We have shown previously that ADP released upon platelet adhesion mediated by α1β3, integrin triggers accumulation of phosphatidylinositol 3',4'-bisphosphate (PtdIns(3,4-P$_2$)) (Gironcel, D., Racaud-Sultan, C., Payratte, B., Haricourt, M., Borchert, G., Kieffer, N., Breton, M., and Chap, H. (1996) FEBS Lett. 389, 253-256). ADP has also been involved in platelet spreading. Therefore, in order to study a possible role of phosphoinositide 3'-kinase in platelet morphological changes following adhesion, human platelets were pretreated with specific phosphoinositide 3'-kinase inhibitors LY294002 and wortmannin. Under conditions where PtdIns(3,4-P$_2$) synthesis was totally inhibited (25 μM LY294002 or 100 nM wortmannin), platelets adhered to the fibrinogen matrix, extended pseudopodia, but did not spread. Moreover, addition of ADP to the medium did not reverse the inhibitory effects of phosphoinositide 3'-kinase inhibitors on platelet spreading. Although synthetic dipalmityl PtdIns(3,4-P$_2$) and dipalmitoyl phosphatidylinositol 3',4',5'-trisphosphate restored only partially platelet spreading, phosphatidylinositol 4',5'-bisphosphate (PtdIns(4,5-P$_2$)) was able to trigger full spreading of wortmannin-treated adherent platelets. Following $^{32}$P labeling of intact platelets, the recovery of $^{32}$P labeled PtdIns(4,5-P$_2$) in anti-talin immunoprecipitates from adherent platelets was found to be decreased upon treatment by wortmannin. These results suggest that the lipid products of phosphoinositide 3'-kinase are required but not sufficient for ADP-induced spreading of adherent platelets and that PtdIns(4,5-P$_2$) could be a downstream messenger of this signaling pathway.

Platelets play a key role in hemostasis by their capacities to adhere and to aggregate in response to vascular injury. The most abundant platelet integrin, the α$_{1b}$β$_3$ complex, is largely responsible for platelet aggregation after binding of soluble fibrinogen. Moreover, α$_{1b}$β$_3$ integrin is required for a complete and irreversible platelet adhesion to the subendothelial matrix (1). In this case, its preferential ligand is the von Willebrand factor, but under certain conditions fibrinogen or fibrin could also act as adhesion substrates. In its resting state, α$_{1b}$β$_3$ integrin is able to recognize immobilized fibrinogen. However, the interaction of soluble fibrinogen with α$_{1b}$β$_3$ complex requires a previous conformational change of the integrin due to an inside-out signaling pathway. When platelets adhere in vitro to a fibrinogen matrix, they undergo several irreversible morphological changes such as rounding and spreading. These responses are sustained by a cytoskeletal reorganization including extension of filopodia, lamellipodia, and controlled orientation of stress fibers. It has been shown that a concomitant granular secretion of ADP from adherent platelets was necessary for spreading (2), and that it controlled specific signals, i.e. p125FAK and PtdIns 3-kinase activations (2, 3). Data from Haimovich et al. (4) show that tyrosine phosphorylation of p125FAK tyrosine kinase seems to be correlated with cell spreading upon platelet adhesion to a fibrinogen matrix. On the other hand, PtdIns 3-kinase activity has been involved in cytoskeletal rearrangements occurring during cell motility or platelet aggregation (5-9). Moreover, studies in whole cells have demonstrated an association of PtdIns 3-kinase with p125FAK (10, 11) and the small G proteins Rac and Cdc42 (12), all of them being involved in the regulation of cytoskeleton organization. Taking advantage of specific PtdIns 3-kinase inhibitors, LY294002 and wortmannin (8, 13, 14), we herein demonstrate that PtdIns 3-kinase is involved in the ADP-signaling pathway that controls platelet spreading. Nevertheless, our results suggest that PtdIns(4,5-P$_2$), a phospholipid tightly associated with actin-binding proteins in focal contacts and a key regulator of actin polymerization (15), could be a downstream messenger of this signaling pathway.

