Lysophospholipid Receptors

Signaling and Biochemistry

Edited by
Jerold Chun
Timothy Hla
Wouter Moolenaar
Sarah Spiegel

WILEY
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# CONTENTS

**PREFACE** ix

**CONTRIBUTORS** xi

**1 LYSOPHOSPHATIDIC ACID (LPA) RECEPTOR SIGNALING** 1
 *Hope Mirendil, Mu-En Lin, and Jerold Chun*

**2 SPHINGOSINE 1-PHOSPHATE (S1P) RECEPTORS** 41
 *Bongnam Jung and Timothy Hla*

**3 GLOBAL GENE EXPRESSION PROGRAM OF LYSOPHOSPHATIDIC ACID (LPA)-STIMULATED FIBROBLASTS** 61
 *Catelijne Stortelers and Wouter H. Moolenaar*

**4 IDENTIFICATION OF DIRECT INTRACELLULAR TARGETS OF SPHINGOSINE 1-PHOSPHATE (S1P)** 71
 *Nitai C. Hait, Sheldon Milstien, and Sarah Spiegel*

**5 LYSOPHOSPHOLIPID RECEPTOR SIGNALING PLATFORMS: THE RECEPTOR TYROSINE KINASE–G PROTEIN-COUPLED RECEPTOR SIGNALING COMPLEX** 85
 *Nigel J. Pyne and Susan Pyne*

**6 AUTOTAXIN: A UNIQUE ECTO-TYPE PYROPHOSPHODIESTERASE WITH DIVERSE FUNCTIONS** 103
 *Hiroshi Yukiura and Junken Aoki*
7 STUDIES ON AUTOTAXIN SIGNALING IN ENDOCYTIC VESICLE BIOGENESIS AND EMBRYONIC DEVELOPMENT USING WHOLE EMBRYO CULTURE AND ELECTROPORATION 121
Masayuki Masu, Seiichi Koike, Takuya Okada, and Kazuko Keino-Masu

8 STANDARDIZATION AND QUANTIFICATION OF LYSOPHOSPHATIDIC ACID COMPOUNDS BY NORMAL-PHASE AND REVERSED-PHASE CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY 137
Jeff D. Moore, Shengrong Li, David S. Myers, Stephen B. Milne, H. Alex Brown, and Walter A. Shaw

9 SPHINGOSINE KINASES: BIOCHEMISTRY, REGULATION, AND ROLES 153
Melissa R. Pitman, Kate E. Jarman, Tamara M. Leclercq, Duyen H. Pham, and Stuart M. Pitson

10 FUNCTIONAL AND PHYSIOLOGICAL ROLES OF SPHINGOSINE 1-PHOSPHATE TRANSPORTERS 185
Atsuo Kawahara and Tsuyoshi Nishi

11 LIPID PHOSPHATE PHOSPHATASES AND SIGNALING BY LYSOPHOSPHOLIPID RECEPTORS 201
Ganesh Venkatraman and David N. Brindley

12 LIPID PHOSPHATE PHOSPHATASES: RECENT PROGRESS AND ASSAY METHODS 229
Andrew J. Morris, Susan S. Smyth, Abdel K. Salous, and Andrew D. Renault

13 LYSOPHOSPHATIDIC ACID (LPA) SIGNALING AND CARDIOVASCULAR PATHOLOGY 265
Susan S. Smyth, Anping Dong, Jessica Wheeler, Manikandan Panchatcharam, and Andrew J. Morris

14 SPHINGOSINE 1-PHOSPHATE (S1P) SIGNALING IN CARDIOVASCULAR PHYSIOLOGY AND DISEASE 283
Bodo Levkau

15 SPHINGOSINE 1-PHOSPHATE (S1P) SIGNALING AND THE VASCULATURE 313
Christian Waeber

16 REGULATION OF THE NUCLEAR HORMONE RECEPTOR PPARγ BY ENDOGENOUS LYSOPHOSPHATIDIC ACIDS (LPAS) 349
Ryoko Tsukahara, Tamotsu Tsukahara, and Gabor Tigyi
17 MECHANISMS AND MODELS FOR ELUCIDATING THE CARDIAC EFFECTS OF SPHINGOSINE 1-PHOSPHATE (S1P) 373
Shigeki Miyamoto, Sunny Yang Xiang, Nicole H. Purcell, and Joan Heller Brown

