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Integrin $\alpha_{IIb}\beta_3$ signals lead cofilin to accelerate platelet actin dynamics

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Falet, Hervé, Gregory Chang, Brigitte Brohard-Bohn, Francine Rendu, and John H. Hartwig. Integrin $\alpha_{IIb}\beta_3$ signals lead cofilin to accelerate platelet actin dynamics. *Am J Physiol Cell Physiol* 289: C819–C825, 2005. First published May 18, 2005; doi:10.1152/ajpcell.00587.2004.—Cofilin, in its Ser3 dephosphorylated form, accelerates actin filament turnover in cells. We report here the role of cofilin in platelet actin assembly. Cofilin is primarily phosphorylated in the resting platelet as evidenced by a specific antibody directed against its Ser3 phosphorylated form. After stimulation with thrombin under nonstirring conditions, cofilin is reversibly dephosphorylated and transiently incorporates into the actin cytoskeleton. Its dephosphorylation is maximal 1–2 min after platelet stimulation, shortly after the peak of actin assembly occurs. Cofilin rephosphorylation begins 2 min after activation and exceeds resting levels by 5–10 min. Cofilin is dephosphorylated with identical kinetics but fails to become rephosphorylated when platelets are stimulated under stirring conditions. Cofilin is normally rephosphorylated when platelets are stimulated in the presence of Arg-Gly-Asp-Ser (RGDS) peptide or wortmannin to block $\alpha_{IIb}\beta_3$ cross-linking and signaling or in platelets isolated from a patient with Glanzmann thrombasthenia, which express only 2–3% of normal $\alpha_{IIb}\beta_3$ levels. Furthermore, actin assembly and Arp2/3 complex incorporation in the platelet actin cytoskeleton are decreased when $\alpha_{IIb}\beta_3$ is engaged. Our results suggest that cofilin is essential for actin dynamics mediated by outside-in signals in activated platelets.

STIMULATION OF DISCOID BLOOD platelets with soluble agonists (thrombin, ADP) or by molecules bound to the subendothelial matrix exposed by damage (collagen, von Willebrand factor) results in shape change and secretion. Shape change is mediated by a rapid, yet precise, remodeling of the resting platelet cytoskeleton and the assembly of filamentous actin (F-actin) that is induced by signals unleashed by receptor ligation (18). For example, ligation of thrombin or collagen receptors results in increases in platelet intracellular calcium levels, which activate gelsolin (4, 11). Active gelsolin severs actin filaments, initially reorganizing the cytoskeleton of the discoid platelet (18). Dissociation of gelsolin from the fragmented filaments by newly generated membrane polyphosphoinositides supplies barbed-end nucleation sites that are further amplified by the Arp2/3 complex, leading to a burst of actin assembly that drives the shape change reaction (12, 19, 20). In addition, dissociation of adducin from both spectrin and actin filament barbed ends facilitates the collapse of the membrane skeleton into the center of the spread platelet, potentiating the remodeling reaction (5).

Agonist-induced platelet activation also triggers inside-out signals, which convert the fibrinogen receptor, the integrin $\alpha_{IIb}\beta_3$, into its active, fibrinogen-bound form. Engagement of activated $\alpha_{IIb}\beta_3$ by linking fibrinogen is necessary for platelet aggregation, which induces subsequent outside-in signals to enhance platelet activation. Although platelet actin assembly and shape change per se do not require the participation of $\alpha_{IIb}\beta_3$, actin remodeling is influenced by outside-in signals coming from this ligated receptor (13). Key signaling intermediates involved in integrin-induced remodeling of the actin cytoskeleton are the small Rho family of GTPases (23), which induce actin assembly and the formation of focal adhesions. They also involve the phosphorylatable adaptor protein pleckstrin, recently shown to be important for integrin-induced cell spreading (34), and the tyrosine kinases Src and Syk, which activate downstream signaling intermediates such as the adaptor proteins SLP-76 and Cbl, the tyrosine kinase focal adhesion kinase (FAK), and the guanine nucleotide exchange factor Vav (27, 28). Cbl likely links integrin signaling to phosphoinositide (PI)3-kinase (36), known to be important for platelet spreading, and Vav is a guanine nucleotide exchange factor for Rac (24), a small GTPase that induces lamellipodia formation in activated platelets (2, 19). Finally, FAK is an important component of focal adhesions, which are linked to actin stress fibers in a Rho-dependent manner (7, 40).

