RESEARCH ON THE ROLE of phosphoinositides in cellular signaling accelerated significantly after Michell’s seminal review and has been expanding ever since. Metabolic studies on human platelets have been no exception to this trend. Two of the most important research fronts today for work on signal transduction in platelets concern the regulation of phosphoinositidase C (PI3-K), which has been reviewed extensively, and the factors governing the metabolism of 3-OH-phosphorylated phosphoinositides (3-PPIs), products of the action of phosphoinositide 3-kinases (PI3-Ks). Research in the former area has led to a wealth of information on the regulation of increases in cytosolic Ca2+ and activation of protein kinase C promoting physiologic events such as secretion in response to stimuli such as thrombin, thromboxane A2, collagen, and platelet activating factor. Recent developments in the latter area, particularly with respect to the function(s) of 3-PPIs, are such that it is now possible to review a relatively coherent body of work on platelets and, hopefully, provide inspiration for additional experimental approaches. The focus of this review, therefore, will be on 3-PPIs and PI3-Ks. Hopefully, it will be clear from the results to be described that PI3-K activity plays a role in regulating an important platelet function, ie, aggregation, and is also, in turn, affected by this function. It is likely that PI3-Ks are involved in other events in platelets and platelet progenitors as well. The structures of the 3-PPIs, which are quantitatively a minor subspecies of the phosphoinositide lipid family, along with those of the better-known isomers, are shown in Fig 1. The reader interested in additional information on 3-PPIs and PI3-Ks in a variety of cell types should be alerted to two detailed and thoughtful surveys, in the tradition of Michell’s review of 1975, by Stephens et al and Fry.

Activated platelets display cytoskeletal reorganization, secretion, and integrin-dependent events that, as noted above, increasing evidence links to alterations in the metabolism of inositol-containing species, including 3-PPIs. Platelets, being anucleate, offer a useful model system for dissecting connections among receptor occupancy, metabolism of inositides, and morphologic changes uncomplicated by variations due to protein synthesis. They also can be obtained in quantity, with a good degree of homogeneity of cell type, for metabolic studies. However, unfortunately, because of limited availability of fresh human platelets (protein degradation being a significant risk for outdated platelets), human platelets have not been the system of choice for enzyme purification, and most kinetic and regulatory studies have not been undertaken with purified platelet enzymes. Platelets also significantly frustrate any molecular biologist longing to examine the effects of altered protein expression on metabolic and morphologic events, studies that may be predicated on techniques that allow massive culturing of human megakaryocytes and induced spawning of platelets as well as gene knock-out studies with mice. In an attempt, ultimately, to address issues of 3-PPI regulation at the level of molecular biology, using sufficient numbers of cells for metabolic studies, we have undertaken some work with a leukemic cell line, CHRF-288, derived from megakaryoblasts. These cells, having morphologic and cytochemical features of megakaryoblasts and immature megakaryocytes, were described initially by Witte et al and suspension culturing was reported by Fugman et al. Unlike K562 or HEL, CHRF-288 cells do not express myeloid or erythroid markers. Platelet-like characteristics include a high degree of expression of the integrin αIIβ3, which is necessary for fibrinogen-linked platelet aggregation. For reasons yet to be defined, CHRF-288 cells in suspension do not aggregate in response to thrombin + fibrinogen, but they are responsive in other ways (including secretion) to thrombin and to some agonists that cause platelet aggregation. Experimental results with these and HEL cells will be reviewed here as well. However, the majority of work that will be described has been performed with intact or permeabilized platelets or platelet subfractions, rather than with purified components in reconstitution experiments, and is limited by this constraint. Nevertheless, even...
with such limitations, studies with platelets have afforded insights into stimulus-response coupling, with applications that go beyond platelets themselves.

**SOME HISTORY**

The identification via deacylation and high-performance liquid chromatography (HPLC) analysis of a new phosphorylated phosphoinositide was made by Whitman et al. The compound was characterized further and was found to be a species phosphorylated by a detergent-inhibitable kinase at the D-3 hydroxy position of the inositol ring of phosphatidylinositol. Two additional members of this group were identified in chemotactic peptide (f-met-leu-phe)-stimulated neutrophils as PtdIns(3,4,5)P3 and PtdIns(3,4)P2. A distinctive aspect of these 3-PPIs is that they are very poor substrates for the PTKs described to date. That the 3-PPIs are of relevance to signaling events was implied by early findings that their synthesis are more specifically, that of PtdIns(3,4,5)P3 and PtdIns(3,4)P2, appears to be required in cells other than neutrophils and platelets for cell transformation or growth stimulation in response to mitogenic polyoma virus middle T antigen (mT) or platelet-derived growth factor (PDGF). In these cases, the responsible kinase, PI 3-K, is found complexed in a phosphotyrosine-dependent manner, via the phosphotyrosine-binding src-homology 2 (SH2) domains of a subunit of one form of PI 3-K, to mT/ pp60 c-src or to the receptor tyrosine kinase, PDGFR, respectively. Of interest as well is the observation that the mT complex is associated with cytoskeletal focal contacts (the anchor points for actin stress fibers) and can be immunoprecipitated with anti-vinculin serum. Because cytoskeletal reorganization is one of the earliest changes observed in polyoma-infected cells or in neutrophils exposed to chemotactic peptide, a possible signaling target for the 3-PPIs has been thought to be the cytoskeleton. The platelet, of course, is another example of a cell that undergoes rapid reorganization of the cytoskeletal apparatus upon stimulation by physiologic agonists such as thrombin, even if a proliferative response is not possible. It therefore seemed worthwhile to test whether platelets were capable of generating 3-PPIs when stimulated.