**EXPERIMENTAL PROCEDURES**

**Materials**—Human fibrinogen, ADP, human thrombin, phorbol 12-myristate 13-acetate (PMA), apyrase, pyruvate kinase, phosphoenolpyruvate, wortmannin, PtdIns(4-P), PtdIns(4,5-P$_2$), fatty acid-free bovine serum albumin, and phosphate-buffered saline (PBS) were from Sigma. Lysophosphatidic acid (LPA), the thrombospondin-1 cell binding do...
main peptide H-RFYVMWKOH, and the TXA2 analog U46619 used were, respectively, from Sigma, Bachem (Voisins-le-Bretonneux, France), and Calbiochem (Meudon, France). LY294002 and GF109203X were obtained, respectively, from Biomol (Plymouth Meeting, PA) and Glaxo (Les Ulis, France). Synthetic Di-C16-PtdIns-3,4-P2 (dipalmitoyl t-octadecanoylphosphatidylinositol, 3,4-bisphosphate) and Di-C16-PtdIns-3,4,5-P3 were purchased from Matreya (Pleasant Gap, PA).

Preparation of Platelets—Human platelets were isolated from fresh platelet concentrates (Centre Régional de Transfusion Sanguine, Tou- louse, France) by centrifugation as described previously (16). All washing procedures were performed at 37 °C in the presence of apyrase (1 unit/ml) as an ADP scavenger. In some experiments, platelet-rich plasma was incubated with 100 μM aspirin for 20 min to block cyclooxygenase activity. Platelets were labeled for 90 min with 0.4 μCi/ml [γ-32P]phosphate (Amersham Pharmacia Biotech, Bucks, United King- dom), as described previously (16). They were finally resuspended in modified Tyrode’s buffer (pH 7.4) containing 2.5 mM CaCl2.

Cell Adhesion Assays and Lipid Extract Analysis—Cell culture flasks (75 cm², Greiner Lahorteknik, Poitiers, France) were precoated or not (control) with 100 μg/ml of fibrinogen and were then blocked with fatty acid-free bovine serum albumin (3). The cell adhesion assay was performed using 5 ml of human platelets (3 × 10⁸ platelets/ml) that were added for 60 min at 37 °C to the fibrinogen-coated flasks or to the control flasks. In some experiments, the ADP scavenger pyruvate kinase-phosphoglycerate (14.3 units/ml and 1 mM, respectively, 10 min) or the protein C (PKC) inhibitor GF109203X (12 μM; 60 min) or the PtdIns-3-kinase inhibitors LY294002 (0–25 μM; 10 min) or wortmannin (0–100 nM; 15 min) were added to the platelet suspension before adhesion. GF109203X, wortmannin, and LY294002 were dis- solved in Me2SO, which did not exceed 0.06% (v/v). Recovering of adherent cells, evaluation of the extent of cell adhesion, and lipid extract analysis by HPLC were performed as described previously (3).

Spreading Restoration Assays—After elimination of unattached platelets and two washes with PBS, adherent wortmannin-treated platelets were incubated with different agonists (20 μM ADP, 5 μM LPA, 50 μM H-RFYVMWKOH, 5 μM U46619, 10 mM PMA, or 1 unit/ml thrombin) or phosphoinositides (PtdIns-4-P, PtdIns-4,5-P2, Di-C16-PtdIns-3,4-P2, Di-C16-PtdIns-3,4,5-P3, or a mixture of these lipids, 10–30 μM) in Tyrode’s buffer for 30 min at 37 °C. Before use, phosphoinositides were dried, suspended in 10 mM Hepes (pH 7.0), and sonicated in the absence of carrier phospholipids.