18 NEURAL EFFECTS OF LYSOPHOSPHATIDIC ACID (LPA) SIGNALING 399
Nobuyuki Fukushima

19 WIDESPREAD EXPRESSION OF SPHINGOSINE KINASES AND SPHINGOSINE 1-PHOSPHATE (S1P) LYASE SUGGESTS DIVERSE FUNCTIONS IN THE VERTEBRATE NERVOUS SYSTEM 419
H. Meng and V.M. Lee

20 LYSOPHOSPHATIDIC ACID AND NEUROPATHIC PAIN: DEMYELINATION AND LPA BIOSYNTHESIS 433
Hiroshi Ueda

21 ROLE OF LYSOPHOSPHATIDIC ACID (LPA) IN BEHAVIORAL PROCESSES: IMPLICATIONS FOR PSYCHIATRIC DISORDERS 451
Guillermo Estivill-Torrús, Luis Javier Santín, Carmen Pedraza, Estela Castilla-Ortega, and Fernando Rodríguez de Fonseca

22 SPHINGOSINE 1-PHOSPHATE (S1P) SIGNALING AND LYMPHOCYTE EGRESS 475
Alejandra Mendoza, Lauren A. Pitt, and Susan R. Schwab

23 BIOLOGY REVEALED BY SPHINGOSINE 1-PHOSPHATE (S1P) RECEPTOR GENE-ALTERED MICE 489
Maria L. Allende, Mari Kono, Alkaterini Alexaki, Christina Giannouli, Jiman Kang, Catherine C. Theisen, Eleanor L. Koerner, and Richard L. Proia

24 ROLE OF LYSOPHOSPHATIDIC ACID (LPA) IN THE INTESTINE 507
C. Chris Yun and Peijian He

25 LYSOPHOSPHOLIPID SIGNALING IN FEMALE AND MALE REPRODUCTIVE SYSTEMS 529
Xiaoqin Ye

26 THE GONADS AND THEIR MAGIC BULLET, LYSOPHOSPHATIDIC ACID: PHYSIOLOGICAL AND TOXICOLOGICAL FUNCTIONS OF LYSOPHOSPHATIDIC ACID (LPA) IN FEMALE AND MALE REPRODUCTIVE SYSTEMS 569
Lygia Therese Budnik, Bärbel Brunswig-Spickenheier, and Dieter Müller
CONTENTS

27 LYSOPHOSPHOLIPID REGULATION OF LUNG FIBROSIS 587
Barry S. Shea and Andrew M. Tager

28 LYSOPHOSPATIDIC ACID (LPA) SIGNALING AND BONE 609
Jean Pierre Salles, Sara Laurencin-Dalicieux, Françoise Conte-Auriol, Fabienne Briand-Mésange, and Isabelle Gennero

29 LYSOPHOSPHATIDIC ACID (LPA) SIGNALING AND BONE CANCER 627
Olivier Peyruchaud, Marion David, Timothy L. Macdonald, and Kevin R. Lynch

30 UNDERSTANDING THE FUNCTIONS OF LYSOPHOSPHATIDIC ACID RECEPTORS IN CANCER 641
Nattapon Panupinthinu and Gordon B. Mills

31 LYSOPHOSPHATIDIC ACID RECEPTORS IN CANCER 661
Abir Mukherjee, Jinhua Wu, Yongling Gong, and Xianjun Fang

32 LPA RECEPTOR SUBTYPES LPA$_1$ AND LPA$_2$ AS POTENTIAL DRUG TARGETS 681
Gretchen Bain and T. Jon Seiders

33 CLINICAL INTRODUCTION OF LYSOPHOSPHATIDIC ACID (LPA) AND AUTOTAXIN ASSAYS 709
Yutaka Yatomi, Koji Igarashi, Kazuhiro Nakamura, Ryunosuke Ohkawa, Akiko Masuda, Akiko Suzuki, Tatsuya Kishimoto, Hitoshi Ikeda, and Junken Aoki