**ACCUMULATION OF 3-PPIs IN PLATELETS AND RELATED CELL LINES**

Platelets, indeed, form PtdIns(3,4)P2 in response to thrombin. Platelets also accumulate PtdIns(3,4,5)P3; furthermore, both PtdIns(3,4)P2 and PtdIns(3,4,5)P3 increase in response to the thromboxane receptor agonist U46619 or in permeabilized platelets, GTPγS. The use of an increase in PtdIns(3,4,5)P3 and PtdIns(3,4)P2 as an indication of a change in mass has been validated for U46619-stimulated platelets by mass measurements, and PtdIns(3,4)P2 has been found to have the same distinctive fatty acid profile as that of the other phosphoinositides. The mass of PtdIns(3,4,5)P3 that accumulates in platelets exposed to thrombin has not been quantified directly, but, based on the amount formed in equilibrium labeling experiments with P32P, one can estimate it to be about 1% of the resting mass of PtdIns(4,5)P2 or 8 to 10 pmol/10^9 platelets. Most of the experiments in our laboratory have been undertaken using platelets exposed to aspirin before washing to eliminate effects secondary to thromboxane A2 generation. The elevation in PtdIns(3,4,5)P3 precedes that in PtdIns(3,4)P2. No significant changes in PtdIns(3)P are observed in intact platelets incubated with platelet agonists, and permeabilized platelets show only small increases in PtdIns(3)P, which are minor in comparison with the increases seen in the more highly phosphorylated 3-PPIs. Stimulation of 3-PPI accumulation can also be achieved with the thrombin receptor tethered ligand peptides, SFLLRNPNDKYPEFP or SFLLRN, but it is not a consequence of PDGF secretion, because no form of PDGF accumulation is able to elicit this effect or to potentiate the effects of thrombin. Other agonists tested thus far that increase 3-PPI accumulation in platelets are lysosphosphatic acid (LPA) and adenosine diphosphate (ADP). Each is effective, even when added to aspirin-treated platelets, although they are much weaker agonists than is thrombin. CHRF-288 cells also respond to thrombin receptor stimulation (thrombin or SFLLRN) or exposure to LPA or serum with rapid and highly transient accumulations of PtdIns(3,4,5)P3 and PtdIns(3,4)P2. Similarly, an erythroleukemic cell line (HEL) that shares some characteristics of platelets and megakaryocytes accumulates PtdIns(3,4)P2 in response to thrombin.

**ROUTES OF SYNTHESIS**

Routes for the synthesis and subsequent metabolism of the phosphoinositides are shown in Fig 2 where known and potential enzymatic activities are indicated by the solid arrows and broken arrows, respectively. PI 3-K activities capable of using PtdIns, PtdIns(4)P, and PtdIns(4,5)P2 have been described in fractionated platelets, as has a PtdIns(3)P 4-kinase activity. This 4-kinase activity, in contrast to previously described PtdIns 4-kinase activities, is inhibited by detergents such as Nonidet P-40 and is present in both pellet and cytosolic fractions from platelet homogenates, with the cytosolic entity having an apparent molecular weight of 150 kD. Unfortunately, the relative activities of PtdIns(4)P 3-kinase and PtdIns(3)P 4-kinase have not been compared under the same conditions. In addition, two forms of inositol polyphosphate 3-phosphatase in brain extracts have been described that have the unusual property of hydrolyzing both Ins(1,3)P2 and PtdIns(3)P, and two PtdIns(3,4,5)P3-hydrolyzing 5-phosphatases are present in platelets. However, thus far, no PtdIns(3,4)P2 5-phosphatase has been reported for any cell. This fact is important to consider when evaluating the potential pathways for synthesis of PtdIns(3,4,5)P3. Finally, PtdIns(3,5)P2, although separable from PtdIns(3,4)P2 and PtdIns(4,5)P2 on the HPLC systems used, has not been reported to accumulate in response to platelet agonists and we have not seen such changes.

There is at present some controversy in the literature about the routes by which PtdIns(3,4)P2 and PtdIns(3,4,5)P3 are formed in stimulated cells. Stephens et al, working with f-met-leu-phe–stimulated neutrophils, and Hawkins et al, studying PDGF-stimulated Swiss 3T3 fibroblasts, have concluded that PtdIns(3,4,5)P3 is generated by PI 3-K acting
on PtdIns(4,5)P₂. They attributed the delayed generation of PtdIns(3,4)P₂ either to 5-phosphatase–mediated hydrolysis of PtdIns(3,4,5)P₃, or to direct action of PI 3-K on PtdIns(4)P, although the former route was favored on the grounds that such a 5-phosphatase exists and that PtdIns(3,4,5)P₃ might be the true intracellular signal. Therefore, hydrolysis by 5-phosphatase would therefore be a means of its disposal. In contrast, Cunningham et al¹⁹ and Cunningham and Majerus,³⁸ based on experiments with thrombin-stimulated platelets and PDGF-stimulated NIH 3T3 fibroblasts, respectively, reported PtdIns(3)P + PtdIns(3,4)P₂ + PtdIns(3,4,5)P₃ to be the route of synthesis. For the system in which there would seem to be an overlap between the two laboratories, ie, PDGF-stimulated 3T3 cells, there were several procedural differences. Hawkins et al³⁷ used 16-hour serum-starved Swiss 3T3 cells that were labeled with ³²P for 30 minutes before PDGF addition to be assured of nonequilibrium labeling of the major phosphoinositide species, with equilibrium being achieved after 70 minutes. Incubations were quenched with CHCl₃/MeOH. A time course study to monitor the appearance of labeled PtdIns(3,4,5)P₃ and PtdIns(3,4)P₂ in response to 30 ng/mL PDGF showed that PtdIns(3,4,5)P₃ was formed very rapidly, achieving a maximum level within 60 seconds, whereas the appearance of PtdIns(3,4)P₂ was delayed by about 20 seconds. Incubations with PDGF to generate products for digestive analysis were performed for 35 seconds. The products were rigorously identified. Because, under nonequilibrium labeling conditions, the hottest phosphate is the last phosphate added to the inositol ring, determinations of the specific activity of the phosphates in the various positions of the inositol ring yield information about which kinase is acting on which substrate. The analyses showed that the percentage of distribution of ³²P in each position of PtdIns(3,4,5)P₃ was #1 = 0.45%; #3 = 41.3%; #4 = 26.0%; and #5 = 32.0%. Because PtdIns(4,5)P₂ is
formed by 5-kinase acting on PtdIns(4)P and the phosphodiester (1) phosphate position would be labeled only slowly by de novo or by PI cycle (Fig 3, pathway 7) synthesis, one must conclude from these data that PI 3-K acts on PtdIns(4,5)P2 to yield PtdIns(3,4,5)P3. Cunningham and Majerus31 used NIH 3T3 cells grown overnight in 0.5% fetal calf serum. The cells were washed and labeled with 32Pi for 60 minutes at 37°C. Incubations with 50 ng/mL PDGF were terminated after 5 minutes with 1N HCl. It was not reported how closely the 60-minute labeling period approached that for equilibrium, but if these cells are similar to Swiss 3T3 cells, one would expect equilibrium to be virtually achieved, and therefore minimal differences detectable for positions 3, 4, and 5. Nevertheless, positional labeling differences were observed. Time-course data for the production of PtdIns(3,4,5)P3 and PtdIns(3,4)P2 in 3T3 cells responding to PDGF were not provided in this study. Positional analyses of the phosphates on Ins(1,3,4,5)P4 derived from PtdIns(3,4,5)P3 yielded an average distribution in two experiments of #1 = 2.5%; #3 = 24.5%; #4 = 31.5%; and #5 = 41.5%. One would conclude from these studies that PtdIns(3)P → PtdIns(3,4)P2 → PtdIns(3,4,5)P3. The observations of Stephens et al10 and Hawkins et al13 that the increases in labeled PtdIns(3,4,5)P3 precede those in labeled PtdIns(3,4)P2 and that labeled PtdIns(3)P does not increase significantly in the period preceding (or during) that in which PtdIns(3,4,5)P3 and PtdIns(3,4)P2 levels increase would both tend to favor the route of a stimulated PI 3-K acting on PtdIns(4,5)P2, and activation of PI 3-K showing substrate preference for PtdIns(4,5)P2 has been observed in several stimulated cell types. All of this would be difficult to reconcile with PtdIns(3)P 4-kinase activity using a cold, endogenous pool of PtdIns(3)P.