One protein that may be implicated in platelet actin remodeling is cofilin (8). Cofilin belongs to the actin-depolymerizing factor/cofilin family of small (18–21 kDa) actin-binding proteins that accelerate actin filament turnover by increasing filament end numbers (9). Human platelets express nonmuscle cofilin isoform 1 (31). Cofilin binds to or near the pointed ends of actin filaments, where it can sever filaments and/or promote actin monomer release (6, 33). Both of these functions accelerate actin filament turnover because newly released monomers are recycled for rounds of polymerization at filament barbed ends and because severing of actin filaments increases the number of barbed ends available for binding of the Arp2/3 complex, which amplifies the actin polymerization response (15, 21, 25). Cofilin is negatively regulated through phosphorylation on Ser3 (1, 3), and it has been established in platelets that dephosphorylation of cofilin accompanies activation by agonists including thrombin and phorbol esters (8). Herein we establish a central role for sustained signals generated by $\alpha_{IIb}\beta_3$ in maintaining cofilin in its active dephosphorylated form in aggregating platelets.

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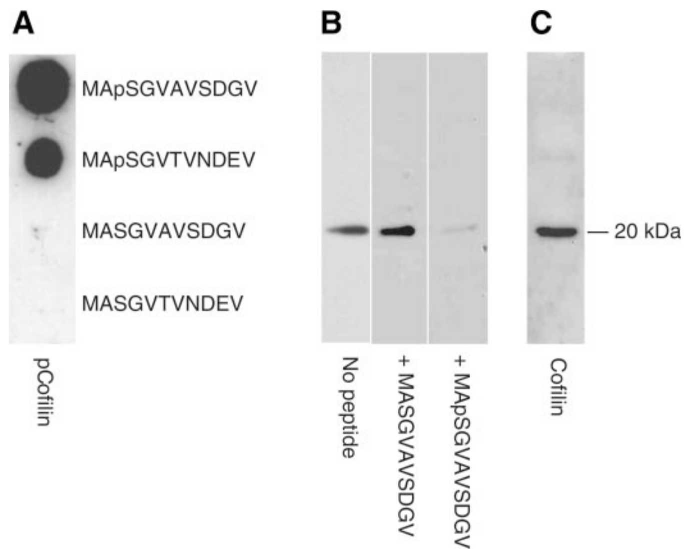

Fig. 1. Specificity of the rabbit anti-human phosphocofilin antibody. *A*: peptides (1 μg; pS, phosphoserine) corresponding to the Ser3-phosphorylated and dephosphorylated forms of human cofilin isoforms 1 and 2 were probed with rabbit anti-phospho (p)cofilin antibody by dot blot analysis as indicated. *B*: resting platelet lysates were subjected to 15% SDS-PAGE and probed with rabbit anti-phosphocofilin antibody in the presence or absence of peptides corresponding to the Ser3-phosphorylated and dephosphorylated forms of human cofilin 1 as indicated. *C*: resting platelet lysates were subjected to 15% SDS-PAGE and probed with chicken anti-human cofilin 1 antibody.

MATERIALS AND METHODS

Materials. Peptides MASGVAVSDGV and MApSGVAVSDGV (where pS means phosphoserine), corresponding to the 11 NH_2-terminal amino acids of human nonmuscle cofilin isoform 1, and MASGVTVNDEV and MApSGVTVNDEV, corresponding to the 11 NH_2-terminal amino acids of human muscle cofilin isoform 2, were synthesized at the Tufts University Core Facility (Boston, MA). Sepharose 2B and CNBr-activated Sepharose 2B were purchased from Amersham Biosciences (Piscataway, NJ). Goat antibody directed against Arp3 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Chicken antibody directed against recombinant human cofilin 1 was provided by Dr. Peter Marks (Div. of Hematology, Brigham and Women's Hospital, Boston, MA). Purified cofilin was provided by Dr. Marie-France Carlier (Centre National de la Recherche Scientifique, Gif-sur-Yvette, France). All other reagents were of the highest purity available (Sigma, St. Louis, MO, and Bio-Rad, Hercules, CA).

Development of anti-phosphocofilin antibody. Peptide MApSGVAVSDGV (500 μg) was injected subcutaneously in multiple sites into rabbits. After 2 wk, rabbits were boosted with injections of Freund's incomplete adjuvant containing the phosphopeptide and serum was collected. Sepharose 2B affinity columns were prepared according to the manufacturer's instructions. Rabbit anti-phosphocofilin serum was passed three times over the column, and bound antibodies were eluted with 2.5 M glycine, pH 2.7, in tubes containing sufficient 1 M phosphate buffer to return the pH to >6. Fractions containing the IgG antibodies were dialyzed overnight in Tris-buffered saline and subsequently tested for phosphospecificity on dot blots against phosphorylated and nonphosphorylated cofilin peptides (Fig. 1*A*). Rabbits were maintained and treated as approved by the Harvard Medical Area Standing Committee on Animals and according to National Institutes of Health standards as set forth in the Institute for Laboratory Animal Research *Guide for the Care and Use of Laboratory Animals*.