34 ANTIBODIES TO BIOACTIVE LYSOPHOSPHOLIPIDS 737
Roger A. Sabbadini, Jonathan M. Wojciak, Kelli Moreno, James S. Swaney, and Barbara Visentin

INDEX 753
Lysophospholipids are simple phospholipids that arise from cell membranes and related compartments. They are epitomized by two well-known species, lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P). Studies on these lipids have shown a dramatic increase in number, from comparatively rare reports before the 1990s to what is now a vibrant and expansive scientific literature encompassing thousands of publications that range from fundamental lipid biochemistry and cell signaling to physiologies and pathophysiologies of virtually every organ system. A galvanizing event for this field’s expansion was the discovery of related cell surface G protein-coupled receptors (GPCRs) for LPA and S1P, which served to bring together scientists from many different areas. This book grew out of a desire to capture the dynamism of this field, representing both a snapshot of current knowledge as well as a single source of information for backgrounds, techniques, and literature references that encompass the current field of lysophospholipid signaling.

The book can be considered to have two parts, the first covering receptors and enzymes (Chapters 1–12), and the second covering physiology and pathophysiology (Chapters 13–34). Efforts have been made, where feasible, to pair themes common to LPA and S1P signaling, such as the receptors themselves or cardiovascular effects, in an effort to provide readers new to the field with a sampling of themes from both lipids. Complementing elements common to both lipids, such as degradative pathways mediated by lipid phosphate phosphatases, are noted, as well as distinguishing features that could provide a basis for molecular selectivity. The comprehensive index will aid access to specific topics, including methodologies.

The depth and breadth of the lysophospholipid signaling field precludes an all-encompassing treatment, and the reader is encouraged to use the provided chapters as a starting point to explore the primary literature for more
thorough and timely reports. All scientific fields contain controversies and inconsistencies that are also represented within this book; rather than enforce a single viewpoint, the decision was made to provide balance with alternative views, the validity of which awaits independent study, as seen in some non-GPCR lysophospholipid mechanisms, or physiological mechanisms of lipid-targeted antisera. That said, the richness of possibilities as well as emerging data from the primary literature represent a prime example of the field’s activity and dynamism.

As important, the decision was made to leave out areas that have been superbly and extensively treated in recent reviews. A key example is the FDA approval of fingolimod—known in the scientific literature as FTY720 and commercially as Gilenya™ (Novartis AG, Basel) as the first oral treatment for relapsing forms of multiple sclerosis. Now approved worldwide, fingolimod is phosphorylated to become a non-selective S1P receptor modulator and represents the first compound targeting lysophospholipid receptors that has become a human medicine. Basic mechanisms relevant to fingolimod’s activity are, however, discussed in receptor and immunology chapters, and examples can be found in the index. Other recent areas without representation include the structural biology of lysophospholipid GPCRs, particularly the S1P receptor S1P1, as well as emerging data on newly identified lysophospholipid GPCRs for other lysophospholipid species, particularly lysophosphatidyl serine and lysophosphatidyl inositol. These topics represent areas for any future iteration of this book.

The myriad details and logistical challenges of creating this book required the efforts of many, who deserve both credit and thanks. First, this project required the efforts and vision of all of the contributors, who are integral members of the larger community of scientists whose work involves lysophospholipid signaling. Many of us were brought together through the biennial FASEB Summer Research Conferences as well as other venues such as Keystone Symposia or the ASBMB meetings; we were the organizers and sponsors of these important gatherings. Second, easily an equal number of other potential authors could have written chapters, and we thank them for both their willingness to contribute and apologize for not being able include so many worthy authors because of time and space constraints. Third, the tireless and painstaking efforts of Danielle Letourneau deserve special kudos, as she juggled every phase of this project while still handling the many demands of an active laboratory. This book would not exist without her. Fourth, we thank Anita Lekhwani, Kris Parrish, Cecilia Tsai, and all of their staff at John Wiley for their interest and infinite patience in the many—at times very slow—steps toward completing this project, particularly during Hurricane Sandy with its flooding and power outages in Hoboken and New York. Finally, we thank you, the reader, for your interest and future contributions to this growing field, and hope that this book provides you with useful and stimulating information that will lead to new scientific and medical advances through the field of lysophospholipid signaling.

