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MINIREVIEW

Lysophosphatidic Acid Receptors

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ABSTRACT

Lysophosphatidic acid (LPA) is a simple bioactive phospholipid with diverse physiological actions on many cell types. LPA induces proliferative and/or morphological effects and has been proposed to be involved in biologically important processes including neurogenesis, myelination, angiogenesis, wound healing, and cancer progression. LPA acts through specific G protein-coupled, seven-transmembrane domain receptors. To date, three mammalian cognate receptor genes, lp_{A1}/vzg-1/*Edg2*, lp_{A2}/*Edg4*, and lp_{A3}/*Edg7*, have been identified that encode high-affinity LPA receptors. Here, we review current knowledge on these LPA receptors, including their isolation, function, expression pattern, gene structure, chromosomal location, and possible physiological or pathological roles.

Lysophosphatidic acid (LPA; 1-acyl-2-*sn*-glycerol-3-phosphate) is a naturally occurring lysophospholipid (LP) that activates diverse cellular actions on many cell types (Fig. 1). It is also an intermediate in de novo biosynthesis of membrane phospholipids. Although all cells contain small amounts of LPA associated with membrane biosynthesis, some cellular sources can produce significant amounts of extracellular LPA such as activated platelets, which account for the LPA found in serum (Eichholtz et al., 1993). Sphingosine-1-phosphate (S1P) and sphingosylphosphorylcholine (SPC) also activate cellular responses in many cell types (Spiegel et al., 1998). LPA, S1P, and SPC each activate specific members of the G protein-coupled receptor (GPCR) superfamily.

Lysophospholipid GPCRs are encoded by the *lp* genes (also referred to by various orphan receptor names such as *vzg/edg/mrec/gpcr26/h218/agr16/nrg-1*), of which there are currently eight known members (Fig. 2). Three of these genes ($lp_{A1–3}$) encode high-affinity LPA receptors (Hecht et al., 1996; An et al., 1997b, 1998a; Fukushima et al., 1998; Bandoh et al., 1999; Im et al., 2000b). The other five, lp_{B1} through lp_{B4} and lp_{C1}, encode high-affinity S1P or SPC receptors (An et al., 1997a; Lee et al., 1998b; Zondag et al., 1998; Zhang et al., 1999; Im et al., 2000a; Van Brocklyn et al., 2000), with one study reporting that LP_{B1}/EDG1 can also serve as a low-affinity LPA receptor (Lee et al., 1998a). In addition to the LP receptors, a dissimilar, putative LPA receptor (PSP24) has also been reported in *Xenopus* (Guo et al., 1996), although independent confirmation of this identification has yet to emerge. This review will focus on the three confirmed mammalian LPA receptors.

Cellular Effects of LPA

The proliferative effects of LPA were first recognized in the mid-1980s (Moolenaar et al., 1986; van Corven et al., 1989). In these reports, serum-starved quiescent Rat-1 or human foreskin fibroblasts were found to respond to LPA with increased [^{3}H]thymidine incorporation, inhibition of adenylyl cyclase (AC), increased inositol phosphates and intracellular calcium ($[Ca^{2+}]_i$), increased protein kinase C activity, and arachidonic acid release. The proliferation and AC responses were completely inhibited with pertussis toxin (PTX) pretreatment, which specifically inactivates $G_{i/o}$-type G proteins.

Changes in cell morphology in response to LPA were first demonstrated in the early 1990s (Dyer et al., 1992; Jalink and Moolenaar, 1992; Ridley and Hall, 1992; Tigyi and

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ABBREVIATIONS: LPA, lysophosphatidic acid; LP, lysophospholipid; S1P, sphingosine-1-phosphate; SPC, sphingosylphosphorylcholine; GPCR, G protein-coupled receptor; AC, adenylyl cyclase; PTX, pertussis toxin; PCR, polymerase chain reaction; EST, expressed sequence tag; MAP kinase, mitogen-activated protein kinase; OCC, ovarian cancer cell lines; ORF, open reading frame; OSE, ovarian surface epithelial cells; PLC, phospholipase C; SRE, serum-responsive element; TMD, transmembrane domain; kb, kilobases.

Miledi, 1992; Jalink et al., 1993). One group demonstrated that LPA induced actin cytoskeletal rearrangement of 3T3 fibroblasts, forming stress fibers through activation of the small GTPase, Rho, as demonstrated by complete inhibition of this response with *Botulinum* C3 toxin (Ridley and Hall, 1992). Other groups independently showed that LPA caused neurite retraction/cell rounding in cell lines of neural origin (Dyer et al., 1992; Jalink and Moolenaar, 1992; Ridley and Hall, 1992; Jalink et al., 1993).

Since these initial studies, proliferative and morphological responses to LPA have been shown in many other cell lines. Numerous other cellular and biochemical responses to LPA have also been documented (reviewed in Moolenaar et al., 1997, 1999; Chun, 1999; Chun et al., 1999). The actual mechanisms through which this detergent-like molecule acted were long uncertain, however available evidence supported the involvement of specific GPCRs (Moolenaar et al., 1997). The search for LPA receptors was hampered by a lack of specific receptor antagonists, difficulty in ligand-binding experiments, and the ubiquitous presence of LPA responsiveness in many cell types (reviewed in Chun, 1999); these factors contributed to the prolonged absence of cloned receptors for any lysophospholipid.

