Phosphatidylinositol 4-phosphate 5-kinase (PIP5K) catalyzes the rate-limiting step in the production of phosphatidylinositol 4,5-bisphosphate (PIP₂), a signaling phospholipid that contributes to actin dynamics. We have shown in transfected tissue culture cells that PIP5K translocates from the cytosol to the plasma membrane following agonist-induced stimulation of Rho family GTPases. Nonetheless, it is unclear whether Rho GTPases induce PIP5K relocalization in platelets. We used PIP5K isoform-specific immunoblotting and lipid kinase assays to examine the intracellular localization of PIP5K in resting and activated platelets. Using differential centrifugation to separate the membrane skeleton, actin filaments and associated proteins, and cytoplasmic fractions, we found that PIP5K isoforms were translocated from cytosolic to actin-rich fractions following stimulation of the thrombin receptor. PIP5K translocation was detectable within 30 s of stimulation and was complete by 2–5 min.

This agonist-induced relocalization and activation of PIP5K was inhibited by 8-(4-parachlorophenylthio)-cAMP, a cAMP analogue that inhibits Rho and Rac. In contrast, 8-(4-parachlorophenylthio)-cGMP, a cGMP analogue that inhibits Rac but not Rho, did not affect PIP5K translocation and activation. This suggests that Rho GTPase may be an essential regulator of PIP5K in platelets. Consistent with this hypothesis, we found that C3 exotoxin (a Rho-specific inhibitor) and HA1077 (an inhibitor of the Rho effector, Rho-kinase) also eliminated PIP5K activation and trafficking into the membrane cytoskeleton. Thus, these data indicate that Rho GTPase and its effector Rho-kinase have an intimate relationship with the trafficking and activation of platelet PIP5K. Moreover, these data suggest that relocalization of platelet PIP5K following agonist stimulation may play an important role in regulating the assembly of the platelet cytoskeleton.

Phosphatidylinositol 4-phosphate 5-kinase (PIP5K) catalyzes the synthesis of phosphatidylinositol 4,5-bisphosphate (PIP₂), a signaling phospholipid, by phosphorylating PI4P at the D-5 position of the inositol ring. In turn, PIP₂ serves as a precursor for diacylglycerol and inositol triphosphate in signal transduction cascades (1). Three classical isoforms of PIP5K have been identified, designated α, β, and γ, with molecular masses of ~60 kDa (PIP5Kα and PIP5Kβ) and 90 kDa (PIP5Kγ) (2–4). Recently, the fourth member of the family, phosphatidylinositol phosphate kinase homolog, was identified by Chang et al. (5), although it apparently lacks intrinsic catalytic activity. The differential regulation and/or subcellular localization of the PIP5K isoforms have been reported. PIP5Kα was found to specifically localize to membrane ruffles (6) and PIP5Kβ to localize near endosomes (7), whereas PIP5Kγ was found to target to focal adhesions (8, 9) and nerve terminals (10).

Actin assembly and vesicle trafficking are controlled by PIP₂. Some of these actin signaling pathways depend on intact PIP₂ rather than on the products of its hydrolysis (11, 12). This is thought to be due to the binding and displacement of various actin regulatory proteins from actin filaments by PIP₂, thus allowing the polymerization of these filaments (13). Consistent with an important role for PIP₂ in the organization of the actin cytoskeleton, overexpression of PIP5K has been shown to modulate actin cytoskeletal dynamics and to induce stress fibers (14) (15), membrane ruffles (16), microvilli (17), and motile actin comets (18).

The relationship between PIP5K isoforms and actin is complex but appears to be regulated by small GTPases of the Rho family. A physical association between RhoA and PIP5K has been demonstrated (24) that may be mediated by Cdc42. Recently, Oude Weernink et al. (26) showed that all three PIP5K isoforms are positively regulated by expression of RhoA, Rac1, and Cdc42 in HEK-293

This paper is available on line at http://www.jbc.org
cells, and this results in enhanced cellular PIP$_2$ levels. Lastly, Honda et al. (16) have suggested that activation of another small GTPase, Arf6, is actually the critical step in PIP5K activation. Thus, there is evidence that PIP5K activity may be regulated by Rho, Rac, or Arf6.