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1.1. INTRODUCTION

Lysophosphatidic acid (LPA) is a simple phospholipid that has been shown to act as a potent lipid-signaling molecule. LPA acts through defined G protein-coupled receptors (GPCRs) in many developmental and adult processes involving most, if not all, vertebrate organ systems. All LPA molecules contain a phosphate head group attached to a glycerol backbone that is attached to a single aliphatic chain of varied length and saturation, typically ester-linked (with other linkages existing, e.g., alkyl-LPA) (Fig. 1.1). LPA species are present in all eukaryotic tissues at relatively low concentrations that include both structural as well as signaling pools, the latter of which can evoke myriad physiological responses in a wide variety of cell types (1–4).

LPA was long known as a minor component of membrane phospholipid metabolism (5, 6). Hints of LPA's possible actions as a bioactive lipid were suggested in reports dating from the early 1960s that examined smooth muscle effects including influences on blood pressure (7, 8). The chemically defined LPA species involved emerged years later with LPA's isolation from soybeans (9). This chemical identity raised mechanistic questions on how it might function, and many theories were proposed that included physical perturbation of the membrane (10), calcium chelation (11), second messenger signaling (12), intracellular receptors (13), and cell surface receptors (14). These competing theories to explain the effects of extracellularly applied LPA as well as other lysophospholipids were clarified upon identification of the first lysophospholipid receptor: a GPCR from the brain initially named “ventricular zone gene-1” because of its expression in the embryonic neuroproliferative layer of the cerebral cortex (15) and which is now known as LPA\textsubscript{1} (15, 16). The cloning
and functional identification of this receptor gene led to the deorphanization of other putative receptor genes in the databases based upon their homology to one another (17–19). This collective group of orphan receptors was known by many different receptor names (20), the first of which was “endothelial
differentiation gene” (EDG). This EDG group contained both LPA and sphingosine 1 phosphate (S1P) receptors, which underscored the significant homology among LPA and S1P receptors. At the time of the initial identification, S1P1 had greatest homology to LPA1 but was still an orphan receptor, while a homologous known receptor to LPA1 was the cannabinoid receptor CB1 (encoded by CNR1) that itself interacts with endogenous lipid molecules anandamide and 2-arachidonyl glycerol (21, 22). More recently, three somewhat divergent LPA GPCRs have been identified (LPA4–6) (23–27), which belong to the P2Y purinergic receptor family (Fig. 1.2), providing evidence for the existence of dissimilar clusters of receptors mediating the effects of the same ligand. Other species of bioactive lysophospholipids are also currently being assessed for matching receptors, though none has been identified as of yet (28). An additional dimension to LPA receptor interactions is the likelihood that different chemical forms of LPA may bind preferentially to LPA receptor subtypes (29), although the extreme difficulty of doing classical receptor binding experiments with LPA has prevented direct assessments of this possibility, relying instead on secondary readouts of receptor activity that do support ligand selectivity. All six LPA receptors are type I, rhodopsin-like GPCRs with seven transmembrane domains. Each receptor can couple to one or more of four heterotrimeric $G_{\alpha}$ proteins ($G_{12/13}, G_{q/11}, G_{i/o}$, and $G_{s}$) (Fig. 1.3), resulting in the activation of a wide range of downstream signaling pathways and resulting in diverse physiological and pathophysiological effects documented for LPA signaling.

1.2. LPA METABOLISM

LPA is produced both intracellularly and extracellularly from membrane phospholipids (Fig. 1.1). Intracellular LPA is thought to be structural (6) or an intermediate for phospholipid biosynthesis, so it is less likely that it functions as an extracellular pool of signaling molecules (30). Additional LPA-producing pathways also exist (31). The term LPA, at least in an extracellular signaling context, generally refers to 1-acyl-2-hydroxy-sn-glycero-3-phosphate, but distinct chemical forms exist, such as 1-alkyl- or 2-acyl-LPA (32, 33). The acyl chain length and degree of saturation generally depend on the precursor phospholipid, with the most abundant forms of LPA in plasma being 16:0-, 18:2-, and 18:1-LPA (34). The 18:1-LPA form is perhaps the most commonly used LPA species in the laboratory for signaling studies.

