CMLS, Cell. Mol. Life Sci. 57 (2000) 1978–1985
1420-682X/00/141978-08 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Lysophosphatidic acid: receptors, signaling and survival

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Received 13 March 2000; received after revision 25 April 2000; accepted 25 April 2000

Abstract. Though the mitogenic activity of lysophosphatidic acid (LPA) has been well established through classical studies, its mechanism of action was long obscure. Recent identification and cloning of LPA-specific receptors has led to the elucidation of the G-proteins and signaling pathways through which this molecule functions. In addition to its mitogenic properties, recent reports have suggested that LPA may also promote cell survival. This review will summarize the current literature regarding LPA signaling and its role as an antiapoptotic factor.

Key words. G-protein-coupled receptors; apoptosis; mitogen; calcium mobilization; smooth muscle contraction; phosphatidylinositol.

Introduction

Lysophosphatidic acid (LPA) is a lipid mitogen present in serum (2–20 μM) bound to specific high-affinity sites for long-chain fatty acids on serum albumin [1–3]. Considerable evidence suggests that albumin-bound LPA is an intracellular signaling molecule which contributes to the biological activity of whole serum [4, 5]. LPA has been shown to mediate a range of effects, including smooth muscle contraction, changes in blood pressure, Ca^{2+} mobilization, chemotaxis, neurite retraction, stimulation of cell proliferation, platelet aggregation, tumor invasion, differentiation, and formation of focal adhesion and stress fibers [6–9].

LPA is generated as an intermediate in the de novo synthesis of phospholipids [10], including phosphatidic acid (PA). Structurally, LPA consists of a glycerol moiety containing a fatty acyl chain, a hydroxyl group and a phosphate group in the *sn*-1, *sn*-2 and *sn*-3 positions (1-acyl-*sn*-glycerol-3-phosphate), respectively. It is formed by the acylation of *sn*-glycerol-3-phosphate by acyl coenzyme A in the membrane of the endoplasmic reticulum; and a second acylation by acyl coenzyme A generates PA, which is converted to LPA by phospholipase A2. Alternatively, LPA is generated and released by activated platelets [11] following hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) into inositol-1,4,5-triphosphate (IP_3) and 1,2-diacylglycerol (DAG). The subsequent phosphorylation of DAG by DAG kinase generates PA, which is converted to LPA by phospholipase A2 [10].

Overview of LPA activities

Early observations showed that LPA induces smooth muscle contraction [12–14]. The key event in contractile changes induced by LPA is activation of the G-protein Rho [15]; inactivation of Rho by C3 ADP-ribosyltransferase inhibits LPA-induced cytoskeletal effects and phosphorylation of focal adhesion kinase (FAK) [16, 17]. Rho activation precedes the appearance of stress fibers, phosphorylation of myosin light chain and focal adhesions with recruitment of viniculin, talin, paxillin and $p125^{FAK}$ [15, 18, 19]. Other cytoskeletal responses to LPA include growth cone collapse, neurite retraction

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and morphological changes in neuronal cell body [20, 21].

Subsequent work has demonstrated that LPA functions as a mitogen [6, 7, 9, 22, 23] and induces Ca^{2+} release from intracellular stores [5, 17–23]. It may also be a key regulator of coagulation and wound healing [9]. As a mitogen, LPA stimulates proliferation via the serum response element (SRE)-dependent enhancement of transcription of immediate-early genes coupled to growth [22, 24]. This influences proliferation indirectly by inducing production and release of peptide growth factors which function as mitogens [25] or which act synergistically with LPA to stimulate proliferation [26]. Recently, breast and ovarian cancer cell lines have been shown to proliferate in the presence of OCAF (ovarian cancer activating factor) [27, 28], which was later determined to be LPA. LPA is significantly elevated in patients with various gynecologic malignancies, including ovarian (early and late stage), cervical, and endometrial carcinomas, suggesting LPA functions in a positive feedback loop and represents a potential biomarker for gynecologic cancers [29]. Ascites fluid from patients with ovarian cancer stimulates the proliferation of ovarian cancer cells in vitro and in vivo [30]. Indeed, elevated levels of LPA in the ascites and plasma of these patients may have an immunoregulatory role as well, as LPA triggers release of intracellular Ca^{2+}, stimulates proliferation and enhances IL-2 production in conjunction with phorbol ester in Jurkat T cells [27, 31].