Although reports about the association of PIP5K with small GTPases have been controversial, there is agreement that small GTPases regulate the activity of PIP5K isoforms, and this contributes to actin dynamics (14, 27, 28). The apparent discrepancies about which GTPase is critical for PIP5K activation might be attributable to cell type-specific differences. In this study, we used intact human platelets to investigate the mechanism by which small GTPases regulate platelet PIP$_2$ formation by PIP5K. We demonstrate that platelet PIP5K is mostly regulated by Rho and its effector Rho-kinase. We also show that the activated PIP5K translocates to the platelet actin cytoskeleton, where it is available to initiate actin assembly.

**EXPERIMENTAL PROCEDURES**

**Materials**—The [γ-32P]phosphate was obtained from Amersham Biosciences. The Rho-kinase inhibitor, HA-1077, was from Upstate Biotechnology (Lake Placid, NY). The hirudin (2000 AU) was obtained from Roche Diagnostics. The anti-RhoA and anti-Rac antibodies and GST fusion proteins were purchased from Cytoskeleton, Inc. (Denver, CO). Triton X-100 (Surfact-Amps X-100) and Brij 58 (Surfact-Amps 58) were from Pierce. The two rabbit polyclonal antisera directed against PIP5K isoforms were further purified using a protein A affinity column (29). All other reagents were obtained from Sigma-Aldrich.

Recombinant botulinum C3 exoenzyme (C3) was expressed in bacteria as a glutathione S-transferase fusion protein. Following lysis of the bacteria, the C3 protein was affinity purified with glutathione-Sepharose 4B beads. The C3 exoenzyme was cleaved from GST by thrombin (86 U/mL enzyme) overnight at 4°C in a reaction mixture containing 0.25 M Na$_2$SO$_4$ and 0.1 M dithiothreitol. Recombinant C3 protein was resuspended in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM MgCl$_2$, and 0.1 mM dithiothreitol.

**Platelet Preparation**—Blood was drawn from healthy volunteers and collected into 1 volume of 0.15 M sodium citrate. Platelet-rich plasma was obtained by centrifugation at 200 g for 10 min and adjusted to pH 6.5 with 0.15 M citric acid to initiate platelet activation during further isolation. After a second centrifugation at 800 g for 10 min, the pellet was washed twice with ACD (74.8 mM sodium citrate, 41.6 mM citric acid, 138.8 mM dextrose, and 145 mM NaCl). The washed platelet pellet lysates were fractionated by established methods described previously (80). After boiling for 3 min, proteins were resolved on SDS polyacrylamide gel and then transferred to polyvinylidene difluoride. The membrane was blocked overnight at 4°C in Tris-buffered saline containing 5% (w/v) bovine serum albumin. The proteins were detected by blotting with the appropriate monoclonal or polyclonal antibodies in Tris-buffered saline, 0.02% Tween, 2% bovine serum albumin followed by incubation with either anti-mouse or anti-rabbit IgG antibody coupled to horseradish peroxidase. Detection was achieved using a chemiluminescent substrate (Amersham Biosciences). Protein concentrations were determined using the micro BCA protein assay (Pierce) with bovine serum albumin as standard.

**In Vitro Kinase Assay**—Phosphoinositide kinase activity was determined as described previously (33). Briefly, PIP5K activity was assayed in 50 μl reactions containing 50 mM Tris, pH 7.5, 0.1 mM MgCl$_2$, 1 mM EGTA, 1 μM DTT, 2 mCi [γ-32P]ATP (4 μCi/μl assay). Reactions were stopped after 20 min by adding 80 μl of 1 N HCl and then 160 μl of CHCl$_3$/MeOH (1:1). Lipids were separated by TLC using CHCl$_3$/MeOH:H$_2$O:NH$_4$OH (70:100:25, v/v). Lanes containing commercial standards of phosphatidylinositol phosphate or PIP$_2$ were stained with iodine vapors. After overnight exposure of film to the plates, the radioactive spots were visualized and quantitated by a PhosphorImage STORM 820 (Amersham Biosciences).

**Lipid Raft Preparation**—Lipids were isolated by density through a step gradient of 0–40% (w/v) sucrose as described previously (37–39). Briefly, resting or stimulated platelets (2 × 10$^5$ in 100 μl of HEPES-Tyrode’s buffer) were lysed in 200 μl of ice-cold buffer (20 mM Tris, 150 mM NaCl, 10 mM EGTA, 1 mM MgCl$_2$, 1 mM Na$_3$VO$_4$, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 μg/ml pepstatin A, and 0.1% Triton X-100). The lysates were then centrifuged at 800 × g for 10 min, the supernatant was washed twice with ACD (74.8 mM sodium citrate, 41.6 mM citric acid, 138.8 mM dextrose, and 145 mM NaCl). The washed platelet lysates were resuspended in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM MgCl$_2$, and 0.1 mM dithiothreitol.