LPA Receptor Gene Cloning

The first LPA receptor cDNA, lp_{A1}, was isolated using degenerate PCR from a mouse cerebral cortical neuroblast cDNA template (Hecht et al., 1996). It was named *ventricular zone gene-1* (*vzg-1*) because of its predominant expression in the neurogenic ventricular zone of the embryonic cortex. This receptor gene encoded the first identified, high-affinity LPA receptor based on multiple criteria (Hecht et al., 1996). Several other groups also identified this gene from other species as an orphan receptor of unknown ligand specificity or function (reviewed in Chun 1999; Chun et al., 1999). Identification of this gene as encoding a LPA receptor received independent support (An et al., 1997b; Erickson et al., 1998). However, perhaps reflecting the historical difficulty in identifying a receptor, skepticism from some persisted about its identity (Allard et al., 1998; Hooks et al., 1998). Definitive studies utilizing heterologous expression in mammalian cells (Fukushima et al., 1998) or genetic deletion of lp_{A1} in mice (Contos et al., 2000) have eliminated such concerns. This functional information, combined with sequence and genomic structure analyses (Contos and Chun, 1998) provided a straightforward way to identify similar genes, which led to the subsequent identification of two other LPA receptors.

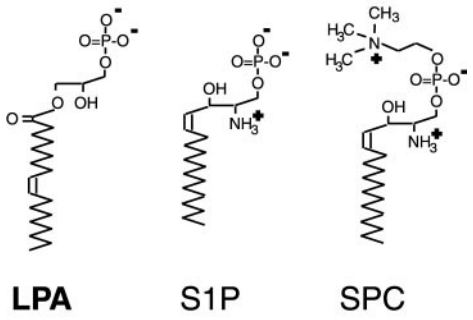

Fig. 1. Chemical structure of several natural bioactive lysophospholipids. All lysophospholipids contain one long hydrocarbon chain (shown here as the unsaturated oleoyl group for LPA) on a three carbon backbone containing a phosphate group.

The second LPA receptor gene, lp_{A2}, was identified through both an EST (expressed sequence tag) and genomic clones in the GenBank database by virtue of its substantial similarity to lp_{A1} (An et al., 1998a; Contos and Chun, 1998). Using homology searches, An et al. (1998a) identified two ESTs from the same cDNA clone in tumor cell libraries, and based upon functional studies, the encoded protein was determined to be another LPA receptor. The gene was called *Edg4*, based on its similarity with *"endothelium differentiation genes* (*Edgs*)". At the same time, exons of the human gene were identified (Contos and Chun, 1998), and additional studies have indicated that the reported and functionally assessed *Edg4* receptor is actually a mutant distinct from that encoded by lp_{A2} (discussed further below). Nevertheless, both mutant and wild-type genes have general properties of functional LPA receptors.

The third LPA receptor gene, lp_{A3}, was identified through degenerate PCR strategies similar to those used to isolate lp_{A1} (Hecht, 1996; US patent #6,057,126, filed in 1997). More recent analyses of this gene utilized cDNAs isolated by PCR from human Jurkat T cell (Bandoh et al., 1999) and human

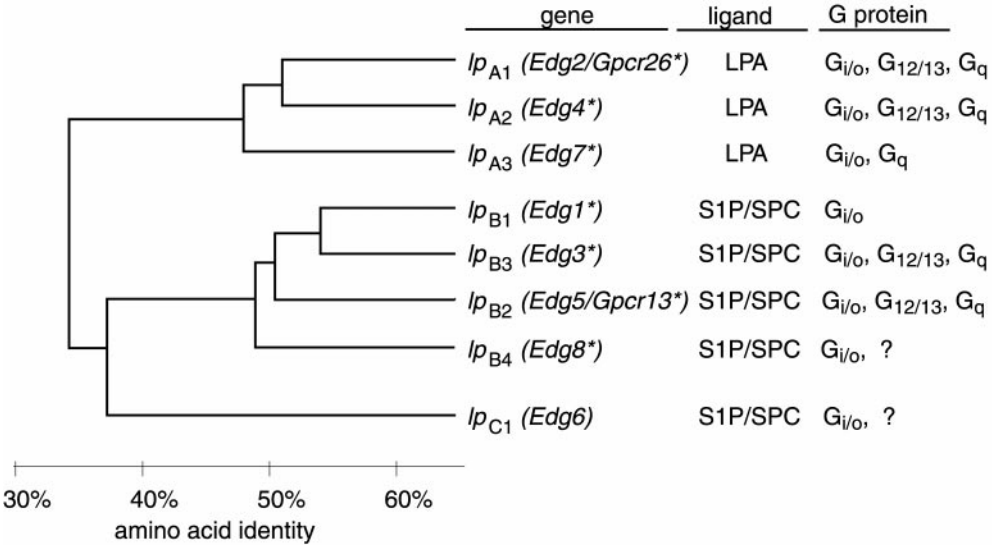

Fig. 2. Properties of lysophospholipid receptor genes. The dendrogram is based on percentage of amino acid identities among mouse LP receptor sequences (except lp_{B4}, which is from rat) and shows the predicted evolutionary divergence of the genes. Common synonyms are indicated in parentheses, and mouse gene symbols are denoted with asterisks. Probable G protein partners for each of the encoded receptors are shown, based on receptor expression studies that indicate PTX-sensitive cellular responses ($G_{i/o}$ coupling), PTX-insensitive cytoskeletal rearrangement/Rho activation ($G_{12/13}$ coupling), or PTX-insensitive intracellular calcium mobilization and/or inositol phosphate production (G_q coupling).

embryonic kidney 293 cell (Im et al., 2000b) cDNA to support its identification as a third LPA receptor.

