**Physical and Functional Interactions of the Lysophosphatidic Acid Receptors with PDZ Domain-containing Rho Guanine Nucleotide Exchange Factors (RhoGEFs)**

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Lysoosphatidic acid (LPA) is a serum-derived phospholipid that induces a variety of biological responses in various cells via heterotrimeric G protein-coupled receptors (GPCRs) including LPA₁, LPA₂, and LPA₃. LPA-induced cytoskeletal changes are mediated by Rho family small GTPases, such as RhoA, Rac1, and Cdc42. One of these small GTPases, RhoA, may be activated via G₁₂/₁₃α-linked Rho-specific guanine nucleotide exchange factors (RhoGEFs) under LPA stimulation although the detailed mechanisms are poorly understood. Here, we show that the C terminus of LPA₁ and LPA₂, but not LPA₃, interact with the PDZ domains of PDZ domain-containing RhoGEFs, PDZ-RhoGEF, and LARG, which are comprised of PDZ, RGS, Dbl homology (DH), and pleckstrin homology (PH) domains. In LPA₁- and LPA₂-transfected HEK293 cells, LPA-induced RhoA activation was observed although the C terminus of LPA₁ and LPA₂ mutants, which failed to interact with the PDZ domains, did not cause LPA-induced RhoA activation. Furthermore, overexpression of the PDZ domains of PDZ domain-containing RhoGEFs served as dominant negative mutants for LPA-induced RhoA activation. Taken together, these results indicate that formation of the LPA receptor/PDZ domain-containing RhoGEF complex plays a pivotal role in LPA-induced RhoA activation.

Lysoosphatidic acid (LPA)¹ is a serum-derived phospholipid that induces a variety of biological responses in various cells (1–5). LPA is also the prototypic G protein-coupled receptor (GPCR) ligand that activates MAP kinase, phospholipase C, and small GTPases, etc., via heterotrimeric G proteins (1–5). Three distinct G protein-coupled receptors for LPA have been identified, termed LPA₁, LPA₂, and LPA₃ (previously Edg2, Edg4, and Edg7, respectively). LPA signals induce actin rearrangements via the Rho family GTPase, RhoA, Rac1, and Cdc42 (1, 5–6). Rho family GTPases have GDP-bound inactive and GTP-bound active forms, the cycle of which is regulated by Rho guanine nucleotide exchange factors (RhoGEFs) that stimulate the exchange of GDP for GTP (7). Members of the RhoGEF family have a Dbl homology (DH) domain that catalyzes the exchange reaction and a pleckstrin homology (PH) domain immediately C-terminal to the DH domain (8). The PH domain is responsible for both cellular localization and modulation of DH domain function (8). Recently, it has been recognized that the G₁₂/₁₃α family mediates signaling from the LPA receptor to RhoA and that RhoGEFs containing regulators of G protein signaling (RGS) domains are involved in these processes (9–10). RGS domain-containing RhoGEFs have been described p115-RhoGEF, PDZ-RhoGEF, and leukemia-associated RhoGEF (LARG) (11–13). The activation mechanisms of RGS domain-containing RhoGEFs induced by extracellular signals are well known in the case of p115-RhoGEF (14–15). The RGS domain of p115-RhoGEF stimulates the intrinsic GTPase activity of the G₁₂ or G₁₃α subunit, and activated G₁₂ or G₁₃α subunits bind to the RGS domain of p115-RhoGEF, thereby enhancing its ability to catalyze guanine nucleotide exchange of RhoA. On the other hand, PDZ-RhoGEF and LARG, but not p115-RhoGEF, both have N-terminal PDZ domains. The PDZ domain is known as a modular domain that binds to specific C-terminal peptide sequences of many membrane proteins (16). PDZ domain-containing proteins function as mediators of clustering of neurotransmitter receptors and ion channels, and then are involved in asymmetric distribution of receptors in epithelial cells (17–20). PDZ-RhoGEF and LARG also bind to the G₁₁ or G₁₃α subunit via RGS domains in a manner similar to p115-RhoGEF, although the molecular mechanisms controlling the GEF activity are not yet fully understood (8, 21–23). Recently, we and other groups have reported that the PDZ domains of PDZ-RhoGEF and LARG interact directly with the C-terminal domain of Plexin-B1, a Semaphorin-4D (Sema-4D) receptor, and/or the insulin-like growth factor (IGF-1) receptor (24–29). Stimulation of Sema-4D or IGF-1-induced RhoA activation through the complex of Plexin-B1 or IGF-1 receptors with PDZ-RhoGEF or LARG. These observations suggest that the PDZ domain-mediating interaction between PDZ domain-containing RhoGEFs and Plexin-B1 and/or the IGF-1 receptor may play an important role in the regulation of RhoGEF activity.

