TOLC: The Bacterial Exit Duct for Proteins and Drugs, *Vassilis Koronakis, Jeyanthy Eswaran, Colin Hughes*	467
ROLE OF GLYCOSYLATION IN DEVELOPMENT, *Robert S. Haltiwanger, John B. Lowe*	491

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STRUCTURAL INSIGHTS INTO THE SIGNAL RECOGNITION PARTICLE, *Jennifer A. Doudna, Robert T. Batey* 539

PALMITOYLATION OF INTRACELLULAR SIGNALING PROTEINS: Regulation and Function, *Jessica E. Smotrys, Maurine E. Linder* 559

FLAP ENDONUCLEASE 1: A Central Component of DNA Metabolism, *Yuan Liu, Hui-I Kao, Robert A. Bambara* 589

EMERGING PRINCIPLES OF CONFORMATION-BASED PRION INHERITANCE, *Peter Chien, Jonathan S. Weissman, Angela H. DePace* 617

THE MOLECULAR MECHANICS OF EUKARYOTIC TRANSLATION, *Lee D. Kapp, Jon R. Lorsch* 657

MECHANICAL PROCESSES IN BIOCHEMISTRY, *Carlos Bustamante, Yann R. Chemla, Nancy R. Forde, David Izhaky* 705

INTERMEDIATE FILAMENTS: Molecular Structure, Assembly Mechanism, and Integration Into Functionally Distinct Intracellular Scaffolds, *Harald Herrmann, Ueli Aebi* 749

DIRECTED EVOLUTION OF NUCLEIC ACID ENZYMES, *Gerald F. Joyce* 791

USING PROTEIN FOLDING RATES TO TEST PROTEIN FOLDING THEORIES, *Blake Gillespie, Kevin W. Plaxco* 837

EUKARYOTIC mRNA DECAPPING, *Jeff Coller, Roy Parker* 861

NOVEL LIPID MODIFICATIONS OF SECRETED PROTEIN SIGNALS, *Randall K. Mann, Philip A. Beachy* 891

RETURN OF THE GDI: The GoLoco Motif in Cell Division, *Francis S. Willard, Randall J. Kimple, David P. Siderovski* 925

OPIOID RECEPTORS, *Maria Waldhoer, Selena E. Bartlett, Jennifer L. Whistler* 953

STRUCTURAL ASPECTS OF LIGAND BINDING TO AND ELECTRON TRANSFER IN BACTERIAL AND FUNGAL P450S, *Olena Pylypenko, Ilme Schlichting* 991

ROLES OF N-LINKED GLYCANS IN THE ENDOPLASMIC RETICULUM, *Ari Helenius, Markus Aebi* 1019

ANALYZING CELLULAR BIOCHEMISTRY IN TERMS OF MOLECULAR NETWORKS, *Yu Xia, Haiyuan Yu, Ronald Jansen, Michael Seringhaus, Sarah Baxter, Dov Greenbaum, Hongyu Zhao, Mark Gerstein* 1051

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