Optical Microscopy—At the end of the adhesion step or the spreading restoration assay, unattached platelets were removed by washing with PBS and the buffer was replaced by 1% glutaraldehyde in 0.1 M Na2HPO4. Fixation was continued at room temperature for 15 min. After washing, adherent platelets were examined by interference light microscopy with a Reichert EMF4 microscope. Micrographs were taken at original magnification ×1250.

Immunoprecipitation of Talin—Adherent platelets (4.5 × 10⁸ plate- lets) were scraped off at 4 °C in a lysis buffer containing 20 mM Tris- HCl, pH 8, 137 mM NaCl, 10% glycerol, 1 mM Na3VO4, 1 mM PMSF, 10 μM pepstatin, 10 μg/ml leupeptin, and 1% (v/v) Triton X-100. Resting platelets in suspension (4.5 × 10⁸ platelets) were centrifuged and re- suspended in 600 μl of the lysis buffer. After sonication (20 kHz for 2 × 10 s) and centrifugation (12,000 × g for 10 min at 4 °C), the soluble fraction was collected and subsequently preincubated for 30 min at 4 °C with protein G-Sepharose 4B fast flow. The immunoprecipitates were then washed twice with the same buffer without Triton.

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Lipid Extraction and Western Blotting on Talin Immunoprecipi- tates—For lipid analysis, immunoprecipitation of talin was performed after plating of [32P]-labeled platelets as described above. Lipids were extracted as described previously (3) and separated by TLC following the procedure established by Pignataro and Ascoli (18). Briefly, lipid extracts were applied on oxalate-EDTA-impregnated silica gel plates, which were then developed twice for 120 min with CH3CN, CH3OH, 9 μM phosphoric acid, and 1% NH4OH (40:40:15). Individual lanes containing commercial standards PtdIns-4-P or PtdIns-4,5-P2 were stained with iodine vapors. After exposure of the plates for 3–7 days, the radioactive spots were visualized and quantitated by a PhosphorImager 445 SI (Molecular Dynam- ics, Inc). Quantification was also performed after scraping the appro- priate areas of the plate and counting in a liquid scintillation counter.

For protein analysis, anti-talin immunoprecipitates were solubilized, separated on 7.5% SDS-polyacrylamide gels, and blotted onto nitricel- lulose as described previously (11). Immunodetection of talin was performed with the mouse monoclonal anti-talin antibody 8d4 from Sigma. Antibody reaction was visualized using the ECL chemiluminescence system (Amersham Pharmacia Biotech). Quantification of the different bands was performed by a densitometric analysis, which determines the pixel volume in each area (Gel Doc 1000, Bio-Rad).

RESULTS

Wortmannin and LY294002 Inhibit PtdIns-3,4-P2 Synthesis Triggered upon Platelet Adhesion—Wortmannin and LY294002 have been largely used in platelets as specific inhibitors of PtdIns 3-kinase, at nanomolar (10–100 nM) and micro- molar (25 μM) concentrations, respectively (8, 9, 19, 20). Since we have shown previously that platelet adhesion triggers ac- cumulation of [32P]PtdIns-3,4-P2 (3), we first assessed the inhibitory effect of wortmannin and LY294002 on the production of [32P]PtdIns-3,4-P2 as a reflection of PtdIns 3-kinase activation. 32P-Labeled platelets were preincubated with increasing doses of wortmannin or LY294002, and cells were then plated on the fibrinogen matrix for 60 min. After washing, adherent cells were scraped off and their lipid extract was analyzed by HPLC after desalination. [32P]PtdIns-3,4-P2 was quantified as described under “Experimental Procedures.”

FIG. 1. Dose-dependent inhibition of adhesion-induced [32P]- PtdIns-3,4-P2 synthesis by wortmannin and LY294002. Washed platelets were pretreated with wortmannin (15 min) or LY294002 (10 min) at different concentrations and then were plated on the fibrinogen matrix for 60 min. After washing, adherent cells were scraped off and their lipid extract was analyzed by HPLC after desalination. [32P]PtdIns-3,4-P2 was quantified as described under “Experimental Procedures.”