In this study, the fact that the C terminus of LPA receptors, LPA₁ and LPA₂, also have classical PDZ domain interaction motifs prompted us to examine the interaction of LPA₁ and

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The abbreviations used are: LPA, lysophosphatidic acid; Edg, endothelial differentiation gene; HEK, human embryonic kidney; PDZ, F79/gl5/2ZO-1; GST, glutathione S-transferase; MIBP, maltose-binding protein; MAP, mitogen-activated protein; GPCR, G protein-coupled receptor; GEF, guanine nucleotide exchange factor; DH, Dbl homology; PH, pleckstrin homology; RGS, regulators of G protein signaling.
LPA$_2$ with the PDZ domains of PDZ-RhoGEF and LARG. We found that the C terminus of LPA$_1$ and LPA$_2$ directly interacted with the PDZ domains of PDZ domain-containing RhoGEFs, PDZ-RhoGEF, and LARG. Amino acid mutations in the C terminus of LPA$_1$ and LPA$_2$ abolished LPA-induced Rho activation. Furthermore, LPA-induced RhoA activation was inhibited by overexpression of the PDZ domains of PDZ domain-containing RhoGEFs. These results indicated that PDZ domain-containing RhoGEFs might provide the link between LPA receptors (LPA$_1$ and LPA$_2$) and RhoA activation.

**EXPERIMENTAL PROCEDURES**

**Construction of Expression Vectors**—Various expression vectors were constructed in pFLAG-CMV (Sigma), pCMV-Myc, pMal-C2 (New England BioLabs), and pGEX5-X1 (Amersham Biosciences) using standard molecular biology methods (30). The cDNAs of LPA$_1$, LPA$_2$, and LPA$_3$ were cloned by reverse transcriptase-PCR with mouse kidney cDNA as template using the reverse primer TCTAGCTAAACCACAGATTGGTT, TCTAGATTAAAGGGGGAGTCCATCG and TCTAGATTAGGGGTTTTTATTACCA forward primer GGGCGCG CAATGGGAGTGGCCTCTAC, GGGCGCGCAATGGGAGTCGCTCCTAC, and GGGCGCGCAATGGGAGTCGCTCCTAC. Then the PCR products were subcloned into pFLAG-CMV expression vector. LPA$_1$ and LPA$_2$ mutants, pFLAG-CMV-LPA$_{AAA}$ (C terminus three amino acids Ser, Val, Val replaced with three alanines, Ala, Ala, Ala) were prepared with a PCR fragment amplified from the LPA$_1$ and LPA$_2$ plasmid using the reverse primers TCTAGCTAAACCACAGATTGGTT, TCTAGATTAGGGGTTTTTATTACCA forward primer GGGCGCG CAATGGGAGTGGCCTCTAC, GGGCGCGCAATGGGAGTCGCTCCTAC, and GGGCGCGCAATGGGAGTCGCTCCTAC. Then the PCR products were subcloned into pFLAG-CMV expression vector. LPA$_1$, LPA$_2$, and LPA$_3$ were also cloned by reverse transcriptase-PCR from mouse kidney cDNA using the primers TCTAGCTAAACCACAGATTGGTT, TCTAGATTAGGGGTTTTTATTACCA forward primer GGGCGCG CAATGGGAGTGGCCTCTAC, GGGCGCGCAATGGGAGTCGCTCCTAC, and GGGCGCGCAATGGGAGTCGCTCCTAC. Then the PCR products were subcloned into pFLAG-CMV expression vector. The pdZ domains of PDZ-RhoGEF and LARG were kindly provided from Kazusa DNA Research Institute (Japan) and Dr. Caligiuri, respectively. pCMV-Myc-PDZ-RhoGEF and pCMV-Myc-LARG were prepared as described (24). pGEX-KG-PDS95-PDZ3 and pCMV-Myc-PDS95-PDZ3* (this construct contains Src homology 3 (SH3) and guanylate kinase (GK) domains in addition to the third PDZ domain) were generous gifts from Drs. Y. Hata and Y. Takai.