LPA induces Ca^{2+} release from a variety of cells, including *Xenopus* oocytes [32, 33], neuronal cells [21, 34], platelets [5], Jurkat T cells [31], and fibroblast and epithelial cells [35, 36], but not from peripheral blood leukocytes or mast cells [5, 36, 37]. These unresponsive cells are frequently exposed to LPA during coagulation [9], and may be subjected to as yet undetermined regulatory influences to prevent continued activation. While LPA is not detectable in platelet poor plasma or whole blood [3, 33], during the wound-healing process LPA is released into the circulation, where it may initiate the early events in wound healing [38–40] by stimulating phosphatidylinositol 3-kinase (PI3K)-dependent platelet aggregation [41]. LPA also alters platelet morphology, promoting pseudopod formation and centralization of granules [42, 43]. The appearance of lysophospholipids such as LPA in serum and sphingosine 1-phosphate (S1P) in both plasma and serum following endothelial injury, production by activated platelets or introduction by extravasation of intravascular fluids may have profound effects on inflammation, wound healing and angiogenesis [22]. LPA mediates early events in wound healing, including assembly of the fibrillar matrix at specialized cell surface sites [38] and stimulation of cell-mediated binding and assembly of the extracellular matrix protein fibronectin [39, 40].

LPA signaling

The biological actions of LPA are mediated by ligand-receptor interactions via coupling to multiple classes of heterotrimeric G proteins at the plasma membrane [22, 44]. Early evidence for this mode of signaling (fig. 1) was provided by Jalink et al., who demonstrated that LPA induced the release of intracellular calcium and decreased cyclic AMP (cAMP); the effects were abrogated in the presence of pertussis toxin, suggesting the involvement of $G_{\alpha i}$ [36]. In addition to $G_{\alpha i}$, release of intracellular calcium was later proposed to be mediated by $G_{\alpha q}$ [45], and activation of Rho by $G_{\alpha 12/13}$ [46, 47].

Subsequent studies with putative LPA receptors have further characterized the signaling mechanisms of this lipid [48, 49]. Initially, attempts to identify a specific receptor for LPA were complicated by the high level of nonspecific binding of LPA, making it difficult to identify cDNA clones [34, 37, 50]. Hecht et al. described the cloning of the ventricular zone gene 1 (VZG-1), expressed in the embryonic ventricular zone of the cerebral cortex, which encodes a 41-kDa protein [48]. The human homologue of VZG-1, named endothelial differentiation gene-2 (edg-2), was cloned from a human lung cDNA library and is 96% identical to VZG-1 on the amino acid level and ubiquitously expressed [51]. Recent work identified a second LPA receptor (edg-4), with 46% identity to edg-2, encoded by 1.8-kb and 8.0-kb transcripts with expression limited to white blood cells and testis [52]. The edg-2 and edg-4 receptors are members of a larger family consisting of five homologous proteins encoded by the endothelial differentiation genes 1–5, including the receptors for S1P [44]. The five Edg receptors contain features common to all G-protein-coupled receptors: seven putative transmembrane domains, N-terminal N-linked glycosylation sites, disulfide bonds between extracellular loops, and intracellular phosphorylation sites [44].

The proliferative effect of LPA is dependent on activation of the mitogen-activated protein kinase (MAPK) cascade requiring p21ras and p74raf [53, 54], and is mediated by the $\beta\gamma$ subunits of $G_{\alpha i}$ [55]. Additionally, PI3K has been implicated in mitogenic signaling by LPA since the addition of Wortmannin or a dominant-negative mutant of PI3K regulatory subunit (p85) prevents $G_{\alpha i}$-mediated activation of the MAPK pathway in COS-7 cells [56]. PI3K is a heterodimer consisting of a 110-kDa catalytic subunit and an 85-kDa regulatory or adapter subunit [57, 58], and is activated by both receptor tyrosine kinases [59] and G-protein-coupled receptors such as LPA [60], but the exact mechanism(s) remains unclear [6, 7]. PI3K signaling may also involve the recently characterized p110γ isotype of the catalytic subunit [61]. This isotype does not associate with the p85 adapter protein, but with the newly characterized