In light of the opposing conclusions about the route for synthesis of PtdIns(3,4,5)P3 [and PtdIns(3,4)P2] described above, we considered it important to re-examine the platelet system. Instead of incubating platelets for 60 minutes with 32P, we and others have found that equilibrium labeling is achieved by 60 to 90 minutes), we have exposed platelets to high amounts of 32P, for 10 minutes and incubated the platelets immediately with phosphate-free thrombin for 20 or 60 seconds, terminating the incubations with CHCl3/MeOH/.
HCl, rather than with 1 mol/L HCl and cooling. It is known that thrombin accelerates uptake of Pi by fivefold to 10-fold, and we reasoned that newly taken up $^{32}$Pi would have that thrombin accelerates uptake of Pi by fivefold to 10-

HCl, rather than with a specific protein kinase activity that can phosphorylate the p85 subunit, resulting in inhibition of p85/PI 3-K activity. Antibodies to the $\alpha + \beta$-p85 subunits immunoprecipitate PI 3-K activity due to the associated p110 subunits. Although in resting platelets most PI 3-K is Triton-soluble, a small amount of p85/PI 3-K is associated with the cytoskeleton. In the activated platelet, more p85/PI 3-K (both $\alpha$ and $\beta$) shifts to the cytoskeleton and is sedimentable in Triton buffers at 15,000g for 4 minutes. This cytoskeletal PI 3-K is more active (enzymatic activity/immune-quantifiable enzyme) than either the resting or activated-platelet PI 3-K that remains Triton-soluble. Interference by cytochalasin D with actin polymerization does not block the association of PI 3-K (detected by Western blot or activity) with the activated platelet cytoskeleton; however, treatment of the Triton lystate with DNase I strongly decreases the Triton-insolubility of PI 3-K (as well as actin). We have concluded, therefore, that activated PI 3-K is associated with a component of the membrane skeleton, rather than with newly polymerized actin. We have observed as well that interference by arg-gly-asp-ser (RGDS) with fibrinogen binding to $\alpha_{IIb} \beta_{3}$ impairs recruitment of p85/PI 3-K to the cytoskeleton, implying a role for integrin in at least part of this process. Additional components of the membrane skeleton in the activated platelet include p60* (a tyrosine kinase that itself becomes tyrosine-phosphorylated and more active), $\alpha_{IIb} \beta_{3}$,vinculin, $\alpha$-PKC, and $\epsilon$-PKC. It has also been reported that thrombin induces an association of p85/PI 3-K with p60* and p60* (another, related, tyrosine kinase) in platelets. Because these studies were performed by immunoprecipitating phospho-tyrosine and src/fyn-containing proteins from centrifugally clarified detergent lysates of platelets, it is most likely that (1) the bulk of activated PI 3-K activity had already been removed during centrifugation, which would sediment most of the cytoskeleton and leave smaller complexes in the supernatant; and (2) several components were present in the immunoprecipitates, including not only the two tyrosine-phosphorylated entities (p60* and p60*) and PI 3-K, but also other proteins associated with a light cytoskeletal matrix. Thus, it is not possible to conclude from these data that PI 3-K is bound directly to either tyrosine kinase(s) or a tyrosine-phosphorylated protein or that PI 3-K is itself tyrosine-phosphorylated. Other data, in fact, indicate that p85 of PI 3-K is not phosphorylated at tyrosine in activated platelets. Determinations of the direct binding site(s) for p85/PI 3-K at the membrane skeleton and the mechanism of initial PI 3-K activation await further studies. Some additional information relevant to the possible mechanism of these events is provided by studies from Shibasaki et al. and Guinebault et al. The former lab has observed that p85/PI 3-K binds to $\alpha$-actin (present in platelet cytoskeleton, cross-linking actin) via the p85-subunit, and the latter group has reported that p85/PI 3-K can be activated
directly by interactions between the SH3 domain of p85 and a proline-rich region of p125Syk. FAK (focal adhesion kinase) is a tyrosine kinase that localizes with integrins at the cytoskeleton via a C-proximal focal adhesion targeting (FAT) sequence and an N-proximal sequence that binds to the cytoplasmic domains of β-integrins. In platelets, activation (and tyrosine-phosphorylation) of FAK is stimulated by integrin cross-linking in a manner dependent on PCK activation. Another candidate for p85/PI 3-K targeting is the tyrosine kinase p72^tyk^, which is activated and tyrosine-phosphorylated in platelets that are exposed to thrombin, collagen, TXA^2^, or the low-affinity Fcγ receptor (FcγRIIA). A portion of p72^tyk^ activation is insensitive to blockage of integrin function, but much of p72^tyk^ is phosphorylated/activated soon after agonist-induced or anti-LIBS Fab-induced binding of fibrinogen to αmβ^3^.