The two major pathways involved in LPA production initiate either the sequential activity of phospholipase D (PLD) and phospholipase A$_2$ (PLA$_2$) or of PLA$_2$ and lysophospholipase D (also known as autotaxin, ATX) (Fig. 1.1). The first pathway is mainly involved in cellular LPA production through cell membrane-derived phosphatidic acid hydrolysis, and the second pathway is mainly involved in extracellular LPA production in bodily fluids such as plasma (35). In 1986, it was reported by Tokumura et al. that LPA is produced
**Figure 1.2.** Phylogenetic relationships between known LPA receptors. Non-LPA GPCRs (rhodopsin, SIP₁, and the cannabinoid receptors) are included for reference. Amino acid percent identity to LPA₁ is shown in parentheses.
in submillimolar concentrations from plasma incubated at 37°C for a long period of time (36). The enzyme responsible for this production of LPA was later identified as the previously known gene \textit{Enpp2}, which encodes the ATX protein and possesses lysoPLD activity (37, 38). There are at least two additional pathways that can produce intracellular LPA: acylation of glycerol-3-phosphate by glycerophosphate acyltransferase (GPAT) and phosphorylation of monoacylglycerol by monoacylglycerol kinase (MAG-kinase) (39). LPA degradation involves several different enzymes, including LPA-acyltransferase (LPAAT), lipid phosphate phosphatase (LPP), and various lysophospholipases (40). LPA may be converted back to phosphatidic acid by LPAAT, hydrolyzed by LPP-1, -2, and -3, or converted by lysophospholipases into glycerol-3-phosphate (40, 41).

Since LPA is present in low concentrations in all mammalian cells and tissues, it is important to identify biologically relevant concentrations (based upon the half maximal effective concentration [EC\textsubscript{50}] and/or apparent \(K_d\) values of the six LPA receptors). Current LPA detection methods include enzymatic assays, thin-layer chromatography (TLC)–gas chromatography, high-performance liquid chromatography (HPLC)/tandem mass spectrometry, and liquid chromatography–tandem mass spectrometry (LC/MS/MS) (42, 43). LPA concentrations measured in the blood can range from 0.1 \(\mu\text{M}\) in plasma to over 10 \(\mu\text{M}\) in serum, which is well over the apparent \(K_d\) of LPA\textsubscript{1-5} (31, 44, 45).

**Figure 1.3.** Signaling pathways activated by the six confirmed LPA receptors. (See color insert.)
1.3. AUTOTAXIN

ATX is one of the best-studied enzymes associated with LPA signaling. The first reported activity of ATX was as a cell motility-stimulating factor in human melanoma cells (46). The cell motility effect was originally attributed to ATX’s reported function as a nucleotide phosphodiesterase, since ATX shares structural similarities to the nucleotide pyrophosphatase/phosphodiesterase (NPP) family (47). However, the promigratory effects of ATX were blocked by the addition of pertussis toxin, a G\textsubscript{\alpha}i/o inhibitor (46), and G\textsubscript{\alpha}i/o couples to five of the six identified LPA receptors. It is now clear that the cell motility-stimulating activity of ATX is a result of autocrine signaling from the production of LPA and its action on LPA receptors (30, 48, 49). ATX is present in blood and shows fairly broad tissue expression, with relatively high levels of ATX expressed in the brain (especially the choroid plexus), kidney, and lymphoid organs, which leads to high levels of ATX in cerebrospinal fluid and the high endothelial venules (HEVs) in lymphoid organs (50–52).

The physiological importance of ATX was not fully appreciated until the creation of ATX knockout mice (Enpp2\textsuperscript{−/−} mutants). Enpp2\textsuperscript{−/−} mice die around embryonic day 9.5 with prominent vascular and neural tube defects (53, 54). These mutants also have specific deficits in both yolk sac blood vessel formation and large lysosome biogenesis in yolk sac visceral endoderm cells (55). Enpp2\textsuperscript{+/−} heterozygotes survive to adulthood but, importantly, have LPA plasma levels that are half that of wild-type mice. This confirms that ATX activity is the major source of LPA in plasma and is essential for proper embryonic development.