p101 adapter; p101 markedly stimulates the responsiveness to G$\beta\gamma$ subunits [62] and was recently shown to mediate Ras-independent activation of MAPK [63]. LPA can also activate the MAPK pathway following trans-phosphorylation of receptor tyrosine kinases, including the epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) receptors [64, 65]. Additional signaling intermediates include the non-receptor tyrosine kinases Src [66] and protein tyrosine kinase Pyk2 [67, 68]. Src is a myristilated protein ty-

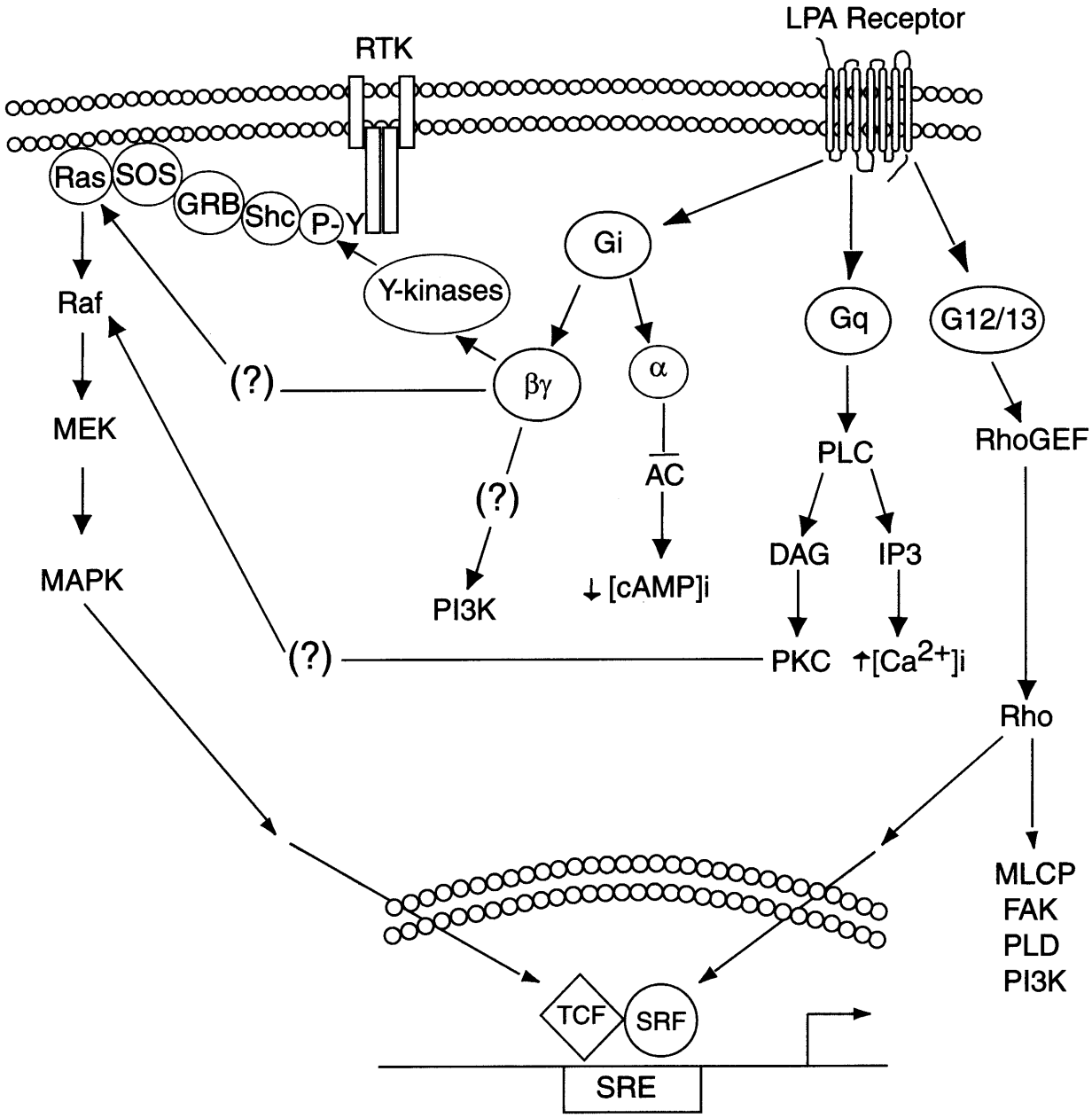

Figure 1. LPA growth factor signaling pathways stimulated by the LPA G-protein-coupled receptor. See text for details. LPA, lysophosphatidic acid; RTK, receptor tyrosine kinase; PLC, phospholipase C; IP_3, inositol triphosphate; DAG, 1,2-diacylglycerol; PKC, protein kinase C; SRE, serum response element; SRF, serum response factor; TCF, ternary complex factor; MAPK, mitogen-activated protein kinase; MEK, MAP kinase kinase; Y-kinase, protein tyrosine kinase; AC, adenylyl cyclase; RhoGEF, Rho guanine nucleotide exchange factor; MLCP, myosin light-chain kinase; FAK, focal adhesion kinase; PLD, phospholipase D; PI3K, phosphatidylinositol-3 kinase.

rosine kinase which can phosphorylate FAK and paxillin and also contributes to the activation of raf-1 and PI3K [69]. Src is known to phosphorylate the EGF receptor at Tyr845, which is required for LPA-induced DNA synthesis [70, 71], and mediates LPA tyrosine phosphorylation of SH_2-containing protein (SHC) and subsequent activation of MAPK pathway [72].

Putative coupling to $G_{\alpha q}$ suggests that LPA may serve as an effector for additional signaling pathways. Receptor-$G_{\alpha q}$ interactions activate phospholipase C (PLC), which catalyzes the formation of IP_3 and DAG from PIP_2, culminating in activation of PKC and mobilization of intracellular calcium. It is well established that PKC can phosphorylate Raf-1 both in vitro and in vivo [73], and it is generally believed the activation of the MAPK pathway by PKC in COS and CHO cells is p21Ras independent [74, 75]. However, it is not clear whether LPA activates the MAPK cascade by this mechanism.