**Chemicals**—Mouse monoclonal antibody to FLAG was purchased from Sigma, and mouse monoclonal antibody to Myc (E910) was purchased from American Type Culture Collection. For Western blot analysis, primary antibodies were used at a 1:2000 dilution. Immunoreactive proteins were incubated with horseradish peroxidase-conjugated antibody (Jackson Laboratories) at a 1:2000 dilution and then visualized using the ECL system (Amersham Biosciences). LPA was purchased from Sigma. Pertussis toxin was purchased from List Biological Laboratories, Inc.

**In Vitro Binding of LPA Receptors to PDZ-RhoGEF and LARG—**HEK293 cells were transfected by the calcium phosphate method with pFLAG-CMV-LPA$_1$, LPA$_2$, or LPA$_3$, or various mutant plasmids of LPA$_1$, LPA$_2$, and LPA$_3$. After 48 h, the cells were lysed in 0.5 ml of lysis buffer (20 mM Tris-HCl, pH 7.5, 1 mM EDTA, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, and 1% Triton X-100), and centrifuged at 100,000 x g for 30 min. 500 ml of the supernatant was incubated with various MBP-fused proteins fixed on 20 ml of amylose-Sepharose 4B beads (New England BioLabs). After the beads were washed with the lysis buffer, proteins on the beads were subjected to the Western blot analysis.

**Immunoprecipitation**—pCMV-Myc-LARG or pCMV-Myc-LARG-PDZ was co-transfected with pFLAG-CMV-LPA$_1$ or -LPA$_2$AAA into HEK293 cells by the calcium phosphate method (31). After 48 h of culture, the cells were lysed with the lysis buffer and centrifuged at 100,000 x g for 30 min. The supernatant was incubated with anti-Myc or anti-FLAG antibodies. The MBP-fused proteins fixed on the beads were subjected to the Western blot analysis with the indicated antibodies.

**Rho Activity Assay**—Various pFLAG-CMV-LPA$_1$, LPA$_2$, or LPA$_3$ receptors and/or pCMV-Myc-PDZ-RhoGEF-PDZor -LARG-PDZ-transfected HEK293 cells were deprived of serum for 24 h. The cells were treated with or without 10 nM LPA for 2.5 min and lysed with buffer (20 mM Tris-HCl, pH 7.6, 1 mM EDTA, 150 mM NaCl, 1 mM Triton X-100, 1 mM Na$_2$VO$_4$, 50 mM NaF, 1 mM phenylmethylsulfonyl fluoride). The soluble supernatant was boiled in sample buffer for SDS-PAGE and subjected to Western blot analysis with anti-RhoA antibody.

**MAP Kinase Activity Assay**—Various pFLAG-CMV-LPA receptors and/or pCMV-Myc-PDZ-RhoGEF-PDZ or-LARG-PDZ-transfected HEK293 cells were deprived of serum for 24 h. The cells were treated with or without 10 nM LPA for 2.5 min and lysed with buffer (20 mM Tris-HCl, pH 7.6, 1 mM EDTA, 150 mM NaCl, 1% Triton X-100, 1% Triton X-100, 1% phenylmethylsulfonyl fluoride). The soluble supernatant was boiled in sample buffer for SDS-PAGE and subjected to Western blot analysis with antiphospho-p44/42 MAPK antibody and p44/42 MAPK antibody (PhosphoPlus p44/42 MAPK antibody kit, New England BioLabs).

**RESULTS**

**LPA Receptors and PDZ Domain-containing RhoGEFs—**LPA signaling is mediated via a family of GPCRs including LPA$_1$, LPA$_2$, and LPA$_3$. Interestingly, LPA$_1$ and LPA$_2$ have a cytoplasmic region with C-terminal amino acid residue motifs of DTL and SSV, respectively, which may be PDZ domain-interacting motifs. PDZ domain-containing RhoGEFs PDZ-RhoGEF and LARG consist of PDZ, RGS, DH, and PH domains, and their RGS domain may interact with the activated G$_{12}$ family causing RhoGEF activation. Because LPA-induced RhoA activation is also known to be mediated by the G$_{12}$ family, the possibility was raised that PDZ domain-containing RhoGEFs may interact with LPA$_1$, LPA$_2$, or LPA$_3$, and play an important role in LPA-induced RhoA activation.

