Activation of Actin Polymerization by Phosphatidic Acid Derived from Phosphatidylcholine in IIC9 Fibroblasts

Kwon-Soo Ha and John H. Exton

Howard Hughes Medical Institute and the Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0295

Abstract. α-Thrombin induced a change in the cell morphology of IIC9 fibroblasts from a semiround to an elongated form, accompanied by an increase in stress fibers. Incubation of the cells with phospholipase D (PLD) from Streptomyces chromofuscus and exogenous phosphatidic acid (PA) caused similar morphological changes, whereas platelet-derived growth factor (PDGF) and phorbol 12-myristate 13-acetate (PMA) induced different changes, e.g., disruption of stress fibers and cell rounding. α-Thrombin, PDGF, and exogenous PLD increased PA by 20–40%, and PMA produced a smaller increase. α-Thrombin and exogenous PLD produced rapid increases in the amount of filamentous actin (F-actin) that were sustained for at least 60 min. However, PDGF produced a transient increase of F-actin at 1 min and PMA caused no significant change. Dioctanoylglycerol was ineffective except at 50 μg/ml. Phospholipase C from Bacillus cereus, which increased diacylglycerol (DAG) but not PA, did not change F-actin content. Down-regulation of protein kinase C (PKC) did not block actin polymerization induced by α-thrombin. H-7 was also ineffective. Exogenous PA activated actin polymerization with a significant effect at 0.01 μg/ml and a maximal increase at 1 μg/ml. No other phospholipids tested, including polyphosphoinositides, significantly activated actin polymerization. PDGF partially inhibited PA-induced actin polymerization after an initial increase at 1 min. PMA completely or largely blocked actin polymerization induced by PA or PLD. These results show that PC-derived PA, but not DAG or PKC, activates actin polymerization in IIC9 fibroblasts, and indicate that PDGF and PMA have inhibitory effects on PA-induced actin polymerization.

Actin is a globular protein which is polymerized into filamentous actin (F-actin).1 The mechanism of actin polymerization is a topic of interest since actin polymerization is known to be important in cell motility, changes in cell morphology, cell division, and intracellular movements (6, 13, 31). There have been several reports on the regulation of actin polymerization, but the mechanism is not clear. Phorbol 12-myristate 13-acetate (PMA) activates actin polymerization in rat basophilic leukemia cells (l) and neutrophils (9), but does not cause activation in human fibroblasts (21). In plasma membranes purified from Dictyostelium discoideum, dioctanoylglycerol (DOG) stimulates actin nucleation, but PMA does not (29). Tyrosine phosphorylation has been also proposed to activate actin assembly in cells such as B lymphocytes (20), platelets (25), and Dictyostelium (14).

Recently, polyphosphoinositides have been reported to be important in the regulation of actin polymerization (4, 6, 11, 16, 31, 37). Polyphosphoinositides have been proposed to bind to actin-binding proteins such as gelsolin (7, 16, 37), profilin (4), and villin (11), resulting in an increase in actin monomers or nucleation sites and a stimulation of actin polymerization. However, there has been one study reporting that the changes in the levels of polyphosphoinositides induced in A431 cells by several agents did not correlate with those of actin-gelsolin complexes (8).

The hydrolysis of phosphatidylcholine (PC) by phospholipase D (PLD) produces phosphatidic acid (PA) in various cell types stimulated with hormones or growth factors (10). PA derived from PC may then be converted into diacylglycerol (DAG) by PA phosphohydrolase (17), leading to activation of certain isozymes of protein kinase C (PKC) (12). PA has also been reported to cause Ca2+ influx (26, 28), induction of mRNA for c-fos and c-myc, stimulation of DNA synthesis (18, 22), and increased protein phosphorylation (3). However, the physiological function of PA is still obscure.

In this report, we present novel results that PA derived from PC but not DAG or PKC, activates actin polymerization.

© The Rockefeller University Press, 0021-9525/93/12/1789/8 $2.00
The Journal of Cell Biology, Volume 123, Number 6, Part 2, December 1993 1789-1796 1789
in IIC9 fibroblasts. These results provide evidence for a possible role of PA in the regulation of actin polymerization.

Materials and Methods

Materials

Culture medium components and polyclonal antibodies raised against synthetic peptides corresponding to specific sequences in PKC isoforms were from GIBCO-BRL. 

Materials of Ha and Extton (12). 

PKC Isozymes c~, ~, and ~" were identified by Dr. Daniel M. Raben, Johns Hopkins University School of Medicine, Baltimore, MD, were grown and maintained according to the methods of 

IIC9 cells, a subclone of Chinese hamster embryo fibroblasts (kindly given by Dr. John R. Falk, University of Texas Southwestern Medical Center, Dallas, TX). 

N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl) phallacidin (NBD-phallacidin) and (+)-1-(5-Isoquinolinesulfonyl)-(2-methylpiperazine (H-7) were purchased from Molecular Probes Inc. 

Materials and Methods

Cell Culture

IIC9 cells, a subclone of Chinese hamster embryo fibroblasts (kindly given by Dr. Daniel M. Raben, Johns Hopkins University School of Medicine, Baltimore, MD), were grown and maintained according to the methods of 

The extracted proteins were separated by 10% SDS-PAGE according to the 

Results

Morphological Changes

Incubation of IIC9 fibroblasts with a~-thrombin caused a change in the appearance of the cells from a semiround to an elongated form. The shape change was accompanied by an increase in the number of intracellular stress fibers and an elongation of these fibers (Fig. 1 b). These changes were evident at 1–2 min, maximal at 15–30 min, and apparent for at least 2–3 h (data not shown). Treatment of the cells with 

Changes in F-actin

In order to understand the biochemical basis for the morphological changes observed with a~-thrombin, changes in the cell content of F-actin were quantified fluorimetrically using NBD-phallacidin. Fig. 2 a shows that a~-thrombin rapidly increased F-actin, with a significant effect at 1 min and a maximal increase at 15 min. The level then decreased slightly over 60 min. 