LPA-induced activation of Rho is mediated by the small G-proteins of the $G_{12/13}$ class of G-α proteins [46, 47] and results in stress fiber formation, assembly of focal adhesions, neurite retraction [33, 76] and modulation of gene transcription [77]. Activated $G_{\alpha 13}$ binds directly to the guanidine nucleotide exchange factor, Lsc/p115RhoGEF, which is capable of activating Rho [47]. In turn, Rho activates such downstream effectors as serine/threonine kinases, phospholipase D, PI3K, p125FAK, myosin light chain phosphatase and serum response factor (SRF) [78]. The growth promoter SRE requires the concerted interaction with SRF and ternary complex factors (TCFs) to stimulate transcription. Because TCFs are activated by the MAPK pathway and SRF is activated by Rho, the full induction of cell proliferation by LPA requires coordinated signaling from both G_i and $G_{12/13}$ pathways [51, 52, 77].

LPA as an antiapoptotic factor

Accumulating evidence has shown that LPA promotes survival of renal proximal tubular cells (MPTs) [79], lymphoblastoma T cells [80], macrophages [81] and neonatal Schwann cells [82] by preventing apoptosis (table 1). Apoptosis is an energy-requiring form of cell death accomplished by specialized cellular machinery, including the Bcl-2 family and caspases. Morphologically, the process is characterized by cell shrinkage, chromatin condensation, DNA fragmentation and formation of membrane-enclosed apoptotic bodies [83–85]. Early evidence that LPA functions as a survival factor was derived from work with MPT cells [79]. Following serum removal, the presence of LPA overrode apoptotic challenge, though apoptosis ensued with the addition of Wortmannin and LY-294002, two structurally dissimilar inhibitors of PI3K [86, 87]. Currently, it is unclear which isoform of PI3K mediates survivability or how activation occurs. LPA also protected human lymphoblastoma T cells from succumbing to apoptotic challenge with anti-Fas, anti-CD2, or a combination of anti-CD28 and CD3.