ATX, through its production of LPA, is significantly involved in vascular development. LPA was found to prevent disassembly of blood vessels in cultured allantois explants (54), supporting a role for LPA signaling in maintenance of existing vasculature in addition to assembly and maturation. LPA additionally acts as a vasoregulator in multiple species (9) and has been implicated in the pathology of posthemorrhagic vasoconstriction (56). ATX expression is induced by vascular endothelial growth factor (VEGF), and induces both proliferation and migration of endothelial cells (57–59). LPA-induced endothelial cell migration in a Matrigel migration assay induced expression of matrix metalloproteinase-2 (MMP-2), which is a proteolytic enzyme involved in endothelial cell migration and matrix remodeling during angiogenesis (60). Because angiogenesis and tissue repair require a variety of bioactive mediators, such as growth factors and cytokines that are released from activated platelets, LPA has been implicated in these processes. LPA is known to be released from activated platelets (34), as well as able to induce platelet activation in a positive feedback loop (61, 62), and this LPA production induces mitogenic and migration effects on many of the cell types involved in angiogenesis and tissue repair (4, 58, 63, 64).

Activation of platelets is also heavily associated with cardiovascular disease. LPA is involved in processes relevant to atherosclerosis during both the early
and late stages of plaque formation involving endothelium dysfunction, monocyte attraction and adhesion, LDL uptake, and proinflammatory cytokine release (65–71). LPA both increases the permeability of endothelial cells and rat mesenteric venules (66, 72) and recruits monocytes to the endothelium (67), implicating LPA in the invasion of reactive macrophages in atherosclerosis. LPA was also found to accumulate in the thrombogenic, lipid-rich core of atherosclerotic plaques (61, 73). LPA’s involvement in atherosclerosis is receptor-dependent, involving both LPA1 and LPA2 signaling, and will be discussed in more detail in the succeeding sections.

One of the major causes of damage to cardiac myocytes during myocardial infarction is ischemia and hypoxia. While LPA clearly plays a role in ischemia and hypoxia, the exact nature of its effects require further clarification. LPA levels are elevated under ischemic conditions (66, 74), and while LPA has been shown to protect hypoxia-induced apoptosis in cardiac myocytes and mesenchymal stem cells (75, 76), LPA3 antagonists were reported to protect renal cells from hypoxia-induced apoptosis (77) in vitro. Treatment with an LPA analog, LXR-1035, of a rat model of retinal ischemia/reperfusion injury resulted in decreased neural cell death and improved functional recovery (78). Yet in porcine cerebral microvascular and human umbilical vein endothelial cells, LPA was found to induce specifically oncotic cell death, which was reproduced in both brain explants and retinas in vivo (79). An LPA1 low-affinity antagonist was able to prevent this oncotic cell death. Recently, it was shown, using an ex vivo cortical culturing system and cell culture, that the cellular neurodevelopmental effects of prolonged hypoxia are ameliorated through antagonism or genetic removal of LPA1 (80), mechanisms that were shown to extend at least in part to maternal hypoxic insult in vivo.

ATX influences on LPA signaling are not only involved in platelet activation, but also function in an immunoregulatory capacity. ATX has been identified as a modulator of lymphocyte trafficking into secondary lymphoid organs, where ATX produced by high endothelial cells (HECs) may bind to activated lymphocytes (52). It is proposed that LPA induces the chemokinesis of T cells via the local production of LPA from ATX bound on the lymphocyte cell surface. ATX activity is also induced in T cells treated with lipopolysaccharide (LPS) (81), and LPA can induce Ca2+ signaling in adult B cells (82), which further implicate ATX and LPA in normal immune cell function.

The effects of LPA can also participate in immune misactivation relevant to various autoimmune diseases, where increases in LPA have been identified in systemic sclerosis patients. Notably, fibroblasts from systemic sclerosis patients are hypersensitive to Cl− current activation during LPA exposure (83, 84). LPA is also involved in arthritis, where a functional single-nucleotide polymorphism (SNP) in the promoter region of LPA1 was shown to increase susceptibility to knee osteoarthritis, possibly via upregulation of LPA1 expression (85). Rheumatoid arthritis patients also exhibited increases in ATX in synovial fluid as well as elevated cytokine production in patient fibroblast-like
synoviocytes treated with LPA (86). These results support the proposal that ATX and LPA are involved in facilitating immune system functioning via modulation of lymphocyte trafficking and sensitization of affected cells during autoimmunity.