**Platelet Activation**—Washed platelets (0.5 ml) were incubated with 200 μg/ml C3 exoenzyme and 10 μg/ml leupeptin. Platelet lysates were incubated for 1 h at 4°C with GST-PAK or GST-Rhotekin already bound to glutathione-Sepharose beads to precipitate GTP-bound RhoA and Rac, respectively. Precipitated complexes were washed three times in lysis buffer and boiled in sample buffer. Total lysates and precipitates were analyzed on Western blots using antibodies against Rac and RhoA.

**C3 Exotoxin, ADP Ribosylation Assay, and HA1077**—A aliquots of cells were taken after the C3 exoenzyme treatment and incubated in 50 μl of the lysis buffer containing 100 μg/ml leupeptin, 10 μg/ml pepstatin A, pH 7.4. A 100 μl reaction containing 50 μM Tris-HCl, 150 mM NaCl, 5 mM MgCl$_2$, 1 mM EGTA, 1 mM MgCl$_2$, and 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 10 μM EDTA, 10 μM leupeptin, 10 μg/ml aprotinin, 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 10 μM EDTA, 10 μM leupeptin, 10 μM DTT, and 5 mM MgCl$_2$. The reaction was then centrifuged at 800 × g for 10 min to pellet all the complexes. The supernatant was transferred to polyvinylidene difluoride. The membrane was blocked overnight at 4°C in Tris-buffered saline containing 5% (w/v) bovine serum albumin. The proteins were detected by blotting with the appropriate monoclonal or polyclonal antibodies in Tris-buffered saline, 0.02% Tween, 2% bovine serum albumin followed by incubation with either anti-mouse or anti-rabbit IgG antibody coupled to horseradish peroxidase. Detection was achieved using a chemiluminescent substrate (Amersham Biosciences). Protein concentrations were determined using the micro BCA protein assay (Pierce) with bovine serum albumin as standard.

**RESULTS**

**Comparison of Relative Abundance of PIP5Kα, PIP5Kβ, and PIP5Kγ in Platelets**—The expression of different PIP5K isoforms in platelets has not been described previously. Using polyclonal antibodies that are specific for PIP5Kα (−60 kDa), or both PIP5Kβ (−60 kDa) and PIP5Kγ (−90 kDa), we examined the presence of each isoform in human platelets (Fig. 1). We found all three isoforms to be present. PIP5Kγ is known to have alternative splice sites that produce an 87-kDa and a 90-kDa (so-called “brain-specific”) variant (4). Because the 90-kDa splice variant has been shown to contribute to focal adhesion formation in tissue culture cells, the 90-kDa PIP5Kγ might play an important role in platelet actin dynamics.
Mechanism of PIP5K Activation in Platelets

Time Course of PIP5K Redistribution in Human Platelets—Consistent with studies of most cells, total cellular PIP$_2$ in platelets does not rise dramatically in response to agonist stimulation of PIP5K (31, 32). This suggests that local increases in the PIP$_2$ concentration may occur at sites of recruited lipid kinases (1). We have demonstrated in transfected COS-7SH cells that stimulation of the PAR1 thrombin receptor induces a translocation of PIP5K$_\alpha$ from the cytosol toward the plasma membrane (33). In these cells, the translocation of PIP5K$_\alpha$ was dependent on both Rac and Rho, although the activation of Rho was essential for the trafficking process. Because the regulation of PIP5K appears to vary in different types of cells, we examined the distribution of the PIP5K isoforms in platelets.

To determine the subcellular location of PIP5K in resting and activated platelets, these cells were fractionated by established Triton X-100 lysis protocols (34, 35) followed by differential centrifugation. This allowed us to obtain the 15,600 g ("low speed cytoskeleton fraction") containing actin filament-rich fraction, a high speed pellet (membrane skeleton fraction), and a soluble (cytoplasmic protein) fraction by centrifugation as described under "Experimental Procedures." Distribution of PIP5K was detected by immunoblotting with antibody against PIP5K. These results show that PIP5K traffics to the actin filament-rich fraction of platelets following stimulation of the thrombin receptor.