Alignment of amino acid sequences for mouse and human LP_A receptors is shown in Fig. 3. Mouse forms of LP_{A1}, LP_{A2}, and LP_{A3} consist of 364, 348, and 354 amino acids, respectively, and molecular weight sizes estimated from the sequences are 41.2, 38.9, and 40.3 kDa, respectively. Human forms of LP_{A1}, LP_{A2}, and LP_{A3} consist of 364, 351, and 353 amino acids, respectively, and estimated molecular weights are 41.1, 39.1, and 40.1 kDa, respectively. Amino acid identities between mouse and human are 97.3% for LP_{A1}, 90.8% for LP_{A2}, and 90.7% for LP_{A3}. Predicted post-translational modification sites are well conserved between species and receptor subtypes, and the modifications may account for differences between the predicted and observed molecular mass of receptor proteins. These receptors can be activated by LPA concentrations around 10 nM, depending on employed assays (Hecht et al., 1996; Fukushima et al., 1998; Bandoh et al., 1999; Goetzl et al., 1999; Ishii et al., 2000).

Functional Studies of LP_A Receptors

The key observation leading to identification of lp_{A1} as encoding a LPA receptor was that overexpression of the receptor in the cortical cells from which it was cloned resulted in an increased percentage of rounded or neurite-retracted cells (Hecht et al., 1996). The ligand for this receptor was determined to be present in serum, used routinely for the growth of these cells, and based on heat stability, specific [^{3}H]LPA binding to plasma membrane preparations and functional responses including AC inhibition, LPA was identified as a ligand (Hecht et al., 1996).

Additional reports provided further information regarding the responses mediated by LP_{A1} (Table 1). Expression of the human ortholog (*Edg2*) caused increased LPA responsiveness in a serum-responsive element (SRE) reporter gene assay in human embryonic kidney 293 cells, increases in specific [^{3}H]LPA binding to plasma membrane preparations in Chinese hamster ovary cells (An et al., 1997b), and in Jurkat T cells, increases in $[Ca^{2+}]_i$ (An et al., 1998b). Human LP_{A1} heterologously expressed in yeast that have neither *lp*-related receptors nor endogenous responses to LPA also resulted in a dose-dependent response to LPA for activating the mitogen-activated protein (MAP) kinase pathway (Erickson et al., 1998).

Mammalian heterologous expression approaches were made possible through the identification of two mammalian cell lines, B103 (rat neuroblastoma) and RH7777 (rat hepatoma), that have undetectable lp_A transcripts and that lack endogenous responses to LPA (Fukushima et al., 1998; Ishii et al., 2000). Cell lines heterologously expressing receptor proteins showed increased specific [^{3}H]LPA binding to plasma membrane preparations and activation of G proteins as detected by GTPγS incorporation (Fukushima et al., 1998). They also became responsive to LPA as manifested by cell rounding, bromodeoxyuridine incorporation, SRE activation, and stress-fiber formation (Fukushima et al., 1998). In B103 cells expressing LP_{A1}, LPA induced activation of phospholipase C (PLC) and MAP kinase, arachidonic acid release, and inhibition of AC (Ishii et al., 2000). These studies confirmed LP_{A1} identity and further demonstrated that a single LPA receptor could activate several distinct signaling pathways.

Several experiments have demonstrated that lp_{A2} also encodes a multifunctional LPA receptor (Table 1). In initial reports, the human mutant lp_{A2} (*Edg4*) was expressed in Jurkat T cells, conferring LPA-specific responses in SRE activation and calcium mobilization assays (An et al., 1998a,b). Bandoh et al. (1999) reported that expression of human lp_{A2} in Sf9 insect and rat PC12 cells conferred $[Ca^{2+}]_i$ increases and MAP kinase activation, respectively, whereas heterologous expression of murine lp_{A2} within murine B103 cells produced LPA-dependent cell rounding, activation of PLC and MAP kinase, arachidonic acid release, and inhibition of AC (Ishii et al., 2000). Differences in assay systems