In addition to stimulation of platelets via the thrombin receptor (which leads to activation of αmβ^3^ and aggregation), stimulation of the adhesion receptor GpIIb/IX by von Willebrand factor (vWF) leads to increased localization of p85/PI 3-K as well as tyrosine kinase p60^tyk^ and (judging by the mobility of tyrosine-phosphorylated proteins indicated) possibly p72^tyk^ at the cytoskeleton in an αmβ^3^-independent manner. Such recruitment is unaffected by exposure of the platelets to aspirin (and therefore prevention of TXA^2^ formation), although aspirin abolishes vWF-induced dense granule (and presumably ADP) secretion and phosphorylation of p47^tyk^ (ie, plectrin, a target of protein kinase C activity). However, as is true for FcγRIIA activation, it has not been reported whether vWF/ristocetin per se actually causes an increase in the levels of PtdIns(3,4,5)P^3^ and/or PtdIns(3,4)P^2^ in intact platelets.

Recently, a new form of PI 3-K has been described after cloning and expression studies. The molecular weight of this catalytic species (p110^γ^) is also approximately 110 kD, but it lacks a binding site for p85 subunits and, instead, contains an N-terminal pleckstrin homology (PH) domain. Most interestingly, it is activated by βγ subunits of heterotrimeric G-proteins. Using reverse transcriptase-polymerase chain reaction and degenerate oligonucleotide primers to the kinase domain of p110 catalytic subunits of PI 3-K, we have detected p110^γ^ in CHRF-288 mRNA (Rittenhouse and Volinia, unpublished results). βγ-activatable PI 3-K activity and immunoreactive p110^γ^ have been observed for human neutrophils, platelets, and CHRF-288 cells, and PI 3-K has been purified from porcine platelet lysates. We have observed that both immunologically detectable and βγ-activatable PI 3-K (designated PI 3-K^γ^) appears in the cytoskeleton of thrombin-stimulated platelets and is not present in the cytoskeleton of resting platelets. Under resting conditions, it is Triton-soluble and not immunoprecipitated by antibodies to α-p85 or β-p85. Activated PI 3-K^γ^ is associated with βγ, and its activity can be inhibited by binding βγ with the PH domain-containing C-terminal peptide of a β-adrenergic receptor kinase (βARK-PH) lacking protein kinase activity. Like p85/PI 3-K, PI 3-K^γ^ uses all three phosphoinositide substrates but displays a preference for PtdIns(4,5)P^3^.

Because activated PI 3-Ks localize to the membrane skeleton, the products of PI 3-K activity should also be localized in membrane skeleton-proximal regions of the plasma membrane. Thus, a 3-PPI such as PtdIns(3,4,5)P^3^, would be concentrated in a position with increased potential for affecting cytoskeletal organization at membrane linkages, perhaps by modifying a nearby enzyme activity. This is, at least, consistent with the possibility that PtdIns(3,4,5)P^3^ and PtdIns(3,4)P^2^ act in part to affect cytoskeletal organization.

Role of PKC. The increase in 3-PPI levels that follows activation of the thrombin receptor does not seem to be simply a consequence of PKC activation and/or Ca^2+^ mobilization, ie, the activation of PKC (see Fig 3, pathway 6) is not sufficient to cause the full response. β-Phorbol dibutyrate or β-phorbol myristate acetate (β-PMA), alone or together with Ca^2+^ ionophore, at concentrations that stimulate PKC activity and [Ca^2+^] increases equivalent to those achieved by thrombin, does not produce a similar elevation of 3-PPI levels. Nevertheless, PKC stimulation does play a necessary role in the process. Permeabilized platelets exposed to the pseudosubstrate peptide inhibitor of PKC, RFARKGALR-QKNV, show inhibited accumulation of 3-PPIs in response either to thrombin or GTPγS, although this inhibitor does not affect the activity of purified PI 3-K. Furthermore, only the PKC-activating β-isofrom of PMA stimulates 3-PPI accumulation, and activation of platelets with β-PMA causes a shift of α- and β-p85/PI 3-K to the cytoskeleton. In contrast, PI 3-K activity does not increase in the cytoskeleton of β-PMA-activated platelets. Thus, a portion (that which is due to p85/PI 3-K) of the 3-PPI response to agonists such as thrombin seems likely to be dependent on PKC activity (Fig 4). Of potential relevance here, β-PMA also rapidly stimulates tyrosine phosphorylation in human platelets, although the identities of the tyrosine kinases activated are not clear.

Role of αmβ^3^. There is also a role for the platelet integrin αmβ^3^ in modulating the accumulation of PtdIns(3,4)P^3^ in response to thrombin. This is the species that appears relatively later than PtdIns(3,4,5)P^3^, Inhibition (>80%) of fibrinogen binding to platelet integrin by RGDS inhibits by 40% to 50% the late (5 minutes) thrombin-induced increases in PtdIns(3,4,5)P^3^, and a similar effect is achieved by omitting Ca^2+^ from the medium or when platelets from thrombasthenic (αmβ^3^, deficient) patients are incubated with thrombin. However, RGDS does not inhibit 3-PPI accumulation in platelets exposed to β-PMA, implying that the activation of PI 3-K by PKC is not secondary to fibrinogen binding.
Normal platelets and thrombasthenic platelets, when permeabilized in the presence of 0.1 mmol/L Ca$^{2+}$ and exposed to thrombin or GTP$\gamma$S, moreover, form similar amounts of PtdIns(3,4)P$_2$ and PtdIns(3,4,5)P$_3$, as do intact platelets exposed to thrombin in the absence of added Ca$^{2+}$. These are conditions in which $\alpha_{IIb}\beta_3$ is not activated. Furthermore, the accumulation of PtdIns(3,4,5)P$_3$ and PtdIns(3,4)P$_2$ induced by exposure of platelets to LPA for 60 seconds is not affected by the presence of the disintegrin eristostatin, which very effectively blocks platelet aggregation. Platelet integrin may thus modulate the activity of a PtdIns(3,4)P$_2$ phosphatase, PtdIns(3)P 4-kinase, and/or a PI 3-K, but is not necessary for the initial activation of PI 3-K. An effect on PtdIns(3,4,5)P$_3$ 5-phosphatase is not a sufficient explanation, because PtdIns(3,4,5)P$_3$ accumulations do not appear to be increased by such treatments. Interestingly, aggregation of platelets by thrombin leads to the dissociation of PtdIns(3,4,5)P$_3$ 5-phosphatase from p85/PI 3-K at the platelet.
let cytoskeleton. Were the activity of this phosphatase a determining factor, one might have predicted that aggregation would lead to less formation of PtdIns(3,4)P$_2$, rather than more. Obviously, this indicates that we cannot be sure how much of a predictor the cytoskeletal localization of catalyzing enzymes is with respect to 3-PPI accumulations, but it would appear that factors in addition to 3-phosphatase affect PtdIns(3,4)P$_2$ levels.