To define conditions for isolation of platelet rafts, we used established Triton X-100 lysis protocols (34, 35) followed by differential centrifugation. This allowed us to obtain the 15,600 g fraction ("low speed cytoskeleton fraction") containing actin filaments and associated proteins, 100,000 g fraction (containing the membrane skeleton), and the 100,000 g supernatant fraction (containing soluble cytosolic proteins). In resting platelets, PIP5Kb (Fig. 2) was predominantly present in the cytoplasmic fraction. Gently stirred platelets were stimulated with the thrombin receptor-activating peptide (TRAP). TRAP stimulation led to rapid translocation of PIP5Kb (Fig. 2) to the low speed cytoskeleton fraction. A similar result was found for other platelet PIP5K isoforms, PIP5K_y and PIP5Kz (not shown). This trafficking event was seen within 30 s and reached a peak within 5 min. This suggests that similar to our observation in tissue culture cells, PIP5K isoforms relocalize within platelets following agonist stimulation.

FIG. 1. Expression of PIP5K isomers within platelets. Equal quantities of total cell lysates of human platelets were fractionated by 10% SDS-PAGE. Immunoblotting was performed using polyclonal antibodies against either PIP5K or against both PIP5Kb and PIP5Kz.

PIPK5K Does Not Localize within Platelet Lipid Rafts—Cholesterol-sphingolipid-rich membrane microdomains (GEMs or lipid rafts) are sites of active phosphoinositide and tyrosine kinase signaling. Lipid rafts are found in platelets (36, 37), although their functional role is just beginning to be elucidated (18, 38). Rozelle et al. (18) have shown that lipid rafts in Ref52 cells are the preferred platforms for membrane-linked actin polymerization mediated by in situ PIP$_2$ synthesis. Therefore, we investigated whether the PIP$_2$ synthesis by PIP5K occurred in platelet lipid rafts and whether the agonist-induced relocalization of PIP5K involved these microdomains.

To define conditions for isolation of platelet rafts, we used CD36, Lyn, and GM1 ganglioside as markers of this microdomain (Fig. 3A) (36, 39). The soluble fractions containing non-raft-associated membrane proteins and cytosolic proteins were identified by blotting for cytosolic p42/44 ERK (extracellular signal-regulated kinase) (not shown) (38). In the cell fractions derived from either resting or TRAP-stimulated platelets, we tested for PIP5K. As shown in Fig. 3B (left panel), we were unable to detect any PIP5Kb protein by immunoblotting with our polyclonal antibody. Similarly, we were also unable to demonstrate any PIP5K enzymatic activity in raft fractions (Fig. 3B, right panel). Although PIP5K does relocalize to different subcellular domains following stimulation of the platelet thrombin receptor, our data indicate that platelet PIP5K does not traffic into lipid rafts.

Rac Is Not Required for Trafficking or Activation of PIP5K in Human Platelets—Reports from various cell types have suggested that PIP5K is a downstream target of Rho proteins (19, 26, 40). We have previously shown that these GTPases regulate the intracellular localization and catalytic activity of PIP5K in Cos7SH cells (33). Offermanns and colleagues (41) demonstrated that cyclic nucleotide analogues of cAMP and cGMP differentially interfere with thromboxane A$_2$-induced Rac and Rho activation. Thus, we examined whether cAMP and cGMP, via their effect on Rac and Rho, modulated the trafficking of PIP5K in intact platelets.

For the initial experiments, we tested whether analogues of cAMP and cGMP would modulate the thrombin receptor-mediated activation of Rac and Rho in addition to their published effect on thromboxane A$_2$ receptor signaling. Washed platelets were incubated with TRAP for 5 min, and activation of Rac and Rho was analyzed using pull-down assays with fusion proteins composed of GST with either a Rac-binding domain or a Rho-binding domain. Incubation of human platelets with TRAP caused a rapid activation of both RhoA and Rac (Fig. 4). Because our capture assay recognizes both Rac1 and Rac2, we could not discern whether there is preferential activation of either Rac isofrom. In the presence of the cAMP analogue, there was little activation of either Rac or Rho after stimulation of the thrombin receptor. However, in the presence of cGMP, TRAP still induced activation of RhoA, but activation of Rac was almost completely inhibited.