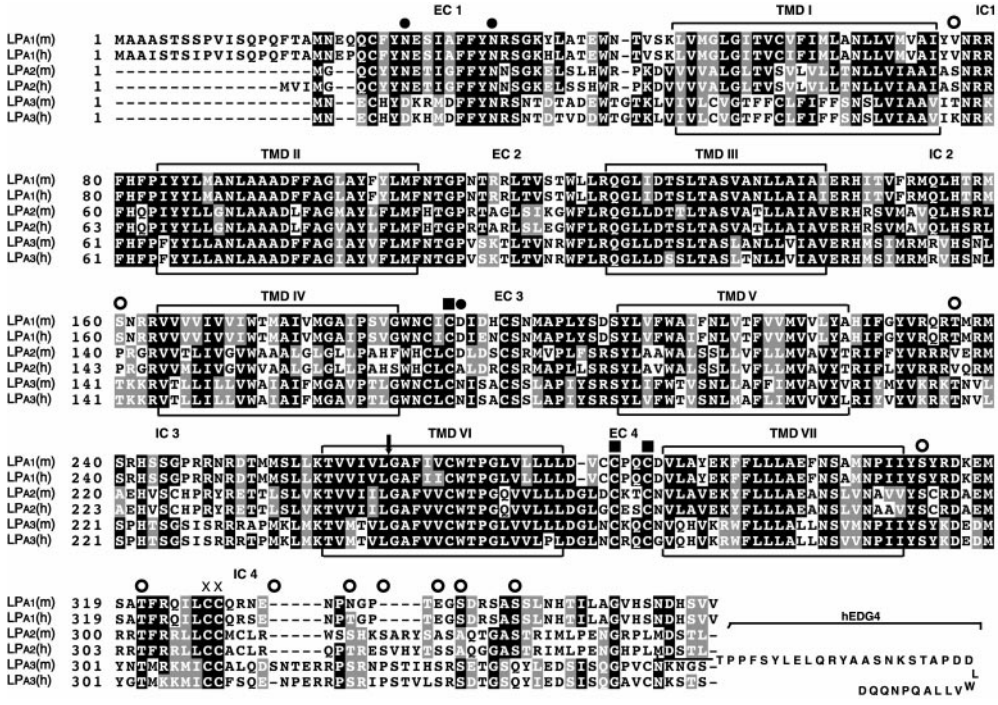

Fig. 3. Alignment of predicted amino acid sequences for mouse (m) and human (h) LP_A receptors. Residues identical in three or more of the sequences are shown in white on a black background, whereas conservative changes are shaded in gray. Approximate locations of the seven putative TMDs are bracketed, and extracellular domains (ECs) and intracellular domains (ICs) are indicated. Sites of putative post-translational modifications in one or several of the receptors are indicated: *N*-linked glycosylation (●), Ser/Thr phosphorylation (○), cysteine palmitoylation (×), and cysteines involved in disulfide bonds (■). Note that in several potential Ser/Thr phosphorylation sites, consensus sequences may only be present on one of the aligned proteins. An arrow (↓) in TMD VI indicates the conserved exon-intron boundary (as yet unknown for human LP_{A3}). Also shown are the 35 divergent C-terminal amino acids of the mutant human *Edg4* cDNA clone. The mrec variant isoform of LP_{A1} begins at the second methionine in the mouse sequence.

may alter outcomes as observed for increased cAMP formation in response to LPA in LP_{A2}-expressing Sf9 cells, contrasting with the decrease that was observed in Edg4-expressing HTC4 cells (An et al., 1998b) or LP_{A2}-expressing B103 cells (Ishii et al., 2000). Others reported that lp_{A2} expression in RH7777 cells conferred LPA-dependent $[Ca^{2+}]_i$ increases but had no effect on cAMP accumulation unlike lp_{A1}-transfected cells where a decrease was observed (Im et al., 2000b).

A third multifunctional LPA receptor is encoded by lp_{A3}, as demonstrated by three independent studies (Table 1). The human gene was expressed in Sf9 cells, resulting in LPA-dependent $[Ca^{2+}]_i$ increases and cAMP accumulation (similar to LP_{A2}) (Bandoh et al., 1999). By comparison, the expression of the human receptor in RH7777 cells mediated LPA-dependent $[Ca^{2+}]_i$ increases without cAMP accumulation (Im et al., 2000b). Mouse LP_{A3} expressed in B103 cells mediated activation of PLC and MAP kinase, arachidonic acid release, and inhibition of AC but not cell rounding (Ishii et al., 2000).

These different responses mediated by the three LPA receptors, as well as the sensitivity of these responses to specific inhibitors such as PTX and *Botulinum* C3 toxin, suggest some differences in G protein-coupling (Figs. 2 and 4). Of the four primary classes of heterotrimeric G proteins, G_s, $G_{i/o}$, $G_{12/13}$, and G_q, LPA receptors apparently couple to all but the G_s types under physiological conditions. LPA stimulates cell proliferation through activation of tyrosine kinase and MAP kinase (Moolenaar et al., 1997). $G_{i/o}$-type proteins are the most likely candidates to mediate these effects of PTX sensitivity. The morphological responses to LPA (e.g., stress-fiber formation, cell rounding) are mediated primarily through Rho activation by the $G_{12/13}$ proteins (Buhl et al., 1995). Rho activates Rho kinases (e.g., ROCK), which in turn phosphorylate cytoskeletal proteins. A specific inhibitor of Rho kinases, Y-27632, is available and has been shown to block morphological responses to LPA (Uehata et al., 1997). PLC activation, which leads to the production of two major classes of second messengers, diacylglycerol and inositol triphosphate, are mediated by the α-subunits of G_q-type proteins (these include G_q, G_{11}, G_{14}, and $G_{15/16}$) and/or the $\beta\gamma$-subunits of $G_{i/o}$ proteins (Exton, 1997). Most studies indicate that the LP_{A1} receptor can couple to the $G_{i/o}$, $G_{12/13}$ and G_q families (Hecht et al., 1996; An et al., 1997a,b; Fukushima et al., 1998; Ishii et al., 2000). LP_{A2} also can couple to the $G_{i/o}$, $G_{12/13}$, and G_q families (An et al., 1998a,b; Bandoh et al., 1999; Im et al., 2000b; Ishii et al., 2000). Similar experiments indicate that LP_{A3} can couple to the $G_{i/o}$ and G_q families (Bandoh et al., 1999; Im et al., 2000b; Ishii et al., 2000). Interestingly, it appears that LP_{A3} does not couple efficiently with $G_{12/13}$, based on the lack of cell rounding in B103 cells expressing this receptor (Ishii et al., 2000).

TABLE 1
Responses mediated by each of the LP_A receptor types in culture

	Responses	Cell Types	References
lp_{A1}	Cell rounding	TSM (immortalized neuroblast)	Hecht et al., 1996
		B103 (neuroblastoma)	Fukushima et al., 1998
		B103	Ishii et al., 2000
	AC inhibition	TSM	Hecht et al., 1996
		HTC4 (hepatoma)	An et al., 1998b
		B103	Ishii et al., 2000
	SRE activation	HEK293 (kidney fibroblast)	An et al., 1997b
		B103	Fukushima et al., 1998
	$[Ca^{2+}]_i$ increase	Jurkat T (lymphoma)	An et al., 1998b
		HTC4	An et al., 1998b
	IP production	HTC4	An et al., 1998b
		B103	Ishii et al., 2000
	MAP kinase activation	Yeast (*S. cerevisiae*)	Erickson et al., 1998
		B103	Ishii et al., 2000
	Stress-fiber formation	RH7777 (hepatoma)	Fukushima et al., 1998
	BrdU incorporation	B103	Fukushima et al., 1998
	Apoptosis inhibition	Primary Schwann cells	Weiner et al., 1999
	Arachidonic acid release	B103	Ishii et al., 2000
lp_{A2}	SRE activation	Jurkat T	An et al., 1998a
	$[Ca^{2+}]_i$ increase	Jurkat T	An et al., 1998b
		HTC4	An et al., 1998b
		Sf9 (insect)	Bandoh et al., 1999
		RH7777	Im et al., 2000b
	IP production	HTC4	An et al., 1998b
		B103	Ishii et al., 2000
	AC inhibition	HTC4	An et al., 1998b
		B103	Ishii et al., 2000
	AC stimulation	Sf9	Bandoh et al., 1999
	MAP kinase activation	PC12 (pheochromocytoma)	Bandoh et al., 1999
		B103	Ishii et al., 2000
	Cell rounding	B103	Ishii et al., 2000
	Arachidonic acid release	B103	Ishii et al., 2000
lp_{A3}	$[Ca^{2+}]_i$ increase	Sf9	Bandoh et al., 1999
		RH7777	Im et al., 2000b
	AC stimulation	Sf9	Bandoh et al., 1999
	AC inhibition	B103	Ishii et al., 2000
	IP production	B103	Ishii et al., 2000
	MAP kinase activation	B103	Ishii et al., 2000
	Arachidonic acid release	B103	Ishii et al., 2000