Platelet preparation and stimulation. Human blood was obtained from healthy volunteers and from a patient with well-characterized Glanzmann thrombasthenia (20). Approval was obtained from the Institutional Review Board of Brigham and Women's Hospital, and informed consent was approved according to the Declaration of Helsinki. Blood was drawn into 0.1 vol of a citrate-based anticoagulant. Platelet-rich plasma was prepared by centrifugation of the blood at 100 *g* for 15 min. Platelets were isolated from plasma with a metrizamide gradient (11). Platelet concentration was adjusted to 3×10^8 cells/ml, and platelets were allowed to rest for 30 min at 37°C before use. Unstirred or stirred platelets were activated by 1 U/ml thrombin at 37°C in a Coulter aggregometer (Havertown, PA).

SDS-PAGE and immunoblotting. Platelets were lysed with 0.1% Triton X-100 in (in mM) 60 PIPES, 25 HEPES, 10 EGTA, and 2 $MgCl_2$, pH 6.9 (PHEM buffer) containing protease inhibitors and 2 μM phalloidin. F-actin was isolated by centrifugation at 100,000 *g* for 30 min at 4°C in a Beckman Optima TL ultracentrifuge (Palo Alto, CA) as described previously (12). Triton X-100-insoluble and -soluble fractions were lysed in SDS-PAGE sample buffer containing 5% β-mercaptoethanol. After being boiled for 5 min, platelet proteins were separated on 15% polyacrylamide gels and transferred onto an Immobilon-P membrane (Millipore, Bedford, MA). Membranes were incubated for 1 h in Tris-buffered saline containing 0.05% Tween 20 and 5% dry milk. Membranes were then incubated overnight with either 5 μg/ml rabbit anti-phosphocofilin antibody or 2 μg/ml chicken

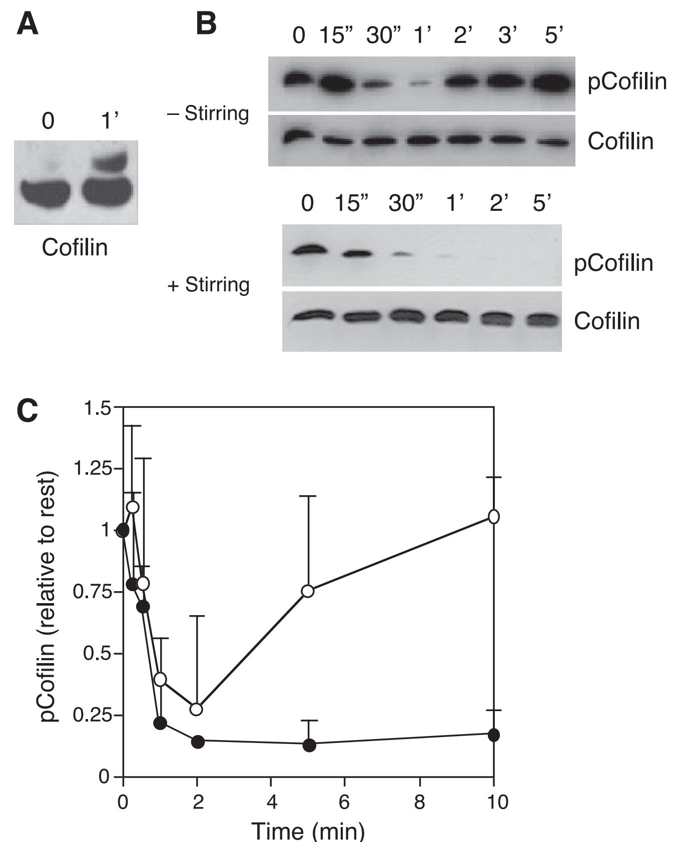

Fig. 2. Cofilin dephosphorylation in thrombin-stimulated platelets. *A*: lysates from resting platelets or platelets stimulated with 1 U/ml thrombin at 37°C for 1 min were subjected to 10% urea-PAGE and probed with chicken anti-human cofilin antibody. Resting platelets primarily contain phosphocofilin. *B*: platelets were activated with 1 U/ml thrombin at 37°C for the indicated times under stirring or nonstirring conditions. Lysates were subjected to 15% SDS-PAGE and probed with rabbit anti-phosphocofilin antibody. The membrane was reprobed with chicken anti-cofilin antibody to control for loading. *C*: platelet phosphocofilin levels relative to resting levels were quantified by performing densitometric analysis of the immunoblots. ●, stirring; ○, nonstirring. Values represent means ± SD of 3 independent experiments.