Wortmannin and LY294002 have been largely used in platelets as specific inhibitors of PtdIns 3-kinase, at nanomolar (10–100 nM) and micro- molar (25 μM) concentrations, respectively (8, 9, 19, 20). Since we have shown previously that platelet adhesion triggers ac- cumulation of [32P]PtdIns-3,4-P2 (3), we first assessed the inhibitory effect of wortmannin and LY294002 on the production of [32P]PtdIns-3,4-P2 as a reflection of PtdIns 3-kinase activation. 32P-Labeled platelets were preincubated with increasing doses of wortmannin or LY294002, and cells were then plated on the fibrinogen matrix before lipid extraction and analysis by HPLC. As shown in Fig. 1, LY294002 and wort- mannin inhibited [32P]PtdIns-3,4-P2 synthesis in a dose-de-
dependent manner with 80% inhibition achieved at 12 μM and 50 nM, respectively. The production of \([^{32}P]PtdIns-3,4-P_2\) was totally abrogated at 25 μM \(LY294002\) and 100 nM wortmannin, at which concentrations platelet adhesion was not significantly affected (Table I). At these concentrations, among other phosphoinositides (PtdIns, PtdOH, PtdIns-4-P, and PtdIns-4,5-P_2), only the PtdIns-4-P level was found to be somewhat decreased, but not significantly in comparison with control MeSO-treated platelets (Fig. 2).

**PtdIns 3-Kinase Inhibitors and ADP-dependent Platelet Spreading**—As described previously (21), upon adhesion, adherent platelets undergo the following steps of morphological changes: disk to sphere shape change, extension of pseudopodia, and a much slower process, cell spreading (Fig. 3, A and B). Pretreatment of platelets with wortmannin or \(LY294002\) inhibited platelet spreading on fibrinogen (Fig. 3, C and D). However, it should be noted that, although pretreated platelets did not fully spread, they still extended pseudopodia. The inhibitory effect of \(LY294002\) and wortmannin on cell spreading was already detectable at 6 μM and 25 nM, respectively. Concentrations of 25 μM \(LY294002\) and 100 nM wortmannin completely prevented platelet spreading. After removing wortmannin and \(LY294002\) from the adhesion medium by two washes, we observed that only the inhibitory effect of \(LY294002\) was reversible after 30 min (data not shown). Indeed, \(LY294002\) is a competitive inhibitor at the ATP-binding site of PtdIns 3-kinase (13), whereas wortmannin induces a covalent modification of the catalytic site of the enzyme (14). In agreement with Haimovich et al. (2, 4), pretreatment of platelets with the ADP scavenger pyruvate kinase plus phosphoenolpyruvate just before the adhesion assay induced the same effects as treatment with the PtdIns 3-kinase inhibitors, i.e. absence of spreading but persistence of pseudopodal extension (Fig. 3E). After washing, ADP (20 μM) was added to adherent platelets to overcome the ADP scavenging system. Addition of ADP restored full spreading of all adherent platelets as shown in Fig. 3F. On the other hand, addition of 20 μM ADP to adherent platelets pretreated with wortmannin did not reverse inhibition of platelet spreading (Fig. 3G). These data demonstrate that PtdIns 3-kinase signaling pathway is required for ADP-induced spreading of adherent platelets.