Expression Patterns of lp_A Genes

A major locus of lp_{A1} expression is within the embryonic cerebral cortex, where it is enriched in the ventricular zone, the zone of neurogenesis (Hecht et al., 1996; Chun, 1999; Dubin et al., 1999; Fukushima et al., 2000). lp_{A1} is also expressed in the adult mouse brain (Fig. 5), where in situ hybridization and Northern blot analyses demonstrate expression in oligodendrocytes, as well as Schwann cells of the peripheral nervous system; these are myelinating cells of the nervous system (Allard et al., 1998; Weiner et al., 1998; Chun, 1999; Weiner and Chun, 1999). Based on Northern blot analysis in adult mouse organs, lp_{A1} is also expressed in many other tissues, including testes, lung, heart, intestine, spleen, kidney, thymus, and stomach (Fig. 5). No expression was detectable in liver. Human lp_{A1} is similarly expressed in many adult organs, including brain, heart, colon, small intestine, placenta, prostate, ovary, pancreas, testes, spleen, skeletal muscle, and kidney (An et al., 1998a). Little or no expression was apparent in liver, lung, thymus, or peripheral blood leukocytes.

Mouse lp_{A2} is expressed most abundantly in testes, kidney, and embryonic brain (Fig. 5; Contos and Chun, 2000). Other organs also express the transcript, including heart, lung, spleen, thymus, stomach, and adult brain, and several have little or no expression, including liver, small intestine, and skeletal muscle (Contos and Chun, 2000). Human lp_{A2} is expressed most abundantly in testes and peripheral blood leukocytes with less expression in pancreas, spleen, thymus, and prostate (An et al., 1998a). Little or no expression was detectable in heart, brain, placenta, lung, liver, skeletal muscle, kidney, ovary, small intestine, or colon.

Mouse lp_{A3}, like lp_{A2}, is expressed most abundantly in testes, kidney, and lung, with moderate levels in small intestine, and low levels in heart, stomach, spleen, and adult and perinatal brain (Fig. 5). Little or no expression was detectable in embryonic brain, liver, or thymus. Human lp_{A3} is expressed most abundantly in prostate, testes, pancreas, and heart, with moderate levels in lung and ovary (Bandoh et al., 1999; Im et al., 2000b). No expression was detectable in brain, placenta, liver, skeletal muscle, kidney, spleen, thymus, small intestine, colon, or peripheral blood leukocytes.

lp_A Structure

The first lp_A gene characterized at the genomic level was lp_{A1} (Contos and Chun, 1998). The primary transcript (represented by the *vzg-1* cDNA clone) is divided among four exons, with the open reading frame (ORF) distributed over the last three exons (Fig. 6). Introns are situated 5′ to the coding region for transmembrane domain I (TMD I) and within the coding region for TMD VI. This finding was unexpected because the majority of GPCR gene ORFs, including the evolutionarily related genes for a S1P receptor, lp_{B1}/*edg1*, and a cannabinoid receptor, *Cnr1*, have uninterrupted ORFs. The presence of an intron in the coding region for TMD VI indicates that it was inserted into the gene after it diverged from the lp_B genes. Interestingly, a cDNA clone variant (mrec1.3) has a completely divergent 5′ sequence from lp_A. This sequence divergence is exactly at the boundary between exons 2 and 3 and was determined to be due to use of an alternative primary exon, located between exons 2 and 3. The coding region of the mrec variant starts at the second ATG of the lp_A ORF, resulting in a protein with 18 fewer amino acids