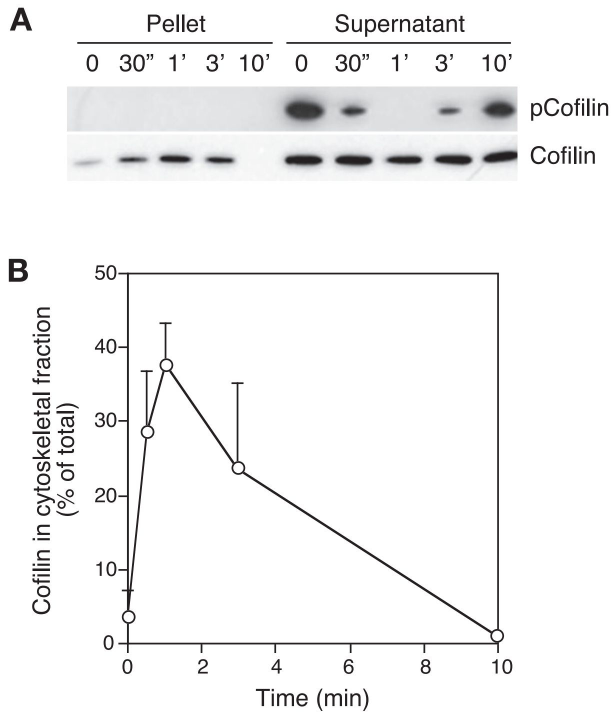

Fig. 3. Cofilin association with F-actin in thrombin-stimulated platelets. *A*: F-actin-associated cofilin was collected by centrifugation of Triton X-100 lysates at 100,000 *g* for 30 min at 4°C. Insoluble and soluble fractions were subjected to 15% SDS-PAGE and probed with chicken anti-cofilin antibody as indicated. Only dephosphorylated cofilin associates with the actin cytoskeleton of the active platelet. *B*: the amount of cofilin in the cytoskeletal fraction relative to total was quantified by densitometric analysis. Values represent means ± SD of 3 independent experiments.

anti-cofilin antibody. Detection was performed with an enhanced chemiluminescence system (Pierce, Rockford, IL).

Cofilin phosphorylation was evaluated by nonequilibrium isoelectric focusing as described previously (29, 30). Gels contained 10% polyacrylamide, 30% Triton X-100, 11.5 M urea, and 3% ampholyte, pH 3–10 (Amersham Biosciences). The cathode (lower) and anode (upper) chambers of the electrophoresis apparatus contained 100 mM NaOH and 20 mM phosphoric acid, respectively. After electrophoresis, gels were equilibrated in SDS-PAGE sample buffer before transfer onto an Immobilon-P membrane as above.

Immunofluorescence. Resting platelets or platelets activated in suspension with 1 U/ml thrombin for 1 or 5 min at 37°C were fixed with an equal volume of 6.8% paraformaldehyde for 10 min, deposited on glass coverslips, and permeabilized with a solution containing 0.1% Triton X-100, 500 nM FITC-phalloidin, and 80 μg/ml anti-phosphocofilin antibody for 1 h at room temperature. Coverslips were washed three times in phosphate-buffered saline containing 1% BSA and incubated in a solution containing 1:200 goat anti-rabbit Cy3-conjugated secondary antibodies for 30 min at room temperature. Coverslips were mounted onto slides, viewed, and photographed on a Nikon epifluorescence microscope (Melville, NY).

RESULTS

Characterization of anti-phosphocofilin antibody. To study cofilin activation in platelets, we developed an antibody specific for the Ser3-phosphorylated form of human nonmuscle cofilin isoform 1. Rabbits were immunized with the peptide MApSGVAVSDGV, corresponding to the 11 NH_2-terminal amino acids of human phosphocofilin 1. The anti-phosphocofilin antibody was affinity purified from the serum and probed for specificity by dot blot analysis (Fig. 1*A*). The antibody recognized peptides corresponding to the Ser3-phosphorylated