**PtdIns-4,5-P_2, but Not Lipid Products of PtdIns 3-Kinase Is Sufficient for a Full Platelet Spreading**—Our previous measurements of PtdIns 3-kinase activation in adherent platelets (3) have shown that, although the PtdIns-3,4,5-P_3 level was not significantly modified between 5 and 30 min of adhesion, PtdIns-3,4-P_2 accumulated as a function of the adhesion time. Time course of PtdIns-3,4-P_2 production closely paralleled platelet spreading upon adhesion (data not shown). In order to determine whether products of PtdIns 3-kinase could be involved in platelet spreading, we used Di-C_16-PtdIns-3,4,5-P_3, which were reported to trigger biologic responses when added to whole cells (7, 22). As shown in Fig. 4A, addition of Di-C_16-PtdIns-3,4-P_2 (20 μM) on adherent platelets pretreated with wortmannin only partially restored platelet spreading. After 30 min of incubation with Di-C_16-PtdIns-3,4-P_2, some adherent platelets have lost their round shape and have undergone pseudopodal and hyalomere extension. Nevertheless, these modifications concerned only a small proportion of adherent platelets (5%), as compared with 35% of spread platelets obtained with non-pretreated control cells (Fig. 4B).

Using amounts of Di-C_16-PtdIns-3,4-P_2 between 10 and 30 μM, we obtained a dose-dependent increase in the response rate (detected as early as 15 min of adhesion) and in the proportion

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**FIG. 2. Effects of wortmannin and LY294002 on \([^{32}P]PtdIns-3,4-P_2\) labeling of various phospholipids from resting and adherent platelets.** Washed platelets were pretreated in absence (A) or presence of MeSO (0.03%; 15 min), wortmannin (100 nM; 15 min) or \(LY294002\) (25 μM; 10 min) and plated on the fibrinogen matrix for 60 min. Control resting platelets (C) were added to a flask without fibrinogen. Control and adherent platelets were then recovered, and their lipid extracts were analyzed by HPLC after deacylation as described under “Experimental Procedures.” Data are from five independent experiments.

**TABLE 1**

|                | A   | MeSO     | LY294002 | Wortmannin |
|----------------|-----|----------|----------|------------|
| Adhesion ± S.E. (%) | 25 ± 5  29 ± 11  20 ± 5  19 ± 5 |
of responsive cells (Fig. 4B). In no case did we observe a full spreading of platelets, even after 60 min of incubation. Addition of Di-C16-PtdIns-3,4-P3 or PtdIns-4-P or both (data not shown) together with Di-C16-PtdIns-3,4-P2 was not more efficient in restoring full spreading (Fig. 4B). Surprisingly, addition of PtdIns-4,5-P2 to adherent platelets pretreated with wortmannin triggered full spreading (Fig. 4, A and B). Moreover, the number of fully spread platelets was increased by addition of both Di-C16-PtdIns-3,4-P2 and PtdIns-4,5-P2 (Fig. 4B). Nevertheless, under these conditions, the amount of fully spread platelets was far below that observed in the control situation.

PtdIns-4,5-P2 effects on cell spreading are unlikely to be dependent on induction of platelet release reaction, as ADP (20 μM), LPA (5 μM), the thrombospondin-1 cell binding domain peptide (H-RFYVWMK-OH; 50 μM), could not restore spreading of platelets treated with 100 nM wortmannin (Table II). By contrast, the TXA2 analog U46619 (5 μM) triggered full spreading of adherent platelets pretreated with 100 nM wortmannin. However, addition of PtdIns-4,5-P2 (20 μM) to adherent platelets pretreated with aspirin (100 μM) and wortmannin (100 nM) was still able to restore full platelet spreading (Table II).