**Interaction of LPA$_1$ and LPA$_2$ with the PDZ Domains of PDZ-RhoGEF and LARG—**We first examined the in vitro binding of LPA$_1$, LPA$_2$, and LPA$_3$ to the PDZ domains of PDZ-RhoGEF and LARG. A, lysates from pCMV-Myc-LPA$_1$, -LPA$_2$, and -LPA$_3$-transfected HEK293 cells were incubated with amylose beads containing MBP-PDZ (PDZ-RhoGEF, LARG) or glutathione beads containing GST-PDZ3 (GST-95). The beads were washed extensively and eluted with SDS-PAGE sample buffer. Each fraction was subjected to SDS-PAGE (10% polyacrylamide gel) followed by Western blot analysis with anti-FLAG antibody. B, lysates from pFLAG-CMV-LPA$_1$, LPA$_2$, or LPA$_3$ were used in the affinity assay as described above. WT, wild type; AAA, three amino acids of the C terminus of LPA$_1$ and LPA$_2$; SSV and STL, were substituted by three alanines, AAA.

![Image](https://example.com/image.png)
domains of PDZ-RhoGEF and LARG but indicated no interaction of LPA₁ with the MBP-PDZ domains (Fig. 1A). We examined the specificity of the PDZ domain of PDZ domain-containing RhoGEFs by using the third PDZ domain of PSD-95, which is a scaffolding protein at the postsynaptic density. The third PDZ domain of PSD-95 failed to interact with the C terminus of LPA₁ and LPA₂ in addition to LPA₃. Because LPA₁ and LPA₂ have cytoplasmic regions with C-terminal amino acid residue motifs of DTL and SSV, respectively, we examined the interaction of the C-terminal LPA₁ and LPA₂ mutants with the MBP-PDZ domains of PDZ-RhoGEF and LARG (Fig. 1B). Substitution of the three amino acid residues with alanine residues at in the LPA₁ and LPA₂ C-terminal regions completely abolished interactions with the PDZ domains of PDZ-RhoGEF and LARG. These results indicated that LPA₁ and LPA₂ interacted with the PDZ domain of PDZ-RhoGEF and LARG and that the C-terminal motifs of LPA₁ and LPA₂ are essential for binding.

Next, we examined the binding of LPA receptors to PDZ domain-containing RhoGEFs in a cellular environment (Fig. 2). HEK293 cells were co-transfected with pFLAG-CMV-LPA₁ or -LPA₂, -LPA₃, and pCMV-Myc-LARG or -LARGΔPDZ. The cell lysates were immunoprecipitated with anti-Myc or anti-FLAG antibodies. Protein A-Sepharose bead-associated immunocomplexes were subjected to SDS-PAGE (10% polyacrylamide gel) followed by Western blot analysis with anti-FLAG or anti-Myc antibodies.

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LPA receptors with PDZ proteins may play an important role in LPA-induced RhoA activation but LPA-induced MAP kinase activation was independent of the interaction of the C terminus of LPA receptors with PDZ proteins.

Because the expression of both PDZ-RhoGEF and LARG has been confirmed in HEK293 cells (33), we speculated that the exogenous PDZ domains of PDZ-RhoGEF and LARG could compete with endogenous PDZ domain-containing RhoGEFs for binding to the C terminus of LPA receptors in HEK293 cells. We next examined the effect of overexpression of the PDZ domains of PDZ domain-containing RhoGEFs on LPA-induced RhoA and MAP kinase activation. HER293 cells were transfected with pFLAG-CMV-LPA1 or -LPA2 in combination with pCMV-Myc-PDZ-RhoGEF-PDZ-LARG-PDZ or -PDZ3-PSD-95. The expression of each protein was determined by Western blot analysis with anti-MAP kinase or antiphospho-MAP kinase antibodies.