Conflicting findings have been published about the activity of fibrinogen binding to $\alpha_{\text{Ib}}\beta_3$ (and of platelet aggregation) to activate 3-PPI accumulation (ie, outside-in signaling) in the absence of the participation of another signal, such as stimulation of PKC by TXA$_2$ or ADP. Kovacs et al. have reported that promoting fibrinogen binding to $\alpha_{\text{Ib}}\beta_3$ induced by an $\alpha_{\text{Ib}}\beta_3$-directed antibody Fab fragment (ligand-induced binding site via anti-LIBS-Fab) leads to a dramatic increase in platelet PtdIns(3,4)P$_2$. In contrast, we have observed only a small (2X) change in the amount of platelet PtdIns(3,4)P$_2$ after exposure to LIBS-Fab + fibrinogen, no change in PtdIns(3,4,5)P$_3$, and a small (1.4X) increase in PtdIns(4,5)P$_2$. Thrombin receptor stimulation can cause up to a 20X increase in PtdIns(3,4)P$_2$ that is 50% inhibitable by blocking fibrinogen binding. An explanation for the discrepancy between the findings from the two labs may lie in our use of aspirin and ayparase or CP/CPK to block the receptor-activating effects of cyclooxygenase products (eg, TXA$_2$) and ADP, respectively, whereas the results of Kovacs et al. may include the potentiating effects of TXA$_2$ + ADP. ADP can cause a 2X increase in PtdIns(3,4)P$_2$ and the activating effects of TXA$_2$ analogues have been described. We suspect that, as is true for the tyrosine-phosphorylation of p72$\text{Tyk}$, $\alpha_{\text{IIb}}\alpha$, and p125$\text{FAK}$, binding of fibrinogen by platelets is a necessary but not sufficient condition for the full response of PtdIns(3,4)P$_2$ accumulation.

Role of GTP-binding proteins. The implication of the stimulatory effects of GTP$\gamma$S (but not of GDP$\beta$S) is that 3-PPI accumulation is under the control of GTP-binding protein(s). If there is one major G-protein responsible for the changes seen, it is apparently not of the G$_{\alpha}$ subclass that regulates adenyl cyclase, because inactivation of G$_{\alpha}$ by pertussis toxin treatment causes only a minor inhibition of 3-PPI accumulation. Similarly, epinephrine, although able to activate G$_{\alpha}$ via the $\alpha_2$ adrenergic receptor, is neither able to stimulate significant increases in 3-PPIs or potentiate increases in 3-PPIs in conjunction with phorbol diester and Ca$^{2+}$ ionophore. One is still left with a wide variety of potential G-protein candidates for this regulatory function, including G$_{\alpha_i}$, G$_{\alpha}$, and G$_{\alpha}$ in the heterotrimeric class, as well as a full palette of small $G$ proteins. Indeed, both small and heterotrimeric G-proteins (including G$_{\alpha}$) are involved in regulating PI 3-K activation, as will be discussed below.

In the context of cytoskeletal reorganization, increasing attention has focused on the small GTP-binding protein Rho. Rho has been found to regulate the assembly of actin stress fibers and focal adhesions (sites of cytoskeletal interaction with the plasma membrane, bearing some similarity to cytoskeletal associated $\alpha_{\text{Ib}}\beta_3$ in growth factor-stimulated fibroblasts. Such assembly is blocked if Rho is ADP-ribosylated by the exoenzyme C3 transferase from Clostridium botulinum, and the blockage can be overcome by the injection of Rho. Because we have shown that the accumulation of 3-PPIs in permeabilized platelets appears to be under the control of GTP-binding protein(s) other than or in addition to G$_{\alpha}$ and because RhoA is present in platelets and is the sole substrate ADP-ribosylated by C3 transferase, which inhibits thrombin-stimulated platelet aggregation, we tested whether Rho participates in regulating platelet PI 3-K. Such a target would provide a potential mechanism for the effects of Rho on cytoskeletal changes.

In examining cytoskeletal preparations from control and thrombin-activated platelets, we have found that most of Rho is Triton-soluble in platelet controls, but the amount of Rho in the cytoskeletal fraction increases threefold to fourfold within 45 seconds of platelet exposure to thrombin. We have also prepared soluble fractions from unstimulated platelets and have been able to activate PI 3-K with GTP$\gamma$S, but not GDP$\beta$S, in these preparations. The activation is blocked by C3 transferase-catalyzed ADP-ribosylation of Rho and rescued by the addition of recombinant exogenous Rho expressed in and isolated from a bacterial host. Therefore, Rho does not need to be posttranslationally modified. Similarly, we have found that GTP$\gamma$S-stimulated PI 3-K activity in lysates of CHRF-288 cells also is inhibited by ADP-ribosylation of Rho. C3 transferase enters healthy platelets and ADP-ribosylates platelet Rho rather poorly. Some improvement is achieved if platelets are stressed by cold and exposure to Tris buffer, but these are not optimal conditions for examining stimulated 3-PPI accumulation in platelets. Therefore, we have studied permeabilized platelets instead. C3 transferase or another Rho-ADP-ribosylating enzyme, EDIN, also partially inhibits the activation of PI 3-K in thrombin receptor- or GTP$\gamma$S-stimulated platelets. In contrast with findings for some other cells, ADP-ribosylation of Rho does not affect $\alpha_2$P-labeling of platelet PtdIns(4,5)P$_2$, nor does GTP$\gamma$S or ADP-ribosylation of Rho in platelet lysates affect PtdIns(4)P 5-kinase activity. Platelets may thus lack significant amounts of a Rho-sensitive isoform of PtdIns(4)P 5-kinase. We have concluded that platelet PI 3-K is activated, directly or indirectly, by RhoA. One argument in support of the direct route has been that PI 3-K possesses on its p85 subunit not only the SH2 and SH3 domains shared by a variety of proteins, including PIC and GTPase-activating proteins (GAPs), but also the rho/GAP-like domain that has been shown to be necessary for the GTPase-activating functions of rho/GAP. However, studies designed to evaluate the direct interaction between Rho and PI 3-K have indicated no direct binding of Rho to recombinant p85 or activation of recombinant p85/PI 3-K activity. Because Rho is immunoprecipitated from platelet cytosol by p85/PI 3-K-directed antibodies, we suspect that some sort of complex is present involving molecules in addition to p85/PI 3-K and Rho; this possibility is under study. Furthermore, it seems likely that activation of Rho is downstream of PKC activation. Stimulation of p85/PI 3-K activity by exposure of platelets to $\beta$-PMA is inhibited partially by ADP-ribosylation of Rho. This finding is consistent with the observations of Tominaga et al., who have reported that LFA-1–dependent lympho-
cyte aggregation induced by PMA can be inhibited by ADPribosylation of Rho. Thus, two possibly related events downstream of Rho, ie, p85/PI 3-K activation and aggregation, appear to be dependent on PKC activity (Fig 4). The correlation of p85/PI 3-K activation with platelet aggregation will be addressed more directly later in this review.