The fact that strong platelet agonists, such as thrombin (1 unit/ml), the TXA2 analog U46619, and the PKC activator PMA (10 nM), were able to restore spreading of platelets pretreated with 100 nM wortmannin (Table II) might suggest a PtdIns 3-kinase-independent pathway of platelet spreading. In order to determine a possible activation of PKC isoforms by high concentrations of PtdIns-4,5-P2 (23), we used the specific PKC inhibitor GF109203X (24). Preincubation of platelets with GF109203X (12 μM) triggered inhibition of platelet spreading as described previously by Haimovich and coworkers (4). When platelets had been pretreated by both GF109203X (12 μM) and wortmannin (100 nM) together, exogenous PtdIns-4,5-P2 was still able to restore full spreading of adherent platelets with a similar efficiency as in the case of a pretreatment with wortmannin alone (Table II). These experiments argue in favor of a specific PtdIns-4,5-P2 effect on the restoration of cell spreading that is independent on PKC activation.
The partial rescue of platelet spreading with lipids suggests that there may be additional components to the PtdIns 3-kinase-dependent pathway. We addressed the possibility that the myosin light chain kinase (MLCK) could be involved in platelet spreading. Indeed, inhibitors of MLCK have been shown to disassemble focal adhesions and to reduce their phosphoryrosine staining (25), and wortmannin is a MLCK inhibitor, but at micromolar concentrations (26). Preincubation of platelets with 10 μM ML-7 (specific inhibitor of MLCK from Biomol; Ref. 27) during the 30 min before adhesion did not modify platelet spreading (data not shown). We concluded that MLCK does not seem to be involved in platelet spreading.

**DISCUSSION**

We have shown previously that synthesis of PtdIns-3,4-P_2 in adherent platelets is under the control of the released ADP, since addition of ADP reversed the inhibitory effects of an ADP
scavenger on PtdIns-3,4-P₂ synthesis (3). Our present results, in agreement with those of Haimovich et al. (2, 4), demonstrate that ADP release occurring upon platelet adhesion is required for full platelet spreading. Thus, ADP released by adherent platelets controls both PtdIns-3,4-P₂ synthesis and cell spreading. In conditions where PtdIns3-4-P₂ synthesis was totally abolished by inhibitors of PtdIns 3-kinase, we observed an inhibition of platelet spreading while pseudopodal extension was maintained. We demonstrate here that the PtdIns 3-kinase signaling pathway is required for ADP-induced spreading of adherent platelets.

In order to determine which lipid is responsible for platelet spreading, we have added synthetic PtdIns-3,4-P₂ and PtdIns-3,4,5-P₃ to adherent wortmannin-treated platelets. PtdIns-3,4-P₂ appears to be the most efficient in restoring partial spreading, i.e., pseudopodal and hyalomere extension. However, among all phosphoinositides tested, PtdIns-4,5-P₂ alone was able to trigger the full spreading of wortmannin-treated platelets. Although all phospholipid solutions were prepared under similar conditions, their packing into micelles or vesicles was not characterized. Moreover, differences in the acyl chains of PtdIns-3,4-P₂ (palmitate) and PtdIns-4,5-P₂ (stearate and arachidonate) may have influenced their activity. Nevertheless, our results suggest a role for both PtdIns-4,5-P₂ and lipid products of PtdIns-3-kinase in the signal transduction pathway leading to platelet spreading.

We have observed a trend toward a decrease of [³²P]PtdIns-4-P in adherent platelets pretreated with PtdIns 3-kinase inhibitors. This result suggests that a wortmannin-sensitive PtdIns 4-kinase might exist in platelets, as has been shown in other models (30, 31). Nevertheless, in a cell system, wortmannin was reported to inhibit PtdIns 4-kinase at μM concentration, and LY294002 has not been described as an inhibitor of known PtdIns 4-kinases (12). Thus, PtdIns 3-kinase activity could be upstream of a PtdIns 4-kinase and/or a PtdIns-4-P phosphatase. Finally, even though we have not measured a significant decrease of [³²P]PtdIns-4,5-P₂ in whole platelets pretreated with PtdIns 3-kinase inhibitors, a decrease of PtdIns-4-P level could impair the synthesis of a particular pool of PtdIns-4,5-P₂ required for platelet spreading.