Table 1. Mechanisms of cell survival mediated by LPA.

Cell type	Signaling component	Mechanism
MPT cells	PI3K	unknown
Lymphoblastoma T cells	unknown	reduced Bax protein levels
Peritoneal macrophages	PI3K	$p70^{S6K}$ activation, inhibition of GSK3 and Bad?
Schwann cells	$G_{\alpha i}$/PI3K	unknown

One mechanism by which LPA prevents apoptosis in these cells is through selective repression of protein expression. For instance, LPA reduced intracellular levels of the apoptosis-promoting Bax protein, whereas levels of other proapoptotic proteins (including Bad and Bak) and antiapoptotic proteins (Bcl-2 or Bcl-x_L) remained unchanged. However, protection was also observed at concentrations of LPA below the threshold for Bax repression, indicating that other mechanisms must be involved [80]. In Jurkat T cells, S1P inhibited activation of the proapoptotic caspases 3, 6 and 7; however, regulation of caspases by LPA must be studied in this and other cell types to evaluate the importance of this potential mechanism for preventing apoptosis [88].

LPA was recently shown to be a potent macrophage survival factor, exerting protective effects comparable to serum or monocyte-colony stimulating factor (M-CSF) [81]. Since LPA is not a mitogen for differentiated peritoneal macrophages, enhanced viability is thought to be accomplished solely through inhibition of apoptosis. Addition of LY294002 and Wortmannin prevented LPA-induced survival, suggesting PI3K involvement through undetermined pathways. Furthermore, addition of rapamycin (a $p70^{S6K}$ inhibitor) only partially blocked LPA effects, implicating other downstream effectors of PI3K signaling [81]. LPA promotes survival of neonatal Schwann cells in the absence of growth factor stimulation. This protection is ostensibly mediated by G_i/G_o GTP binding protein, as it is sensitive to pertussis toxin and is dependent on PI3K and Akt activation [82]. The effects of LPA on these cells probably centers on the activation of PI3Kγ by $\beta\gamma$ subunits, but this remains to be definitively determined.

Akt is a cytoplasmic serine-threonine kinase whose aberrant expression is implicated in tumorigenesis [89].

Indeed, signaling through Akt is a recurring theme in preventing apoptosis [90], and this protein may be a key regulator of LPA-mediated viability. PI3K catalyzes the phosphorylation of the inositol ring of phosphatidylinositols, generating PI(3)P, PI(3,4)P and PI(3,4,5)P [90]. Activation of Akt results from homodimerization (through NH_2-terminal pleckstrin domains) induced by interaction with PI(3,4)P, leading to phosphorylation at Thr-308 and Ser-473 by phosphatidylinositol-dependent kinases (PDKs)-1 and -2 [91]. Ultimately, activation of Akt leads to regulation of GSK-3, Bad and $p70^{S6K}$, which have all been implicated in apoptosis [90, 92].

While it is unknown which G-protein is responsible for LPA-dependent viability induced by activation of PI3K, $G\beta\gamma$ subunits are likely candidates [61, 62]. However, resistance to apoptosis may be mediated by different pathways in various cell types. In fibroblast cells, activation of Akt by LPA was shown not to be mediated by $G_{\alpha i}$ or by phorbol-ester-induced PKC activation [93]. However, Akt was activated in COS cells by signals emanating from $G_{\alpha i}$, $G_{\alpha q}$ and $\beta\gamma$, but not from $G_{\alpha 12}$ [92]. These results, in addition to the finding that Edg-2 (which does not couple to $G_{\alpha q}$) activates PI3K [44], suggest that $\beta\gamma$ subunits and possibly $G_{\alpha i}$ are involved. This assertion is supported, in part, by the apparent ability of LPA to activate the PI3K-110γ isoform in peritoneal macrophages [81], and this isoform has been previously shown to be activated by $G\beta\gamma$ [62]. Studies by Koh et al. showed that both PI3Kα and PI3Kγ isoforms were activated by LPA in these cells [81]. However, it has yet to be determined which isoform of PI3K mediates survival and whether or not the Rho inhibitor (C3 exoenzyme) negates the antiapoptotic effects of LPA in macrophages.