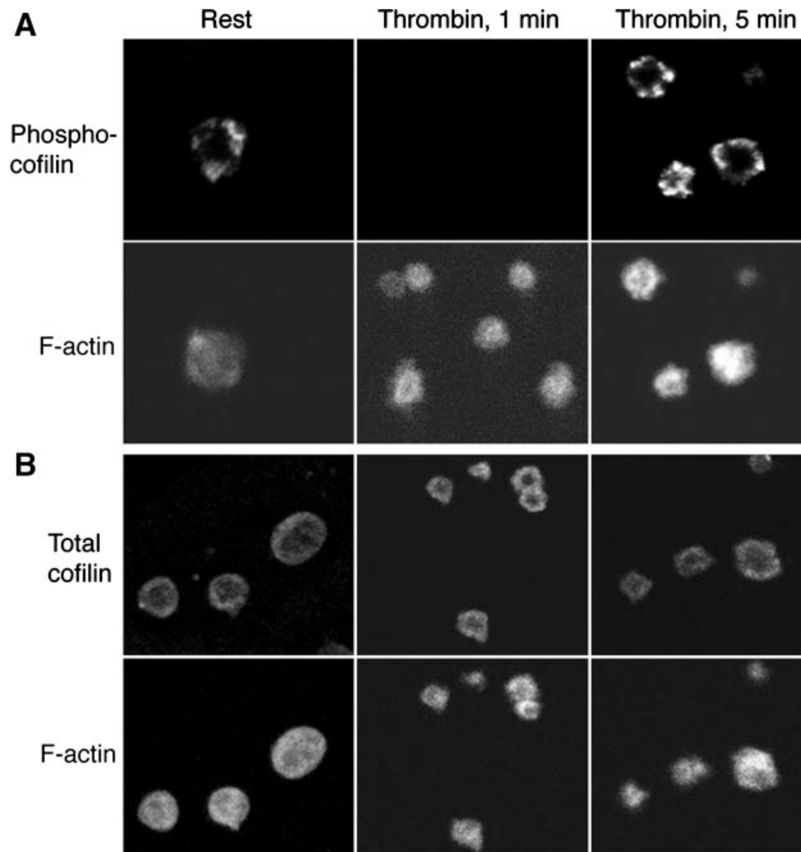

Fig. 4. Cofilin distribution in platelets. Platelets were activated with 1 U/ml thrombin at 37°C under nonaggregating conditions for the indicated times, centrifuged onto glass coverslips, and permeabilized with 0.5% Triton X-100. The actin cytoskeleton was labeled with rabbit anti-phosphocofilin antibody and visualized with Cy3-conjugated anti-rabbit antibody (*A*) or labeled with chicken anti-cofilin antibody and visualized with tetramethylrhodamine isothiocyanate-conjugated anti-chicken antibody (*B*). In addition, the coverslips were exposed to FITC-phalloidin for F-actin staining as indicated.

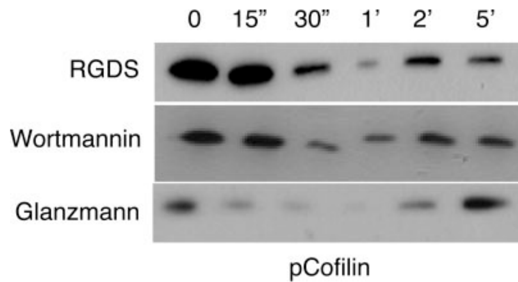

Fig. 5. $\alpha_{IIb}\beta_3$ is required to maintain cofilin in an active, dephosphorylated state. Platelets isolated from a healthy volunteer or from a patient with Glanzmann thrombasthenia were preincubated or not with 300 μg/ml Arg-Gly-Asp-Ser (RGDS) peptide or 50 nM wortmannin as indicated. Platelets were activated with 1 U/ml thrombin at 37°C under stirring conditions for the indicated times. Lysates were subjected to 15% SDS-PAGE and probed with rabbit anti-phosphocofilin antibody.

forms of both cofilin isoforms 1 and 2, but not the nonphosphorylated peptides. Immune sera from these rabbits also specifically recognized a 20-kDa protein in resting platelet lysates (Fig. 1*B*). The phosphocofilin, but not the nonphosphocofilin, peptide blocked recognition by the antibody. This 20-kDa protein comigrates with cofilin, as evidenced by immunoblot using a chicken antibody directed against human cofilin 1, which recognizes cofilin in both its phosphorylated and nonphosphorylated forms (Fig. 1*C*). By performing densitometric analysis of immunoblots against total cofilin using purified human cofilin 1 as a standard, we estimated that platelets contain 28 μM cofilin (data not shown). This is in good agreement with previous estimates based on Coomassie brilliant blue staining relative to actin (8).

Cofilin activation in thrombin-stimulated platelets. We further determined the amount of phosphocofilin in resting platelets using nonequilibrium isoelectric focusing in which the phosphorylated and nonphosphorylated forms of cofilin were resolved by charge difference (Fig. 2*A*; Refs. 29, 30). Densitometric analysis revealed that 87% (SD 7; $n = 4$) of cofilin is phosphorylated on Ser3 in resting platelets. Dephosphorylation was observed in platelets stimulated for 1 min with 1 U/ml thrombin under nonaggregating conditions.

The kinetics of cofilin dephosphorylation in activated platelets under nonaggregating conditions was further investigated by immunoblot analysis using the rabbit anti-phosphocofilin antibody (Fig. 2, *B* and *C*). Cofilin dephosphorylation was rapid and transient, with complete dephosphorylation occurring after 1–2 min of stimulation with thrombin. By 5–10 min, phosphocofilin levels exceeded those found in the resting platelet. Cofilin dephosphorylation and its subsequent rephosphorylation in response to thrombin were not affected by pretreatment of platelets with 50 nM wortmannin to block PI3-kinase or with 1 μM okadaic acid to block protein serine/threonine phosphatases 1 and 2A (data not shown). Similar results were obtained when platelets were stimulated with the anti-CD9 antibody Syb, which activates platelets through the low-affinity receptor for IgGs, FcγRIIA (CD32A) (data not shown; Refs. 32, 35). In this case, however, platelet preincubation with wortmannin blocked cofilin initial dephosphorylation, consistent with a crucial role of PI3-kinase in signaling downstream of FcγRIIA (data not shown; Ref. 17). In platelets stimulated by thrombin under aggregating conditions, cofilin was dephosphorylated with the same time course observed in nonaggregating conditions, but rephosphorylation did not occur (Fig. 2, *B* and *C*).