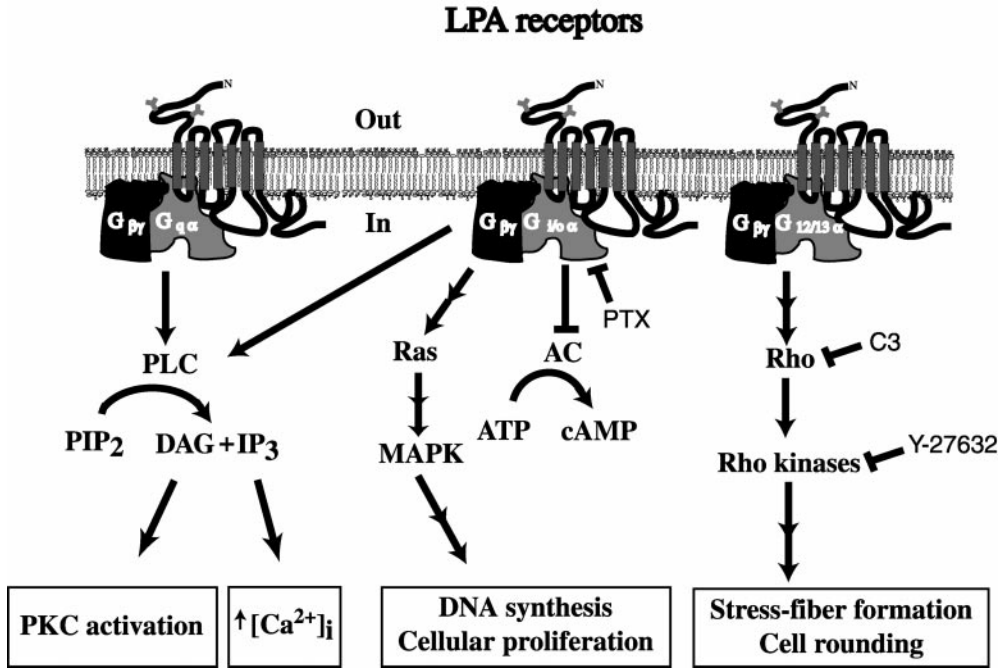

Fig. 4. G protein-coupled receptor signal transduction pathways activated by LPA. Lines with arrowheads illustrate activation paths, whereas inhibition effects are illustrated by lines with crossbars. Sequential arrows indicate multiple signaling steps that are not illustrated. Activation of $G_{i/o}$ inhibits AC and thus cAMP production. This pathway also activates the Ras/MAP kinase (MAPK) cascade, which is primarily responsible for increased proliferation. In addition, $G_{i/o}$ activates PLC via its $\beta\gamma$-subunits, which results in generation of diacylglycerol (DAG) and inositol triphosphate (IP_3) from phosphatidylinositiol diphosphate (PIP_2). DAG activates protein kinase C (PKC), and IP_3 mobilizes $[Ca^{2+}]_i$. All $G_{i/o}$-mediated signaling is specifically inhibited by PTX. $G_{12/13}$ proteins are responsible for activation of the small GTPase, Rho, which can be specifically inhibited by *Botulinum* C3 exoenzyme (C3). Activated Rho stimulates Rho kinases, inducing cytoskeletal and morphological changes. Rho kinases are directly inhibited by Y-27632. The α-subunits of G_q proteins are the primary effectors of PLC activation.

(Fig. 3, beginning with the MNE. . .). The function of these two different isoforms of LP_{A1} remains unclear. Recent experiments indicate that the two transcript forms are produced from alternative promoter usage rather than alternative splicing (J. J. A. Contos and J. Chun, unpublished observation). The human gene has a 4-exon structure similar to the mouse gene (Allard et al., 1999). However, no human counterpart to the mrec exon has been identified in over 150 cDNA clones analyzed.

Both mouse (Fig. 6) and human lp_{A2} genes are divided among three exons (Contos and Chun, 2000). The structure is very similar to that of the mrec variant of lp_{A1}. Both have start and stop sites in the second and third exons, respectively, and introns located just upstream of the start codon and within the coding region for TMD VI. In both mouse and human, two transcript sizes are evident from Northern blot analysis (Fig. 5; An et al., 1998a). In human, these are ~1.8 kb (found primarily in testes, prostate, and pancreas) and ~10 kb (found in leukocytes, spleen, and thymus), whereas in mouse they are ~3 kb (found in all expressing tissues) and ~7 kb (found in kidney, testes, and embryonic brain). Although the smaller transcript sizes are expected from the gene structures, the function of the larger transcript is not known.

Analysis of the mouse lp_{A3} genomic clone (J. J. A. Contos and J. Chun, submitted for publication) indicates that the gene is also divided among three exons in a structure very similar to lp_{A2} (Fig. 6). Introns are located just upstream of the start codon and within the middle of the coding region for TMD VI. Reverse transcription-PCR analysis with primers within exons 1, 2, and 3 indicates that the three exons were spliced in all tissues that were shown to express the transcript by Northern blot analysis (Fig. 5).

lp_A Chromosomal Location

Chromosomal location of each mouse LP_A receptor was determined by linkage analysis. The lp_{A1} gene was localized to proximal chromosome 4 at a location indistinguishable from the vacillans gene (*vc*) (Contos and Chun, 1998). These results are in disagreement with localization for the lp_{A1} isoform *mrec1.3* where the gene was placed at distal chromosome 4 (Macrae et al., 1996). The contrasting results might