LPA has also been investigated as a modulator of constructive wound healing. Myriad factors are released from platelets following tissue trauma, including LPA. Treatment of “wounded” endothelial monolayers in vitro with LPA resulted in closure repair (58), and application of LPA to in vivo cutaneous wounds promoted enhanced repair processes (87). Moreover, fibroblast migration into the fibrin wound matrix is an essential step in the process of wound healing, and LPA has been shown to regulate migration of mouse embryonic fibroblasts (MEFs) through LPA\textsubscript{1} signaling (49, 88).

There is currently a wealth of data explicitly implicating ATX and LPA signaling in cancer progression. LPA signaling has been associated with many of the dysregulated processes involved in cancer development, including proliferation, survival, metastasis, and promotion of angiogenesis (reviewed in References 3 and 89–91). De Alvarez and Goodnell first suggested the involvement of LPA in cancer in 1964 when lysolecithin (known also as lysophosphatidylcholine, LPC), LPA's precursor, was found to be significantly increased in the serum of patients with ovarian cancer (5). Later, ATX was specifically identified as a motility-stimulating factor for cancer cells (46), although ATX had yet to be identified as having lysoPLD activity. Other early clues to LPA's involvement in cancer included the observation that LPA enhanced invasiveness of lung cancer cells in vitro (92). Myriad other cancer cell lines have shown responsivity to LPA in regards to enhanced proliferation, migration, and survival. These cell lines include ovarian, gastrointestinal, breast, prostate, mesothelioma, pancreatic, liver, and glioma (93–102). LPA levels are increased in the ascites and plasma of ovarian cancer patients (93, 103), and a variety of cancer cell lines (99, 104, 105) and primary tumor tissues have increased ATX expression (106–110). In breast cancer in particular, antagonists against ATX and LPA receptors prevent breast cancer cell (BCC) migration and promote tumor regression in vivo (106, 111, 112). Increased ATX expression in breast cancer and melanoma cells has also been implicated in Taxol resistance (Bristol-Meyers Squibb, New York, New York) (113), and forced expression of ATX promotes bone metastasis through activation of osteoclasts (114), which highlights the importance of developing a better understanding of ATX and LPA signaling in cancer. Indeed, LPA receptor mutations and aberrant expression of receptors have been found in osteosarcoma, colon, lung, and liver cancer cells (115–118), further suggesting roles in aspects of cancer. In addition, many tumors require significantly increased blood flow, and ATX/LPA signaling promotes angiogenesis through VEGF and MMPs (119–121). There is interest and effort in developing ATX inhibitors as anticancer chemotherapeutics (122), and a further understanding of how ATX and LPA affect processes like angiogenesis, metastasis, and cancer proliferation could
aid therapeutic modulation of ATX and LPA in understanding and treating cancer.

1.4. LPA RECEPTORS

The numerous reported physiological effects of LPA are primarily mediated through the six currently recognized LPA receptors, LPA$_{1-6}$. These GPCRs couple to all four G$_{\alpha}$ proteins (G$_{12/13}$, G$_{q/11}$, G$_{i/o}$, and G$_{s}$), which initiate a variety of signaling cascades. The interplay among different LPA receptors, primarily modulated by differential receptor subtypes in specific tissues, drives the many biological and pathological processes noted here as well as in subsequent chapters.

1.4.1. LPA$_{1}$

LPA$_{1}$ was the first receptor identified for any lysophospholipid (15) and is the best studied of the six recognized LPA receptors. LPAR1 (human chromosomal locus 9q31.3) encodes a 41-kDa protein containing 364 amino acids with seven putative transmembrane domains. In mice, the Lpar1 gene encodes five exons with a conserved intron (shared among Lpar1-3) interrupting transmembrane domain 6. There has been one reported variant of Lpar1 (mrec1.3) that results in an 18 amino acid deletion of the N terminus (123), but the biological significance of this variant has not been elucidated. LPA$_{1}$ is highly homologous to LPA$_{2-3}$, sharing a $\sim$50–60% amino acid sequence identity. While there are currently no crystal structures available for any of the LPA receptors, mutagenesis studies have identified several residues in LPA$_{1-3}$ signaling. R3.28 and K7.36A are both important for the efficacy and potency of LPA$_{1}$, while Q3.29 decreased ligand interaction and activation (124), based primarily on secondary readouts.