Cofilin incorporation in active platelet cytoskeleton. The dephosphorylation of cofilin correlates temporally with a transient increase in its association with the actin cytoskeleton (Fig. 3). In resting platelets, <5% of cofilin is associated with the cytoskeleton. After 1–2 min of thrombin stimulation under nonaggregating conditions, ~40% of cofilin associates with the Triton X-100-insoluble fraction. Cofilin subsequently dissociates from the cytoskeleton in a time course that correlates with its rephosphorylation. Phosphocofilin is not detected in the cytoskeletal fractions of resting and activated platelets, confirming in vitro findings that phosphorylated cofilin does not bind to F-actin (33).

Cofilin distribution in platelets. Cofilin distribution in platelets was further investigated by immunofluorescence (Fig. 4). In resting platelets, a diffuse signal was observed throughout the cytoplasm without a prevalence of any specific compartment or structure. After stimulation with thrombin under non-

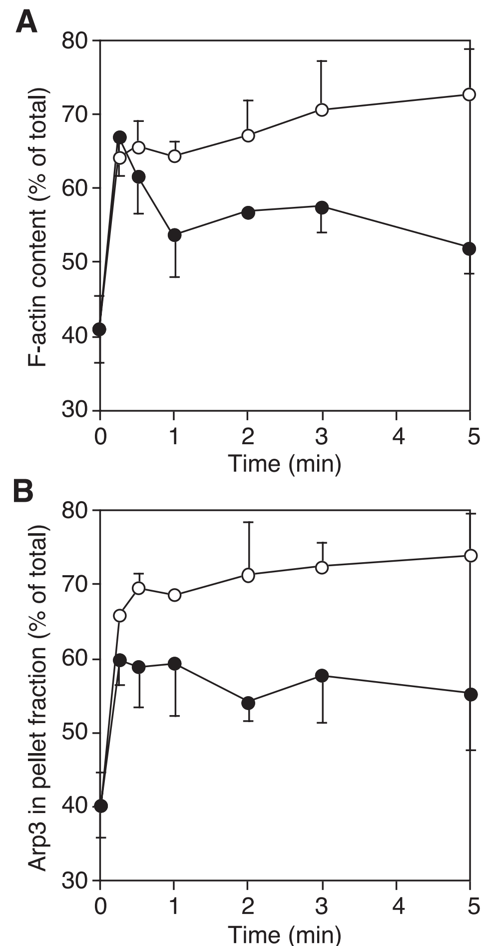

Fig. 6. F-actin content and Arp2/3 complex distribution in activated platelets. Platelets were stimulated with 1 U/ml thrombin at 37°C under stirring (●) or nonstirring (○) conditions. Triton X-100-insoluble and -soluble fractions were collected by centrifugating platelet lysates at 100,000 *g* for 30 min at 4°C and subjected to 10% SDS-PAGE. *A*: actin was probed by Coomassie brilliant blue staining. *B*: the Arp2/3 complex was probed with goat anti-Arp3 antibody. The amount of actin and Arp2/3 complex in the cytoskeletal fraction relative to total was quantified by densitometric analysis. Values represent means ± SD of 3 independent experiments.

aggregating conditions, cofilin concentrated at the cell periphery. Resting cells stained brightly with the anti-phosphocofilin antibody, but staining was reversibly lost after 1 min of activation by 1 U/ml thrombin. After 5 min, significant rephosphorylation occurred, consistent with the biochemical data.

Outside-in signals coming from $\alpha_{IIb}\beta_3$ maintain cofilin in its active state. Cofilin rephosphorylation did not occur in platelets stimulated by thrombin under aggregating conditions (Fig. 2, *B* and *C*). To determine the role of outside-in signals coming from $\alpha_{IIb}\beta_3$ in maintaining cofilin in its active, dephosphorylated state, we next investigated the kinetics of cofilin dephosphorylation in platelets treated with 300 μg/ml Arg-Gly-Asp-Ser (RGDS) peptide to prevent $\alpha_{IIb}\beta_3$ cross-linking or with 50 nM wortmannin to block PI3-kinase (Fig. 5). In these conditions, the percentages of light transmission measured in the platelet aggregometer were 5% (SD 2) and 58% (SD 8), respectively, compared with 65% (SD 4) in the absence of treatment ($n = 6$). After platelet stimulation by 1 U/ml thrombin under aggregating conditions, cofilin was maximally dephosphorylated at 1 min but was rephosphorylated at subsequent time points in the presence of RGDS peptide or wortmannin. Similar results were obtained by using platelets isolated from a patient with Glanzmann thrombasthenia, which express only 2–3% of the normal levels of $\alpha_{IIb}\beta_3$ (20). Together, the data indicate that outside-in signals coming from $\alpha_{IIb}\beta_3$ are necessary to maintain cofilin in its active, dephosphorylated state.