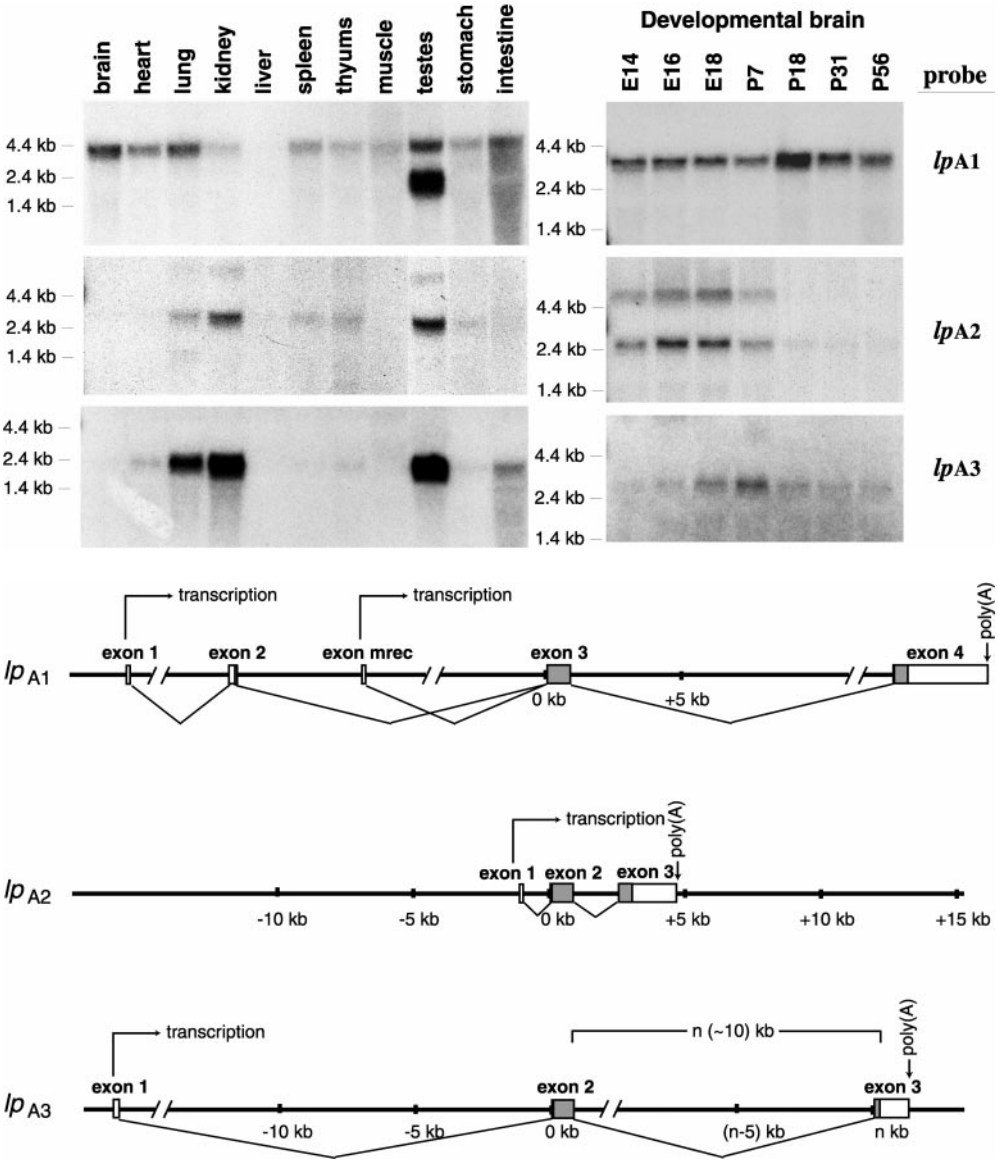

Fig. 5. Expression patterns of lp_A genes in mouse tissues. Total RNAs (20 μg each lane) from various adult organs or whole brain at several embryonic (E) and postnatal (P) ages were examined by high stringency Northern blot analysis using specific probes to mouse lp_{A1}, lp_{A2}, and lp_{A3}. Location of size standard are noted (kb = kilobases).

Fig. 6. Genomic structure of mouse lp_A genes. Boxes represent exons and shadings within them represent open reading frames. The intron between exons 3 and 4 in lp_{A1} and between exons 2 and 3 in lp_{A2} and lp_{A3} is located in the middle of the coding region for transmembrane domain VI. Although the distance between exons 2 and 3 in lp_{A3} has not been determined precisely, genomic Southern analysis indicates that the intron is approximately 10 kb. Human lp_{A1} and lp_{A2} structures are nearly identical, except that no exon mrec is present.

be explained by the unusual finding that exon 4 is duplicated on chromosome 6 in *Mus spretus* (Contos and Chun, 1998) and usage of different lp_{A1} regions in segregation analyses. Vacillans refers to the gene(s) mutated and responsible for a phenotype characterized in the 1950s (Sirlin, 1956). Although the segregation pattern of *vc* has been determined, the genes responsible have not been characterized. Thus, mutations in lp_{A1} might be related to the *vc* phenotype. Vacillans was named because the homozygous mutant (*vc*/*vc*) mice would "vacillate" or waddle when walking, indicating problems with motor control. These mice also displayed violent tremors, less aggressive behaviors, smaller overall sizes, approximately half-normal muscular strength, a mortality rate of 50% by weaning, and delayed male sexual maturity. Some of the phenotypes might be explained by problems in brain development and function, peripheral nerve conduction, and testes development. The expression pattern of lp_{A1} in embryonic brain, myelinating cells (i.e., oligodendrocytes), and testes, suggests that mutations in lp_{A1} might be responsible for *vc*. Unfortunately, neither the *vc* mice nor their DNA remain, making further analyses impossible. Targeted deletion of lp_{A1} in mice shows cellular and growth defects that overlap with some of these *vc* phenotypes (Contos et al., 2000).

Using backcross analysis, mouse lp_{A2} was localized to central chromosome 8 at a location indistinguishable from the myodystrophy (*myd*) gene and very close to the "kidney anemia testes" (*kat*) gene (Contos and Chun, 2000). The expression pattern of lp_{A2} supports a relationship between lp_{A2} and *kat* but not to *myd*. The *kat* phenotype includes polycystic kidney disease, anemia, and male sterility (Janaswami et al., 1997). However, no mutations in lp_{A2} exons could be found in *kat*/*kat* mouse genomic DNA (J. J. A. Contos, unpublished observation). In segregation analyses, *kat* localizes between *DMit128* and *DMit129* markers, whereas lp_{A2} localizes outside of this interval. Thus, mutations in lp_{A2} are unlikely to be related to the *kat* phenotype.