PAGE (10% polyacrylamide gel) followed by Western blot analysis with 10 μM LPA for 2.5 min. The lysates were incubated with GST-rhotekin beads. The bound activated RhoA was subjected to SDS-PAGE (16% polyacrylamide gel) followed by Western blot analysis with the anti-RhoA antibody. These transfected cells were serum-starved for 24 h and then stimulated with 10 μM LPA for 2.5 min. The lysates were subjected to SDS-PAGE (10% polyacrylamide gel) followed by Western blot analysis with anti-MAP kinase or antiphospho-MAP kinase antibodies.

We next examined the effect of overexpression of the PDZ domain-containing RhoGEFs on LPA-induced RhoA and MAP kinase activation. HER293 cells were transfected with pFLAG-CMV-LPA1 or -LPA2 with or without the PDZ3 domains of PDZ-RhoGEF, LARG, and the third PDZ domain of PSD-95 (Fig. 4A). After LPA stimulation, cell lysates were prepared and used in the Rho activity assay and MAP kinase assay. LPA-induced RhoA activation was completely inhibited by overexpression of the PDZ domains of PDZ-RhoGEF and LARG. Whereas overexpression of the third PDZ domain of PSD-95, which did not interact with C terminus of LPA1 and LPA2 receptors (Fig. 1A), had no effect on LPA-induced RhoA activation in pFLAG-CMV-LPA1- and pFLAG-CMV-LPA2-transfected cells (Fig. 4B, data not shown). On the other hand, MAP kinase activation was not altered by overexpression of any PDZ domains (Fig. 4C). It was probable that the exogenous PDZ domains of PDZ domain-containing RhoGEFs could inhibit interaction of LPA receptors with endogenous PDZ domain-containing RhoGEFs and served as dominant negative mutants for LPA-induced RhoA activation. Taken together, these results suggest that LPA-induced RhoA activation but not MAP kinase activation is mediated by PDZ domain-containing RhoGEFs that associate with LPA receptors.

Finally, we examined the effects of the RGS domain of LARG on LPA-induced RhoA and MAP kinase activation. A, HEK293 cells were transfected with pFLAG-CMV-LPA1 or -LPA2 in combination with pCMV-Myc-RGS-LARG. The expression of each protein was determined by Western blot analysis with anti-MAP kinase antibody and anti-Myc antibody. B, these transfected cells were serum-starved for 24 h and then stimulated with 10 μM LPA for 2.5 min. The lysates were incubated with GST-rhotekin beads. The bound activated RhoA was subjected to SDS-PAGE (16% polyacrylamide gel) followed by Western blot analysis with the anti-RhoA antibody. C, these transfected cells were serum-starved for 24 h and then stimulated with 10 μM LPA for 2.5 min. The lysates were subjected to SDS-PAGE (10% polyacrylamide gel) followed by Western blot analysis with anti-MAP kinase or antiphospho-MAP kinase antibodies.

Fig. 5. Effects of the RGS domain of LARG on LPA-induced RhoA and MAP kinase activation. A, HEK293 cells were transfected with pFLAG-CMV-LPA1 or -LPA2 in combination with pCMV-Myc-RGS-LARG. The expression of each protein was determined by Western blot analysis with anti-MAP kinase antibody and anti-Myc antibody. B, these transfected cells were serum-starved for 24 h and then stimulated with 10 μM LPA for 2.5 min. The lysates were incubated with GST-rhotekin beads. The bound activated RhoA was subjected to SDS-PAGE (16% polyacrylamide gel) followed by Western blot analysis with the anti-RhoA antibody. C, these transfected cells were serum-starved for 24 h and then stimulated with 10 μM LPA for 2.5 min. The lysates were subjected to SDS-PAGE (10% polyacrylamide gel) followed by Western blot analysis with anti-MAP kinase or antiphospho-MAP kinase antibodies.
LPA receptors, LPA₁ and LPA₂, coupled to Go_{12/13}-linked RhoA activation and Go₃-linked MAP kinase activation.