Decreased F-actin content and Arp2/3 complex distribution in aggregating platelets. We next determined the role of cofilin and $\alpha_{IIb}\beta_3$ in the platelet actin assembly reaction by quantifying proteins in Triton X-100-insoluble platelet fractions by Coomassie brilliant blue staining (Fig. 6*A*). Platelets were activated with 1 U/ml thrombin in the presence or absence of stirring to induce or prevent $\alpha_{IIb}\beta_3$ cross-linking. Total F-actin in the cytoskeleton rapidly increased from 40% to 65% after 30 s of stimulation under nonaggregating conditions and became maximal at 70% of the total after 1 min of stimulation. Thus filament actin assembly peaked shortly before cofilin activation became maximal.

When aggregation was allowed to maintain cofilin in its active, dephosphorylated form, the F-actin content of the cytoskeleton increased similarly from 40% to 65% in the first 30 s of stimulation but then decreased to 50% at longer times. Similar observations were obtained for the distribution of the Arp2/3 complex in the platelet cytoskeleton (Fig. 6*B*). The amount of the Arp2/3 complex in the Triton X-100-insoluble fraction increased from 40% to 70% in platelets activated in nonaggregating conditions, but to only 55–60% of the total when platelets were activated in aggregating conditions.

Platelet treatment with 300 μg/ml RGDS peptide or with 50 nM wortmannin rescued both the decrease of the F-actin content and the incorporation of the Arp2/3 complex in the actin cytoskeleton observed after 5 min of stimulation, although not to the levels obtained in nonstirred platelets (Fig. 7). The data indicate that $\alpha_{IIb}\beta_3$ outside-in signals modulate platelet actin dynamics by decreasing the F-actin content and/or increasing filament turnover.

DISCUSSION

Signaling to actin assembly and shape change in platelets is unique. A first set of signals is sent into the cytosol to increase the intracellular calcium concentration and activate gelsolin, which severs the actin filaments and caps their barbed ends. A new production of polyphosphoinositides downstream of the small GTPase Rac uncaps the barbed ends, on which actin polymerization occurs and is further amplified by the Arp2/3 complex (12, 18, 19, 38). Signals are also generated to convert the fibrinogen receptor, the integrin $\alpha_{IIb}\beta_3$, into its active, fibrinogen bound form. Once engaged, $\alpha_{IIb}\beta_3$ generates a second round of signals that modulates the platelet actin assembly reaction (27, 28). In the present study, we have found that cofilin, a protein that facilitates actin turnover in cells, is under the control of both signaling pathways. Signals from the thrombin receptors are responsible for an initial dephosphorylation of cofilin, as first reported by Davidson and Haslam (8), and outside-in $\alpha_{IIb}\beta_3$ signals function to maintain cofilin in its active, dephosphorylated state.

Platelets contain 28 μM cofilin, 5% of the estimated molar concentration of actin. We showed that ~90% of cofilin is phosphorylated on Ser3 in resting platelets, suggesting a slow turnover of actin filaments in circulating platelets. This extent