Mouse lp_{A3} was localized to the middle of chromosome 3 in the region of the varitint waddler (*va*) gene (J. J. A. Contos and J. Chun, submitted for publication). Interestingly, this *va* phenotype has several features similar to the *vc* phenotype. Heterozygous (*va*/+) mice have a tinted coat color in various regions (hence the "varitint" name) and moved with a "duck-like" walk (hence the "waddler" name), similar to the vacillation of *vc*/*vc* mice (Cloudman and Bunker, 1945). They are deaf, react violently when disturbed, and run in circles when excited. The homozygous mutation (*va*/*va*) resulted in approximately 80% lethality. Because lp_{A3} expression has not been examined in areas likely defective in *va* mice and possible mutations in lp_{A3} have not been analyzed in *va*/*va* genomic DNAs, lp_{A3} remains a possible candidate for *va*.

Human lp_{A1} was localized to chromosome 9q31.3-32 based on analyses of the presence of the human gene in human x rodent somatic cell hybrid panels and yeast artificial chromosomes mapped to this region (Allard et al., 1999). Human lp_{A2} was identified on genomic clones that were localized to chromosome 19p12 (Contos and Chun, 2000). Mutations in the gene have not been analyzed for genetically inherited disorders that map to this region. However, one possible disorder that may be related to lp_{A2} mutations is a congenital myeloid leukemia that results from a translocation to this region: t(11;19) (q23;p12-13.1) (Huret et al., 1993). Should this translocation disrupt lp_{A2} expression or function, misregulation of myeloid cell proliferation might result. No information has been published on the chromosomal location of human lp_{A3}. However, it appears to be located on chromosome 1, probably at 1p31.2, which is the only area of chromosome 1 syntenic to mouse chromosome 3 (J. J. A. Contos and J. Chun, submitted for publication).

Potential Role of lp_{A2} Mutations in Ovarian Cancer

Several lines of evidence suggest that LPA signaling may have a role in the progression of ovarian cancer. LPA is known to be an "ovarian cancer activating factor" in ascites fluid from ovarian cancer patients (Xu et al., 1995b). Elevated levels of ascites LPA are present both at early and late stages in ovarian cancer; control subject ascites has lower LPA concentrations (Xu et al., 1995a; Westermann et al., 1998). LPA activates ovarian cancer cell lines (OCC) by increasing $[Ca^{2+}]_i$ and stimulating proliferation; this effect was not observed in normal ovarian surface epithelial cells (OSE) (Xu et al., 1995a). LPA also acts as a survival factor for OCC because it antagonizes the programmed cell death effect of the primary chemotherapeutic agent used to treat the disease (Frankel and Mills, 1996). LPA stimulates OCC, but not OSE, to secrete urokinase plasminogen activator, a protein that contributes to metastasis and whose concentration in ascites is inversely correlated with ovarian cancer prognosis (Pustilnik et al., 1999). The source of LPA in ovarian cancer ascites fluid is unclear. Potential intraperitoneal sources include macrophages, mesothelial cells, or ovarian cancer cells themselves (Westermann et al., 1998).

The expression of lp_A genes in OCC and OSE has been investigated. Independent studies demonstrated that lp_{A2} has high expression levels in OCC and low expression levels in normal OSE, whereas lp_{A1} has low or no expression levels both in OCC and normal OSE (Furui et al., 1999; Goetzl et al., 1999; Pustilnik et al., 1999). Although expression of lp_{A3} is not explicitly shown, it was mentioned that its levels were also elevated in ovarian cancer cells (Pustilnik et al., 1999). These results suggest that LP_{A2} and possibly LP_{A3} are involved in mediating the LPA proliferation/transformation signals in ovarian cancer ascites, whereas LP_{A1} is not. In support of these hypotheses is the finding that stimulation of LP_{A2} using an LP_{A2}-specific antibody/phorbol ester combination resulted in proliferation and SRE activation in OCC but not in OSE (Goetzl et al., 1999). In contrast, overexpression of lp_{A1} in OCC induces apoptosis and anoikis, the opposite effects of what would be expected if LPA promotes cancer progression (Furui et al., 1999). Thus, it appears that LP_{A2} could transduce LPA signals from ascites to susceptible cells during oncogenesis, and that mutations in lp_{A2} could cause the transcript and/or protein to be overexpressed in OCC or cause the protein to be constitutively activated.

The first-reported human *Edg4* cDNA clone was derived from an ovarian tumor library (An et al., 1998a) and differed from human lp_{A2} sequences (Contos and Chun, 2000). The predicted Edg4 protein product was 31 amino acids longer at its C terminus relative to the predicted protein product of mouse lp_{A2} cDNA and genomic sequences (Fig. 3). Further analyses of other human genomic and EST sequences revealed that the extra 31 amino acids were specific to the *Edg4* cDNA clone and could be explained by a guanine nucleotide deletion in the fourth-to-last codon (Contos and

Chun, 2000). The extra 31 amino acids in the mutant LP_{A2} protein may alter normal LP_{A2} coupling with G proteins and/or related regulatory proteins such as GPCR receptor kinases, β-arrestins, or internalization proteins. In addition to the guanine deletion in the *Edg4* ovarian tumor cDNA, there are also many sequence variations in the 3′ untranslated regions of multiple ESTs (Contos and Chun, 2000). Such variations might affect message stability. A more comprehensive study of lp_{A2} mutations and transcript levels in multiple ovarian neoplasms could clarify these issues.

Future Directions

Some of the most exciting aspects of LPA receptor studies have moved from receptor identification to determination of gene functions in normal biological and pathological processes. Targeted deletion of each lp_A gene in mice will help to identify in vivo roles of LPA signaling, and initial studies indicate nonredundant and essential roles for signaling by a single LPA receptor (Contos et al., 2000). Receptor subtype-specific agonists and antagonists will be powerful research tools as well as potential clinical drugs, and although not currently available, it is likely that such reagents are on the horizon. Receptor-based studies, as well as those determining how mutations in lp_A genes might contribute to human genetic disorders and to other pathological processes such as cancer, will likely provide new insights on the roles of this simple lipid in the near future.

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