Whereas Rho participates in the regulation of p85/PI 3-K activity, it does not alter the activity of PI 3-K.\(^1\) Instead, this form of PI 3-K is activated by the $\beta\gamma$ subunits of heterotrimeric G-proteins (Fig 4). Such $\beta\gamma$ subunits dissociate from $\alpha$ subunits when seven-transmembrane domain receptors, such as the thrombin receptor, are occupied by the appropriate ligand. It is now known that the thrombin receptor can activate at least two types of heterotrimeric G-protein: $G_i$ and $G_{12.3}$. We have observed, as noted earlier, that binding of $\beta\gamma$ by exogenous $\beta$ARK-PH prevents activation of PI 3-K by platelet cytoskeletal fractions or supernatants.\(^2\)\(^3\)\(^4\)\(^5\)\(^6\)\(^7\)\(^8\)\(^9\)\(^0\) When added to permeabilized platelets, $\beta$ARK-PH inhibits a portion of thrombin receptor-stimulated 3-PII accumulation.\(^3\)\(^5\) Approximately 60% of thrombin receptor-activated 3-PII accumulation is attributable to PI 3-K, with the remainder apparently a result of Rho-dependent p85/PI 3-K activation.\(^2\)

**Role of pleckstrin.** One of the earliest events observed to follow platelet (and PKC) activation in response to a variety of agonists is the phosphorylation of pleckstrin (also designated p40 or p47). Pleckstrin is a cytosolic protein that contains two different PH domains separated by a 150-residue peptide.\(^1\)\(^2\)\(^3\)\(^4\) The phosphorylation sites are at Ser\(^13\), Thr\(^114\), and Ser\(^117\) of the interdomain region.\(^5\)\(^6\)\(^7\) PH domains have been reported to interact both with PtdIns(4,5)P\(_2\) and $\beta\gamma$.\(^8\)\(^9\)\(^0\) We have examined the effects of recombiant and endogenous pleckstrins on platelet PI 3-K and p85/PI 3-K activities and found that, depending on its phosphorylation/charged state, pleckstrin inhibits PI 3-K but not p85/PI 3-K.\(^8\)\(^9\)\(^1\) Phospho-pleckstrin–mediated inhibition of PI 3-K is overcame by excess $\beta\gamma$ and is restricted to PtdIns(4,5)P\(_2\), as substrate, ie, pleckstrin does not inhibit phosphorylation of PtdIns(4)P or PtdIns by PI 3-K.\(^8\)\(^9\)\(^1\) It thus seems likely that an interaction of phospho-pleckstrin with PtdIns(4,5)P\(_2\) promotes the binding of $\beta\gamma$ by phospho-pleckstrin. Consistent with this, activation of PKC by exposure of platelets to $\beta$-PMA before lysis (to increase endogenous pleckstrin phosphorylation) causes inhibition of soluble $\beta\gamma$-stimulable PI 3-K activity only when assayed with PtdIns(4,5)P\(_2\) substrate. As noted above for purified PI 3-K and exogenous phospho-pleckstrin, inhibition is overcome by increasing concentrations of $\beta\gamma$. Furthermore, we have observed that treatment of platelets with $\beta$-PMA before exposure to the thrombin receptor agonist SFLLRN selectively inhibits PI 3-K activity in intact platelets.\(^8\)\(^9\)\(^1\)\(^0\) As will be seen below, this appears to constitute a form of feedback inhibition (Fig 4), because 3-PII, like PIC–generated diacylglycerol (Figs 3 and 4), can promote pleckstrin phosphorylation by stimulating protein kinase activity.\(^2\)\(^4\)\(^9\)\(^0\) This is, in a way, analogous to another feedback phenomenon recently proposed,\(^9\) based on experiments in which p85/PI 3-K association with a tyrosine-phosphorylated membrane receptor (and therefore, presumably, access to substrate) was inhibited by PtdIns(3,4,5)P\(_3\) through competition of PtdIns(3,4,5)P\(_3\) for the SH2 domains of p85. However, it is not clear whether a feedback event like this for p85/PI 3-K occurs in platelets. It is also not known whether p85/PI 3-K activity in platelets in vivo is regulated by the interaction of p85/SH2s with phospho-tyroisines.