LPA$_{1}$ couples with three types of G$_{\alpha}$ proteins: G$_{i/o}$, G$_{q/11}$, and G$_{12/13}$ (Fig. 1.3). These form heterotrimeric G proteins that initiate signaling cascades through downstream molecules such as mitogen-activated protein kinase (MAPK), phospholipase C (PLC), Akt, and Rho. LPA$_{1}$ activation induces a variety of cellular responses, including altered cell–cell contact through serum response element activation, cell proliferation and survival, cell migration and cytoskeletal changes, Ca$^{2+}$ mobilization, and adenylyl cyclase inhibition (reviewed in References 4, 20, and 125).

Expression of Lpar1/LPAR1 is widely distributed in both adult mice and humans, including in the brain, uterus, testis, lung, small intestine, heart, stomach, kidney, spleen, thymus, placenta, and skeletal muscle (17, 125, 126). Expression of Lpar1 is more spatially restricted during embryonic development, but is enriched in parts of the brain, limb buds, craniofacial region,
somites, and genital tubercle (127). In the developing nervous system in particular, Lpar1 expression is regulated both spatially and temporally (reviewed in References 4 and 125). During embryogenesis, central nervous system (CNS) expression is enriched in the neocortical neurogenic region called the ventricular zone (VZ) and superficially in a layer that includes the marginal zone and meninges (15). The VZ disappears just prior to birth, at the end of cortical neogenesis, but Lpar1 expression continues in oligodendrocytes, particularly within the white matter tracks of the postnatal brain and this expression coincides with myelination.

Much of what is known regarding LPA signaling during neurodevelopment has been gleaned from the use of Lpar1−/− mice. Of the four LPA receptor-null mouse lines that have been reported (Lpar1-4−/−), Lpar1−/− mice are the only ones to demonstrate obvious neurodevelopmental defects. These mice show 50% perinatal lethality because of a defect in suckling behavior (128), which could be attributable to olfactory deficits. Surviving Lpar1−/− mice have a significantly reduced body size, craniofacial dysmorphism with blunted snouts, and increased apoptosis in sciatic nerve Schwann cells (SCs) (129). During colony expansion of the original Lpar1−/− line, a variant arose spontaneously that was dubbed “Málaga” (maLPA1) for its geographic location in Spain (130). The maLPA1 variant exhibits more severe developmental brain defects than the Lpar1−/− line, yet has negligible perinatal lethality. Defects in maLPA1 neurodevelopment include reduced proliferative populations, increased cortical apoptosis, and premature expression of neuronal markers (130), as well as similar effects on adult hippocampal neurogenesis (131).

Most LPA receptors are expressed in the nervous system, and LPA is abundantly present in the brain. LPA signaling can influence many neurodevelopmental processes, including cortical development and function (130, 132), growth and folding of the cerebral cortex (133), growth cone process retraction (134–136), cell survival (133), migration (137), adhesion (129), and proliferation (128, 133). LPA1 signaling was first reported to influence proliferation and differentiation of primary neuroprogenitor cells (NPCs) and neurosphere cultures (128, 138, 139). Nonmammalian models have also demonstrated LPA1 effects in the CNS, where analogs of both LPA1 and LPA2 were reported to regulate normal cortical actin assembly in Xenopus embryos (140). A number of in vitro experiments have demonstrated the effect of LPA stimulation on NPC cultures, as well as a variety of neuronal cell lines and primary neurons. These studies reported LPA-induced neurite retraction, growth cone collapse, and migration (136, 137, 141–144).

In addition to NPC and neuronal cell types, LPA1 signaling is involved in the biology of glial cell types. Astrocytes are the most abundant type of glia and play a significant role in developmental, functional, and pathological processes. Astrocytes express LPA1–5 (145) and, upon treatment with LPA, initiate a wide range of effects in vitro, including morphological changes and stabilization of stress fiber (146, 147). These responses are potentially relevant to neurodegeneration, where astrogliosis can be prominent. Injections of LPA