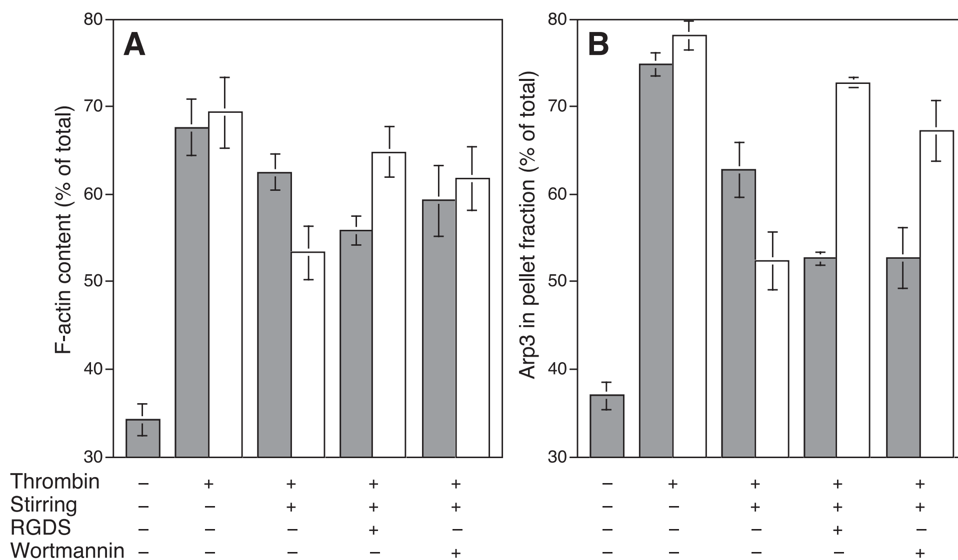

Fig. 7. $\alpha_{IIb}\beta_3$ signals accelerate platelet actin dynamics. Platelets were preincubated or not with 300 μg/ml RGDS peptide or 50 nM wortmannin and stimulated with 1 U/ml thrombin over 1 (gray bars) or 5 (open bars) min at 37°C under stirring or nonstirring conditions as indicated. The amount of actin (*A*) and Arp2/3 complex (*B*) in the cytoskeletal fraction relative to total was quantified as in Fig. 6.

of phosphorylation is much greater than that reported earlier (8). The most likely explanation for this difference is the metabolic radiolabeling approach used in the earlier study. The rate of cofilin phosphorylation/dephosphorylation in resting platelets may be slow to maintain cofilin in an inactive, phosphorylated state. Nevertheless, the rate of phosphorylation exceeds dephosphorylation in the resting platelet. After platelet stimulation by thrombin, cofilin dephosphorylation is rapid, reaching a maximal extent of 75% in 1–2 min. Cofilin dephosphorylation parallels its incorporation into F-actin but temporally follows the peak of maximal actin filament assembly. These findings indicate that in platelets, cofilin activity is not primarily involved in the initial boost of actin assembly, a process dominated by calcium-activated gelsolin, but accelerates the turnover of actin filaments under the control of $\alpha_{IIb}\beta_3$.

Our findings show that cofilin becomes maximally rephosphorylated at Ser3 2 min after stimulation with thrombin under nonaggregating conditions, exceeding resting levels by 5–10 min. However, cofilin activity is extended by signals coming from the $\alpha_{IIb}\beta_3$ integrin. When $\alpha_{IIb}\beta_3$ signaling is disrupted by RGDS peptide or wortmannin, cofilin is rapidly rephosphorylated. This finding was directly confirmed by using platelets isolated from a patient with Glanzmann thrombasthenia, which express only 2–3% of normal $\alpha_{IIb}\beta_3$ levels and failed to delay cofilin rephosphorylation. Cofilin phosphorylation is effected by LIM kinase and can be reversed by the Slingshot phosphatase (1, 10, 26, 37, 39). Rho and its downstream effector, Rho-activated kinase, are upstream in the LIM kinase activation pathway, and Rac has been shown to link to LIM kinase through p21-activated kinase (10). Both Rac and Rho are activated during thrombin stimulation (2, 16, 23), and such activation would be expected to lead to cofilin phosphorylation and inactivation in platelets. LIM kinase is expressed in megakaryocytes and is likely to be responsible for cofilin phosphorylation in platelets (22).

Our findings also indicate that $\alpha_{IIb}\beta_3$ signals modulate cytoskeletal remodeling and actin turnover and decrease stable incorporation of actin and the Arp2/3 complex in the cytoskeleton. Cofilin, maintained in its active, dephosphorylated form in platelets stimulated under aggregating conditions, is likely to be intimately involved in these events. Active, dephosphorylated cofilin binds ADP-bound actin subunits, severs actin filaments, and releases actin subunits from the pointed ends of filaments, which can then be recycled by profilin and β_4-thymosin for new rounds of polymerization (6, 15, 33). Integrin signaling is usually associated with the formation of focal adhesions and stable actin stress fibers. The activation of cofilin after $\alpha_{IIb}\beta_3$ engagement implies that actin filament dynamics are essential for these events. To form stable adhesion sites, platelets may need to recycle actin filaments. Previous studies have suggested that $\alpha_{IIb}\beta_3$ regulates the assembly of actin filaments, not their turnover (13, 14). The most likely explanation for the discrepancy is the lysis buffers used in the earlier experiments, which do not contain enough calcium chelators to block gelsolin severing activity.

In summary, we have characterized the kinetics of cofilin activation/inactivation as platelets convert from their resting to their activated shape. Cofilin activity is under the control of both the initial trigger of activation and the integrin $\alpha_{IIb}\beta_3$. Our findings suggest that cofilin activity is not essential in platelets for the initial polymerization of actin filaments that follows stimulation and leads to shape change, but for actin remodeling mediated by outside-in signals. It remains to be determined exactly how increased actin filament dynamics contribute to platelet spreading and function. Presumably, actin filament turnover is required to continue platelet spreading on the subendothelium to optimally cover breaches in the vascular wall.

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