**EFFECTS OF PI 3-K ACTIVATION AND 3-PIPs**

**Phosphorylation of pleckstrin.** We and others have reported that the phosphorylation of pleckstrin is partially dependent on PI 3-K.\(^2\)\(^4\)\(^9\)\(^0\) Pleckstrin phosphorylation in response to thrombin receptor stimulation is progressively susceptible to inhibition by wortmannin, a potent and specific inhibitor of platelet PI 3-Ks (both p85/PI 3-K and PI 3-K), blocking both the lipid kinase and intrinsic protein kinase activities of this enzyme (see below). Wortmannin achieves 50% to 60% inhibition of pleckstrin phosphorylation 60 seconds after exposure of platelets to SFLLRN, whereas pleckstrin phosphorylation reaches maximum levels within 10 seconds of exposure to SFLLRN. PI 3-K thus seems to play a gradually increasing role in promoting pleckstrin phosphorylation. The IC\(_{50}\) for wortmannin in inhibiting SFLLRN-stimulated 3-phosphorylated phosphoinositide accumulation is 10 nM.\(^2\)\(^4\)\(^1\) and that for inhibiting pleckstrin phosphorylation is similar. Synthetic PtdIns(3,4,5)P\(_3\), when added to saponin-permeabilized (but not intact) platelets, causes wortmannin-insensitive phosphorylation of pleckstrin. PtdIns(3,4,5)P\(_3\) also overcomes the inhibition by wortmannin of thrombin- or GTP\(_\gamma\)S-stimulated pleckstrin phosphorylation.\(^2\)\(^4\)\(^1\) In contrast, PtdIns(4,5)P\(_2\) or Ins(1,3,4,5)P\(_4\) is ineffective in these respects. The pattern of phosphorylation of pleckstrin activated by PtdIns(3,4,5)P\(_3\) is not distinguishable from that of pleckstrin phosphorylated in intact platelets exposed to PKC-activating $\beta$-PMA. These findings implicate a PtdIns(3,4,5)P\(_3\)-activated protein kinase in pleckstrin phosphorylation, rather than the intrinsic protein kinase activity of PI 3-K. Our findings have been confirmed and extended to PtdIns(3,4)P\(_2\), which has also been found to promote pleckstrin phosphorylation, but appears to be less potent than PtdIns(3,4,5)P\(_3\).\(^9\)\(^0\) Several members of the PKC family have been reported recently to be stimulated by PtdIns(3,4,5)P\(_3\), including a mixture of rat brain PKC isozymes,\(^9\) PKC\(_\gamma\),\(^2\) and (by either PtdIns(3,4,5)P\(_3\) or PtdIns(3,4)P\(_2\)) PKCe, \(\delta\), and \(\eta\). Other protein kinases may also be stimulated. The phosphorylation of pleckstrin in response to PtdIns(3,4,5)P\(_3\)/PtdIns(3,4)P\(_2\) (Fig 4) thus offers an alternate route to that provided by the activation of PFC, formation of diacylglycerol, and activation of PKC (Fig 3, pathway 6; and Fig 4). This may serve a redundant function, allowing, eg, a PIC deficiency to have less severe consequences for protein phosphorylation under certain circumstances; it may affect the duration of the PKC response, permitting a more sustained activation after diacylglycerol is metabolized; or it may be relevant to the localization of PKC activation. Furthermore, the localized phosphorylation of pleckstrin, near activated PI 3-K, may cause a feedback inhibition of PI 3-K–mediated production of PtdIns(3,4,5)P\(_3\). PtdIns(3,4,5)P\(_3\) formed by PI 3-K also potentially could compete with phospho-tyroisines for p85/PI 3-K and, in this setting, inhibition of PI 3-K by phospho-pleckstrin might relieve the inhibition by Ptf(3,4,5)P\(_3\) (due to
PI 3-Ky) of p85/PI3K. Our preliminary studies indicate that this is likely.65

Activation of αIIbβ3. Before its recognition as a PI 3-K inhibitor, wortmannin was described as an inhibitor of β2-PMA—induced platelet aggregation.66 Furthermore, p85/PI 3-K in neutrophils has been reported to be more sensitive to wortmannin than is PI 3-Ky.66 Because we have been interested in the role of the two PI 3-Ks in regulating the conversion of the platelet integrin αIIbβ3 during platelet activation to a form that is competent to bind fibrinogen,27,28 a process often referred to as inside-out signaling, we investigated the relative inhibitory effects of wortmannin on αIIbβ3 activation induced by stimulation of the thrombin receptor or by β2-PMA.27 As a control, we studied the effects of wortmannin on induction of αIIbβ3 binding function by anti-LIBS6 Fab (outside-in signaling). We observed that wortmannin (up to 100 nmol/L) does not inhibit the synthesis in intact platelets of PtdIns(4)P, PtdIns(4,5)P2, PtdOH, or the activation of PIC,27 whereas 100 nmol/L wortmannin completely inhibits 3-PPI accumulation in response either to thrombin or to β2-PMA. 3-PMA—stimulated 3-PPI accumulation in platelets is more sensitive to wortmannin (IC50 = 1.3 nmol/L) than is SFLLRN— or thrombin-stimulated 3-PPI accumulation (IC50 = 10 nmol/L), and the activity of p85/PI 3-K in immunoprecipitates or in cytoskeletal fractions is inhibited more potently by exposure of platelets to wortmannin than is the activity of PI 3-Ky. The addition of either β2-PMA or SFLLRN to platelets promotes the conversion of αIIbβ3 into a fibrinogen-binding form required for platelet aggregation (Fig 4), which can be monitored with the fluorescently labeled antibody, PAC1. The PAC1-detectable activation of αIIbβ3 in response to β2-PMA or SFLLRN is inhibited by wortmannin with an IC50 of 1 nmol/L in each case, resulting in a maximum inhibition of 50% to 60%. Wortmannin inhibits neither activation of αIIbβ3 by anti-LIBS6 Fab nor anti-LIBS6 Fab-induced platelet aggregation in the presence of fibrinogen, indicating that this type of outside-in signaling by αIIbβ3 is largely PI 3-Ky-independent.27 The inhibition of PI 3-K (and aggregation) by wortmannin or an unrelated synthetic compound, LY294002, impairs neither actin polymerization nor secretion.67

Wortmannin has been shown to inactivate the catalytic pl 10a subunit of p85/PI 3-K by covalently modifying it at Lys805, which is a residue involved in phosphate transfer by this enzyme.68 If the concentration of wortmannin is maintained below micromolar levels, there is apparently no direct inhibition of other kinases, such as PKC69; cAMP-dependent, cGMP-dependent, or calmodulin-dependent protein kinases70; tyrosine kinase71; or mitogen-activated protein kinase.72 It has also been observed that these amounts of wortmannin do not affect resting levels of PtdIns(4)P or PtdIns(4,5)P2 in intact cells,73,74 although at higher concentrations a form of membrane-associated PtdIns 4-kinase is inhibited.103 Another target of low nanomolar concentrations of wortmannin is phospholipase A2,147 an enzyme that is not stimulated in platelets exposed to β2-PMA.103 Based on these observations, it seems most likely that, under our incubation conditions, wortmannin is specifically targeting PI 3-K and thereby inhibiting platelet aggregation; furthermore, p85/PI 3-K, in preference to PI 3-Ky, contributes to the activation of αIIbβ3 when the thrombin receptor or PKC is stimulated (Fig 4). It is not clear what regulates the remaining 40% to 50% of wortmannin-insensitive aggregation, although, in the case of thrombin-induced activation, generation of PtdOH may contribute.148 A similar discrepancy between PI 3-K activity and aggregation with respect to susceptibility to wortmannin is observable when LPA is the agonist, i.e., the aggregation response is inhibited by lower amounts of wortmannin than is full production of 3-PPI.26 It is thus probable that LPA activates both p85/PI 3-K and PI 3-Ky and, as for thrombin-activated platelets, that the former is responsible for promoting platelet aggregation, even though both PI 3-Ks appear in the Triton-insoluble cytoskeletal fraction after platelet activation. It seems likely that these differences reflect different microenvironments for activated PI 3-Ks and αIIbβ3 on the platelets’ cytoskeletal scaffolding.