**DISCUSSION**

**LPA receptors and Their Signaling**—LPA has a wide variety of biological actions, such as cell proliferation, migration, morphological changes, and survival (1). LPA signal transduction is known to mediate via αβγ trimeric G protein-coupled receptors including LPA₁, LPA₂, and LPA₃ (1–5). LPA receptors couple to three distinct G proteins, G₁, G₂₁₁, and G₂₃₃ (5–6). The main signaling pathways include: (1) G₁-mediated stimulation of MAP kinase cascade in cell proliferation; (2) G₂₁₁-mediated stimulation of phospholipase C in Ca²⁺ mobilization; (3) G₂₃₃-mediated activation of RhoA in morphological changes. Recently, it has been identified that the RGS (regulator of G protein signaling) domain-containing RhoGEFs are involved in LPA-induced RhoA activation because RGS domains especially interact with activated α subunit of G₁₂/₁₃ (11–13). However, the detailed mechanisms remain unclear.

**PDZ Domain-containing RhoGEFs as LPA Receptor-interacting Molecules**—In this study, we showed that PDZ domain-containing RhoGEFs interacted with LPA receptors, LPA₁ and LPA₂, through their PDZ domains. The PDZ domains of PDZ domain-containing RhoGEFs seem to be classified as class I, selecting peptides with a hydroxyl amino acid at position –2 (34). The class I PDZ domains interacts preferentially with the C-terminal amino acid sequence (S/T/X/V/L) (X represents any amino acid), and PDZ domains bind to the peptides that terminate in a hydrophobic amino acid such as Val, Ile, or Leu. Because the three amino acids of the C terminus of LPA₁ and LPA₂ are SSV and STL, respectively, our finding is consistent with this prediction. Recently, reports have been accumulated that GPCRs interact with PDZ domain-containing proteins. For example, β₂-adrenergic receptor (35), 5-HT2 serotonin receptor receptor (36), dopamine receptor (37), and LPA₂ receptor (38) have PDZ domain-binding motifs at the C terminus and interact with PDZ domain-containing proteins including the Na⁺/H⁺ exchange factor (NHEF), GIPC, PSD-95, etc. These interactions are essential for physiologic signaling, receptor trafficking, and receptor targeting. Thus, the interaction of GPCRs with PDZ domain-containing proteins has recently become the subject of considerable interest.

**Role of PDZ Domain-containing RhoGEFs in the LPA-induced RhoA Activation**—In this study, we showed that LPA did not induce RhoA activation in LPA₁ and LPA₂ mutants, in addition to LPA₂, lacking interaction with PDZ domain-containing RhoGEFs-transfected HEK293 cells. We also showed that overexpression of the PDZ domains of PDZ-RhoGEF and LARG inhibited LPA-induced RhoA activation. These data suggest that the interaction of LPA receptors with PDZ domain-containing RhoGEFs may be necessary for LPA-induced RhoA activation. It has been reported that RGS domain-containing RhoGEFs such as p115RhoGEF, PDZ-RhoGEF, and LARG may play an essential role in cellular RhoA signaling by LPA (9–10). However, it is not clearly understood how these RhoGEFs regulated LPA-induced RhoA activation. The current data indicate that both PDZ-RhoGEF and LARG but not p115RhoGEF are signaling intermediates between the LPA receptor and RhoA activation. Therefore, one model predicts that LPA₁ and LPA₂ can bring associated PDZ domain-containing RhoGEFs into proximity with the appropriate G protein, such as the G₁₂ family, resulting in RhoA activation. Consequently, it can be assumed that PDZ domain-containing RhoGEFs may be more efficient than p115RhoGEF for LPA-induced RhoA activation.

**The Limited Inhibitory Effects of Overexpression of RGS Domains of LARG**—We have shown that overexpression of the RGS domain of LARG partially inhibited LPA-induced RhoA activation. Why did RGS domain of LARG have an only insufficient effect on inhibition of LPA-induced RhoA activation in distinction from its PDZ domain? The regulation of subcellular localization of RGS domain-containing proteins is seen to be important for their physiological activities. It has been reported that several RGS domain-containing proteins have additional regulatory motifs, such as PDZ, PX, PTB (phosphotyrosine binding), and GGL (G protein γ subunit-like) domains, and each domain is known to be involved in the regulation of subcellular localization (39). Actually, the interaction of PDZ domains of PDZ-RGS3 with the C terminus of ephrin-B, which is a transmembrane ligand for Eph, was required for its GAP action on CXCR4-mediated G protein signaling (40). Therefore, the limited inhibitory effects of the RGS domain of LARG may be due to the absence of targeting of the RGS domain to the inherent subcellular localization.