We next tested whether the analogues of cAMP and cGMP affected the redistribution of PIP5K. Stimulation of platelets with TRAP induced the translocation of PIP5K, RhoA, and Rac into the low speed actin filament-rich fraction (Fig. 5). A dose of cAMP that simultaneously inhibited the translocation of both Rac and Rho also completely eliminated the trafficking and activation of PIP5Kb (Fig. 5). In contrast, preincubation of platelets with the cGMP analogue inhibited Rac but had minimal effects on the translocation or activation of either Rho or PIP5K. Recent
Fig. 3. Platelet PIP5K does not localize within lipid rafts. A, lipid rafts were fractionated from 1% Brij 58 platelet lysates and dot-blotted onto nitrocellulose. Fractions containing rafts were identified by the presence of GM1 ganglioside, Lyn kinase, and CD36. B, lipid raft fractions (lanes 2 and 3 in A) and non-raft lipid fractions (lanes 6 and 7 in A) were analyzed for PIP5Kβ by immunoblotting (left panel) or by an in vitro lipid kinase assay using PI4P as the exogenous substrate (right panel). This demonstrates that PIP5Kβ protein and PIP5K activity do not redistribute into lipid rafts following platelet activation.

Fig. 4. Cyclic nucleotides affect thrombin receptor-induced activation of Rac and Rho GTPases. Washed human platelets (1 × 10^9/ml) were preincubated for 10 min at 37 °C with or without preincubation of 1 mM 8-pCPT-cAMP or 8-pCPT-cGMP before the addition of 60 μM for 5 min. The platelets were lysed, and GTP-loaded Rho or Rac were affinity purified from a portion of the lysates using a “pull-down” assay with either a GST-Rac-binding protein or GST-Rho-binding protein. Shown are anti-Rac and anti-Rho immunoblots of total cell lysates or affinity purified GTP-loaded proteins. This shows that 8-pCPT-cAMP inhibits TRAP-induced activation of Rac and Rho, whereas 8-pCPT-cGMP inhibits the activation of Rho alone.

We also tested whether the activation of PIP5Kβ in total (unfractionated) platelet lysates was affected by pharmacologic inhibition of RhoA. Lysates derived from platelets stimulated by their thrombin receptor had 2.5-fold higher PIP5K activity compared with lysates derived from resting platelets (Fig. 7). However, this PIP5K activation was completely eliminated by cAMP or C3 exotoxin. This demonstrates that activation of RhoA is critical for the regulation of PIP5K activity in platelets.

Many of the effects of RhoA are mediated by Rho-kinase (also designated as ROCK or Rho-associated kinase). In addition, the Rho-mediated regulation of PIP5K has been speculated to require ROCK kinase (15, 28, 46). Therefore, we tested whether the Rho-kinase inhibitor, HA1077, affected PIP5K trafficking or activity. This Rho-kinase inhibitor reduced PIP5K translocation and activation (Figs. 6B and 7). This suggests that the translocation of active PIP5K toward the actin cytoskeleton is regulated by both Rho and its effector, Rho-kinase.

**DISCUSSION**

The goal of this study was to investigate the signaling pathway within primary platelets initiated by stimulation of the thrombin receptor and leading to the activation of PIP5K and production of PIP2. These observations extend our previous data on the subcellular localization of PIP5Kα expressed in tissue culture cell lines (33). In that report, stimulation of the thrombin receptor induced the translocation of PIP5Kα near the plasma cell membrane through a pathway dependent on Rho. Our current findings demonstrate a pathway for activation of PIP5K in platelets that is dependent on both Rho and Rho-kinase. This signaling pathway simultaneously results in the trafficking and enzymatic activation of PIP5K. We hypothesized that both relocation and biochemical activation of PIP5K are critical for its ability to regulate actin dynamics within platelets, a process critical for adherence under shear conditions of the arterial system.

Publications by Divencha and colleagues (47, 48) previously showed that the “Type C” isoforms of PIP5K migrated to the platelet membrane cytoskeleton after thrombin stimulation. Since that report, it is now accepted that PIP5K C is actually a phosphatidylinositol 5-phosphate 4-kinase (PIP4K) (1). This enzyme, which phosphorylates phosphatidylinositol 5-phosphate, may be involved in an alternative PIP2 synthesis pathway. It is intriguing that PIP5K and PIP4K, both enzymes...
speculated to generate platelet PIP₂, may compartmentalize to a similar location within platelets. Schwartz and colleagues (20) were the first to demonstrate that PIP5K is regulated by small GTP-binding proteins. They found that PIP5K catalytic activity in cell lysates increased in the presence of GDP-bound Rho but not in the presence of either GDP-bound Rho or GTP-bound Rac. Since that publication, various reports have presented evidence that Rac, CDC42, or Arf6 may be the small GTPases responsible for activation of PIP5K in various cell types (16, 18, 24–26). Our results show that inhibition of Rho completely blocks trafficking and activation of PIP5K in thrombin receptor-stimulated platelets. Although activation of Rac is clearly not required for these two processes in platelets following stimulation of the thrombin receptor, our data do not exclude the possibility that Rac contributes to the regulation of platelet PIP5K under certain circumstances. It is also likely that different cell types utilize different GTPases for the activation and perhaps trafficking of PIP5K.