CHRFR-288 cells: proliferation and morphology. We have also studied thrombin-stimulated morphologic changes and the activation of PI 3-K in CHRFR-288 cells.28 We observed that these cells, when exposed to thrombin or SFFLLRN, rapidly change shape, forming membrane blebs detectable by differential interference contrast or confocal microscopy as well as radiolabeled [3H]-myoinositol PtdIns(3,4,5)P3 and PtdIns(3,4)P2. The blebs are distinguishable from ruffles or lamellae because they do not contain phallolidin-detectable actin. Studies with permeabilized CHRFR-288 indicate that PI 3-K is activated synergistically by thrombin + GTPyS. Two forms of PI 3-K, ie, PI 3-Ky and p85/PI 3-K, regulated by βγ subunits and Rho, respectively, are present in these cells, as is true for platelets. Wortmannin inhibits thrombin-stimulated 3-PPI accumulation in a dose-dependent manner (IC50 ~ 10 nmol/L) without affecting PIC activation. Pretreatment of CHRFR-288 cells with either wortmannin (100 nmol/L) or an unrelated synthetic PI 3-K inhibitor, LY294002 (50 nmol/L), abolishes thrombin receptor-stimulated blebbing. These results suggest that thrombin receptor-stimulated accumulation of 3-PPI (s) is required for the shape change response in CHRFR-288 cells. LPA and serum are two other agonists for 3-PPI accumulation in CHRFR-288 cells, neither of which causes membrane blebbing.28 However, unlike stimulation of CHRFR by thrombin or LPA, stimulation by serum promotes proliferation of these cells, and such proliferation is inhibited by wortmannin (IC50 = 15 to 20 nmol/L), implying that PI 3-K activity is necessary, but not sufficient, for agonist-stimulated cell growth or shape change. Additional factors may include the type and localization of PI 3-K activated in response to a given agonist.

CONCLUSION

The preceding findings indicate that PtdIns(4,5)P2, a quantitatively minor lipid in the plasma membrane, acts uniquely as the precursor for three ubiquitous signaling compounds: Ins(1,4,5)P3, DG, and PtdIns(3,4,5)P3 (Fig 3, pathways 1 and 6; and Fig 4). Much attention has been devoted in the last 10 years to the mode of action of the first two second messengers. It is clear that the focus will now be on the third, as well as on PtdIns(3,4)P2, a possible metabolite of LPA.
PtdIns(3,4,5)P$_3$ in vivo (Fig 3, pathway 4). Because synthetic 3-PPIs have become available recently, such studies have become much more feasible. With respect to potential targets for PtdIns(3,4,5)P$_3$, the proposal of Downes and Carter\textsuperscript{14} seems the most likely, i.e., rather than, for example, interacting stoichiometrically with cytoskeletal proteins, PtdIns(3,4,5)P$_3$ would be more effective as a modulator of the activity of an enzyme, such as a kinase, that can amplify its signal. Studies with PKCs make this proposal increasingly plausible.\textsuperscript{71, 130} Conceivably, 3-PPIs might modulate a tyrosine kinase (or phosphatase) or some other enzyme activity. Numerous questions remain. Is PtdIns(3,4)P$_2$ synthesized by PtdIns(4)P 3-K, PtdIns(3)P 4-K, and/or by hydrolysis of PtdIns(3,4,5)P$_3$? (Fig 2)? Why is its synthesis delayed? Our studies with phos-pleckstrin indicate circumstances in which PI 3-Ky acting on PtdIns(4,5)P$_2$ would be inhibited, whereas PI 3-Ky acting on PtdIns(4)P would not. Furthermore, PtdIns(3,4,5)P$_3$, rather than PtdIns(3,4)P$_2$, can inhibit binding of p85PI 3-K to phospho-tyrosines;\textsuperscript{91} this also might favor p85/PI 3-K action on PI(4,5)P$_2$, because the product of that activity, PtdIns(3,4)P$_2$, would not impair localization of p85/PI 3-K and its further activity. Why is accumulation of PtdIns(3,4)P$_2$ potentiated by occupancy of $\alpha_{o}$? Does PtdIns(3,4)P$_2$ have its own signaling targets, apart from those of PtdIns(3,4,5)P$_3$? Can PtdIns(3,4)P$_2$ act as an antagonist of PtdIns(3,4,5)P$_3$ effects? Of course, the regulation of the activity of PtdIns(3,4)P$_2$ is a major subject for experimental inquiry. How do Rho and PKC exert their effects in stimulating p85/PI 3-K? How might CDC42Hs, another Rho-family small G-protein present in platelets\textsuperscript{35} that is not ADP-ribosylatable but is known to bind recombinant p85/PI 3-K and activate it,\textsuperscript{116} be involved in regulating platelet p85/PI 3-K? How is Rho activated in response to platelet agonists? How many phillin modules, in addition to SH2, SH3, and PH, are involved in targeting and activating PI 3-Ks? How does p85/PI 3-K promote activation of platelet integrin $\alpha_{o}3\beta_{2}$? What is the function of PI 3-Ky? Where are p85/PI 3-K versus PI 3-Ky localized on the activated platelet’s cytoskeletal scaffolding, allowing selective effects on $\alpha_{o}3\beta_{2}$? What other PI 3-Ks are present in platelets and/or platelet progenitor cells, and what functions do they perform? We have indications, for example, of at least two forms, in addition to p110$\alpha$, p110$\beta$, and p110$\gamma$ (and p85$\alpha$ and $\beta$), of PI 3-K in platelets and CHRF-288 cells (S.E. Rittenhouse and S. Volini, unpublished results). One of the two isoforms is most likely a VPS34-type PtdIns 3-kinase,\textsuperscript{106} whereas the other (thus far designated PI 3-Kz; S.E. Rittenhouse and S. Volini, unpublished results) bears some resemblance to new PI 3-Ks such as p170$^{07}$ and Cpk.\textsuperscript{108} It seems likely that this latest chapter in the story of phosphoinositide metabolism’s role in signal transduction will yield surprises at least equal to those encountered earlier.

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Phosphoinositide 3-kinase activation and platelet function

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