Although Akt promotes survival in a variety of cells, the step at which Akt circumvents the apoptotic cascade is unknown [94]. Many of the factors downstream of Akt, including Bad and glycogen synthase kinase 3 (GSK-3), appear to be involved in the regulation of apoptosis. Bad (a Bcl-2 homologue) promotes cell death in part through heterodimerization with survival proteins Bcl-2 and Bcl-x_L [95]. Bad normally resides in the cytosol and migrates to the plasma membrane following phosphorylation at Ser-112 and Ser-136 by Akt [96], and Akt-mediated phosphorylation at the latter site may be necessary and sufficient for Bad inactivation [97]. Phosphorylation of Bad leads to its sequestration by 14-3-3 in the cytosol, which effectively inactivates Bad by reducing its interaction with membrane-bound Bcl-x_L [98]. However, inactivation of Bad by LPA signaling through Akt has yet to be demonstrated.

While much is known about the regulation of GSK-3 by Akt, many questions remain concerning the role of GSK-3 in cell fate determination and differentiation. GSK-3 is constitutively active until phosphorylated at Ser-9 (for GSK-3α) or Ser-21 (for GSK-3β) by Akt following stimulation by insulin or other growth factors [99, 100]. Although there are no known activators of GSK-3, its constitutive activation in unstimulated cells is associated with phosphorylation on Tyr-279 (for GSK-3α) and Tyr-216 (for GSK-3β) [99, 100]. GSK-3 has been implicated as a potential downstream effector of the PI3K/Akt cell survival pathway in neural cells [90, 101, 102], fibroblasts [95, 96] and epithelial cells [103]. The involvement of GSK-3 in regulating apoptosis as pertains to LPA signaling is unknown, and the downstream targets of GSK-3 remain to be elucidated.

The apoptotic cascade has been shown to occur following loss of integrity of the outer mitochondrial membrane, resulting in release of cytochrome C. Cytochrome C induces a conformational change and consequent activation of apoptotic protease activating factor 1 (Apaf-1) which activates caspase-9 and triggers the caspase cascade [84]. Accordingly, it has been proposed that the Bcl-2 family influences apoptosis through regulation of mitochondrial integrity [104]. Akt regulates this process through phosphorylation and inactivation of Bad, thereby preventing its heterodimerization with the antiapoptoitc Bcl-2 members at the mitochondrial membrane [97, 98]. Recently, Akt was shown to promote cell survival by maintaining mitochondrial integrity, inhibiting release of cytochrome c, and preventing alterations in the mitochondrial membrane potential [105]. This process was independent of Bad phosphorylation and caspase regulation. While the implications of this new mechanism of survival are intriguing, the involvement of LPA has yet to be demonstrated in this setting.

Conclusion

The many biological activities of LPA are attributable in part to the activation of multiple G-proteins which regulate a multitude of downstream signaling cascades. In addition to cytoskeletal-dependent effects, such as stimulation of chemotaxis, enhanced adhesion, and initiation of contraction and aggregation, LPA has many growth-related effects. Recently, LPA has been shown to suppress apoptosis in addition to its ability to enhance proliferation and alter differentiation. To completely understand how LPA promotes survival will require a greater understanding of the mechanisms which regulate LPA receptor expression, LPA signaling pathways and elucidation of cellular targets. Future biochemical studies in established cell lines and the discovery of new cells responsive to LPA will provide a better understanding not only of the specific signaling cascades involved and how they influence cell survival, but should also reveal additional pathways and new

downstream cellular targets regulated by LPA. While it is known that activation of PI3K and repression of pro-apoptotic protein expression are two mechanisms of LPA-mediated survival, several questions remain. Determining the PI3K isoforms, uncovering downstream effectors of PI3K signaling, and evaluating the regulation of caspases by LPA as a potential mechanism for preventing apoptosis, are just a few and are all areas of current interest.

Acknowledgements. The authors thank Nicola C. Partridge, Sathyra K. Chinnadurai, John P. Corbett and William S. M. Wold for helpful discussions, and Andria Nickel for assistance in preparing the manuscript.

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