Mechanism of PIP5K Activation in Platelets

Schwartz and colleagues (20) were the first to demonstrate that PIP5K is regulated by small GTP-binding proteins. They found that PIP5K catalytic activity in cell lysates increased in the presence of GTP-bound Rho but not in the presence of either GDP-bound Rho or GTP-bound Rac. Since that publication, various reports have presented evidence that Rac, CDC42, or Arf6 may be the small GTPases responsible for activation of PIP5K in various cell types (16, 18, 24–26). Our results show that inhibition of Rho completely blocks trafficking and activation of PIP5K in thrombin receptor-stimulated platelets. Although activation of Rac is clearly not required for these two processes in platelets following stimulation of the thrombin receptor, our data do not exclude the possibility that Rac contributes to the regulation of platelet PIP5K under certain circumstances. It is also likely that different cell types utilize different GTPases for the activation and perhaps trafficking of PIP5K.

It is unclear how GTP-bound Rho recruits PIP5K to the platelet membrane cytoskeleton. Several investigators have demonstrated that Rho family members shuttle on and off the membrane; and this membrane localization is regulated by their GTP/GDP-bound state (49–51). Because Rho is constitutively bound to PIP5K, one could speculate that GTP loading of Rho merely serves to help translocate the complex to the cell membrane, the predominant location of its lipid substrate, PI4P. However, our results demonstrate that Rho regulates the total platelet PIP5K activity in platelets. This demonstrates that Rho does more than chaperone PIP5K and must also regulate the specific activity of PIP5K within platelets.

The association of Rho with PIP5K is guanine nucleotide-independent, yet only the GTP-bound form of this GTPase can activate PIP5K. This suggests a model whereby a Rho effector, once activated by GTP-Rho, can recruit PIP5K. Our results demonstrate that inhibition of Rac does not prevent PIP5K activation or translocation to the membrane cytoskeleton.
Chinese hamster ovary cells. This suggests that, in addition to Rho-kinase, a PIP2-regulated protein may also be required for relocalization of PIP5K. The identity of this PIP2-dependent protein is currently unknown.

Overexpression of PIP5K does not change total cellular concentrations of PIP2. Yet, wild-type PIP5K overexpression induces actin reorganization through a pathway that requires a functional lipid kinase domain. This suggests that PIP2 production within discrete cellular subcompartments contributes to actin dynamics. Our study demonstrates that PIP5K protein and PIP2 production are not preferentially localized with lipid rafts. This is consistent with the previous report that failed to show PIP2 production within this platelet microdomain (52). However, the results of these platelet experiments are in contrast with the report demonstrating that PIP2 signaling may be preferentially localized to rafts within Ref52 cells (18). Recently, it has been shown in B lymphocytes that PIP5K is recruited into a microdomain that contains the tyrosine kinase, Btk (29). It is currently unknown whether localized high concentrations of PIP2 accumulate within a similar microdomain within platelets. It is tempting to speculate that localized production of PIP2 on the platelet membrane contributes to actin dynamics by binding and regulating profilin or actin-capping proteins such as gelsolin and capZ.

In conclusion, our results demonstrate that platelet PIP5K is tightly regulated by Rho GTPase. Once Rho and its effectors, Rho-kinase, become stimulated, they induce the activation and membrane recruitment of PIP5K to generate PIP2. We believe that this leads to the localized production of PIP2 that regulates actin-binding proteins and contributes to platelet actin dynamics.

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Rho and Rho-kinase Mediate Thrombin-induced Phosphatidylinositol 4-Phosphate 5-Kinase Trafficking in Platelets
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J. Biol. Chem. 2004, 279:42331-42336.
doi: 10.1074/jbc.M404335200 originally published online July 23, 2004

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