Recent studies from the Schlessinger and Rhee laboratories (32, 33) demonstrate that γ isoforms of phospholipase C (PLC) could be activated by PtdIns-3,4,5-P₃, either by targeting to cell membrane through their PH domain or by direct activation through their SH₂ domain. One could thus expect an increase of PtdIns-4,5-P₂ level when adherent platelets have been pre-treated with PtdIns 3-kinase inhibitors. Nevertheless, at least two reasons could explain why in our experiments this variation is not observed. First, in our previous paper (3), we have shown that upon platelet adhesion on a fibrinogen matrix a PLC active on PtdIns-4,5-P₂ was rapidly and transiently stimulated. Maximal increase of PtdOH production and PtdIns-4,5-P₂ decrease was observed as early as 5 min of adhesion. Thereafter, these two metabolites returned gradually to their basal level, and that corroborates the absence of PtdOH and PtdIns-4,5-P₂ variations after 60 min of adhesion, as shown in Fig. 2 of our present article. We thus believe that in the late steps of platelet adhesion PLC activity is not involved. However, it should be of importance to check PLC activity during the early steps of adhesion of platelets treated with PtdIns 3-kinase inhibitors. Second, since our present data show a decrease of PtdIns-4-P-4 level upon platelet treatment with PtdIns 3-kinase inhibitors, an eventual increase of the PtdIns-4,5-P₂ level might be impaired.

PtdIns-4,5-P₂ regulates several actin-binding proteins as profilin, gelsolin, α-actinin, and vinculin (34). One of the major proteins of focal adhesions, talin, has been shown to be involved in cell spreading (28, 29). Its interaction with lipids has been documented in vitro and could be of importance for talin nucleated actin polymerization (34). Here, we show that PtdIns-4,5-P₂ as well as PtdIns-4-P and PtdIns-3,4-P₂ become associated with talin upon platelet adhesion. Moreover, treatment of platelets by wortmannin strongly reduces the amounts of polyphosphoinositides recovered in the anti-talin immunoprecipitate. Even though it remains to be determined whether this association is direct or not, our results support the notion of a possible regulation by PtdIns-3-kinase of a pool of PtdIns-4,5-P₂ potentially involved in cell spreading.

Hartwig et al. (35) have reported that D3 and D4 polyphosphoinositides uncouple F-actin in resting permeabilized platelets. At low concentrations (10 μM), PtdIns-4,5-P₂ and PtdIns-3,4-P₂ are more effective than PtdIns-3,4,5-P₃. Synthesis of PtdIns-4-P and PtdIns-4,5-P₂, which are correlated with the exposure of barbed filament ends, seem to be under the control of the small G protein Rac (35). This small G protein has been shown to regulate extension of peripheral lamellipodia (36), to associate in vivo with both PtdIns 3-kinase and PtdIns-4-P 5-kinase (12), and it was suggested that PtdIns 3-kinase functions upstream of Rac (37, 38). Moreover, PtdIns-4,5-P₂ and PtdIns-3,4-P₂ both regulate, in vitro, the severing and capping of the protein gelsolin (9), whose genetic defect is responsible for the absence of lamellae although the filopod formation is maintained, upon platelet activation (39). Thus, in our model, PtdIns-3-kinase could regulate actin remodeling directly through PtdIns-3,4-P₂ synthesis and/or indirectly through PtdIns-4,5-P₂ synthesis.

Recent results from King et al. (40), showing that spreading of COS7 cells attached to fibronectin is delayed after treatment by wortmannin and LY294002, support the view that the PtdIns-3-kinase signaling pathway is required for cell spreading, as controlled by integrins and/or by tyrosine kinase receptors (41). It has been suggested that ADP released from adherent platelets supports some specific signals such as Vav phosphorylation via an indirect mechanism involving activation of α₁β₃ (42). Furthermore, an integrin-associated protein agonist peptide triggers activation of α₁β₃ integrin resulting in platelet spreading on immobilized fibrinogen (43). Thus, upon platelet adhesion to immobilized fibrinogen, it remains to be clarified whether platelet spreading is secondary to the α₁β₃ integrin engagement.

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