Alternatively, these data raise the possibility that the residual activated RhoA may depend on that activated α subunit of heterotrimeric G protein other than G₁₂/₁₃ because RGS domains of LARG preferentially interacted with the activated α subunit of the G₁₂ family, G₁₂δ and G₁₂ε (12, 22). It has been reported that LPA receptors coupled with Gαᵣ in several cells (4, 41), and activated Gαᵣ also promoted RhoA activation in HEK293 cells (43). Therefore, it is likely that Gαᵣ is also involved in LPA-induced RhoA activation in HEK293 cells. LPA-induced RhoA activation was completely blocked by overexpression of the PDZ domains of PDZ-RhoGEF and LARG, suggesting that PDZ domain-containing RhoGEFs may mediate even the activation of RhoA by Gαᵣ. Recent reports showed that the activated α subunit of Gαᵣ, searly interacted with the RGS domains of PDZ-RhoGEF and LARG, but removal of the N-terminal region including the RGS domain enhanced the interaction of Gαᵣ to PDZ domain-containing RhoGEFs (43). These reports suggested that Gαᵣ could activate RhoA through PDZ domain-containing RhoGEFs by a distinct mechanism from G₁₂ family under LPA stimulation. Anyway, PDZ domain-containing RhoGEFs may play an essential role in LPA-induced RhoA activation in HEK293 cells.

**Determination of Specific Uses of PDZ Domain-containing RhoGEFs**—Recently, it has been reported that thrombin and LPA-induced RhoA activation were mediated by PDZ domain-containing RhoGEFs whereas p115RhoGEF was not involved in either thrombin- or LPA-induced RhoA activation, using the method of oligonucleotide small interfering RNAs (33). These investigators also showed that thrombin and LPA receptors utilized different PDZ domain-containing RhoGEFs, LARG, and PDZ-RhoGEF, respectively. In this study, we showed that the C terminus of LPA receptors, LPA₁ and LPA₂, could interact with the PDZ domains of both PDZ-RhoGEF and LARG and that overexpression of the PDZ domains of PDZ-RhoGEF and LARG inhibit LPA-induced RhoA activation to the same extent. These results suggest that the PDZ domain mediating the interactions of LPA receptors with PDZ domain-containing RhoGEFs may not determine specificity. How do LPA receptors utilize PDZ-RhoGEF rather than LARG? Whereas PDZ-RhoGEF and LARG are structurally similar to each other, only PDZ-RhoGEF contains a proline-rich motif C-terminally adjacent to the DH/PH domain (21). Consequently, PDZ-RhoGEF differs from LARG in terms of the subcellular localization site on the membrane (21, 24). It is likely that this different subcellular localization may be involved in determination of the coupling receptor selection via the PDZ domain.

Alternatively, it has been reported that the thrombin receptor stimulates G₁₂δ subunit and that the LPA receptor stimulates G₁₂ᵣ subunit. The selective coupling of thrombin and LPA receptors to the G₁₂ family were determined by the N terminus...
short sequences of G_{12} and G_{13} (44). Furthermore, evidence has been provided that tyrosine phosphorylation of PDZ domain-containing RhoGEFs may regulate the activation of RhoA by GPCRs (42, 45). The mechanisms remain unclear but recent reports demonstrate that tyrosine phosphorylation of PDZ domain-containing RhoGEFs does not affect their basal RhoGEF activity, but rather changes their regulation by the G_{12} family α subunit (42). The report concludes that G_{13} stimulates non-phosphorylated LARG, although G_{12} stimulates the GEF activity of LARG only when LARG was tyrosine-phosphorylated by Tec tyrosine kinase. Thus, these reports suggest that tyrosine phosphorylation of PDZ domain-containing RhoGEFs, in addition to the structural specificity of the G_{12} family, may determine the selective coupling of thrombin and LPA receptors to the G_{12} family and PDZ domain-containing RhoGEFs.

Finally, the selective utilization between GPCRs, G proteins, and PDZ domain-containing RhoGEFs remains unclear. Further analysis is necessary for understanding of the mode of selective utilization of them.

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REFERENCES
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