Changes in Phosphatidic Acid

Since the preceding results suggested a role for PA, the product of PLD action, in the F-actin changes induced by a~-thrombin, the effects of agents on the level of this lipid were quantified. It had previously been shown that a~-thrombin and PDGF elevated DAG in IIC9 cells (12), but the levels of PA were not measured. Fig. 3 shows that both agonists increased [3H]myristic acid for 2 d in serum-free medium and stimulated with various agonists. After quickly washing with ice-cold PBS, cells were scraped into ice-cold methanol and lipids were extracted by the method of Bligh and Dyer (2). 

PA band was identified, scraped, and counted using a scintillation counter.

Fluorescence Microscopy

Cells were grown on glass coverslips for 2 d, serum starved for 2 d, and then treated with agonists for 15 min in a 6-well plate. The cells were fixed with 3.5% paraformaldehyde on ice, permeabilized, and stained with NBD-phallacidin as explained above for F-actin measurement. Following washing twice with PBS, the coverslip was mounted on a slide and then observed with a fluorescence microscope (Leitz, E. Inc., Rockleigh, NJ).

Results

Morphological Changes

Incubation of IIC9 fibroblasts with a~-thrombin caused a change in the appearance of the cells from a semiround to an elongated form. The shape change was accompanied by an increase in the number of intracellular stress fibers and an elongation of these fibers (Fig. 1 b). These changes were evident at 1–2 min, maximal at 15–30 min, and apparent for at least 2–3 h (data not shown). Treatment of the cells with 

Changes in F-actin

In order to understand the biochemical basis for the morphological changes observed with a~-thrombin, changes in the cell content of F-actin were quantified fluorimetrically using NBD-phallacidin. Fig. 2 a shows that a~-thrombin rapidly increased F-actin, with a significant effect at 1 min and a maximal increase at 15 min. The level then decreased slightly over 60 min. S. chromofuscus PLD produced a similar rapid effect which was sustained for 60 min (Fig. 2 b). In contrast, PDGF produced an increase which peaked at 1 min and then declined to control at 15 min (Fig. 2 c).

Changes in Phosphatidic Acid

Since the preceding results suggested a role for PA, the product of PLD action, in the F-actin changes induced by a~-thrombin, the effects of agents on the level of this lipid were quantified. It had previously been shown that a~-thrombin and PDGF elevated DAG in IIC9 cells (12), but the levels of PA were not measured. Fig. 3 shows that both agonists increased [3H]myristic acid for 2 d in serum-free medium and stimulated with various agonists. After quickly washing with ice-cold PBS, cells were scraped into ice-cold methanol and lipids were extracted by the method of Bligh and Dyer (2). 

PA band was identified, scraped, and counted using a scintillation counter.

Lack of Effect of Diacylglycerol

Because of the evidence that PMA and DOG promote actin polymerization in some cells (1, 9), the possibility that the effects of a~-thrombin and PLD on F-actin were secondary to
the production of DAG from PA was explored. First, F-actin content was measured after incubating cells with PMA or DOG to test for possible activation of actin polymerization. However, PMA had no significant effect (data not shown), and DOG had no effect except at 50 μg/ml (Fig. 4a). In addition, neither PMA nor DOG induced cell elongation or stress fiber formation in morphological studies (Fig. 1f for PMA and data not shown for DOG). These results suggest that DAG and PKC do not activate actin polymerization or that actin polymerization requires other factors in addition to PKC in IIC9 fibroblasts.

To test whether endogenously generated DAG is also without effect on actin polymerization, cells were incubated for 15 min with \textit{B. cereus} PC-specific PLC. This enzyme increases DAG (12) with no significant change in PA (Fig. 3) and activates PKC ε translocation (12). It did not significantly change the F-actin amount at any concentration from 0.5 to 10 U/ml (Fig. 4b).

\textit{Lack of Effects of Protein Kinase C Inhibitor and of Down-regulation}

Lack of activation of actin polymerization by PKC was further supported by studies employing PKC down-regulation.

\textit{Figure 1. Changes in F-actin organization of IIC9 fibroblasts.} Cells were grown on coverslips, serum starved, and then treated with no agonist (a), 500 ng/ml α-thrombin (b), 5 U/ml \textit{S. chromofuscus} PLD (c), 1 μg/ml PA (d), 50 ng/ml PDGF (e), or 160 nM PMA (f) for 15 min. The cells were fixed, permeabilized, stained with NBD-phallacidin, and photographed as described in Materials and Methods. The changes are representative of those seen in three separate studies.
Figure 2. Time course of changes in F-actin content. II9 cells, grown and serum starved on 6-well plates, were treated with 500 ng/ml α-thrombin (a), 5 U/ml S. chromofuscus PLD (b), or 50 ng/ml PDGF (c) for the times indicated. F-actin content was measured as described in Materials and Methods. Data are means ± SE from three independent experiments.

Figure 3. Changes of PA content in response to various agonists. II9 cells were grown on 100-mm culture dishes and labeled with 10 μCi/dish [3H]myristic acid for 2 d in serum-free medium. The cells were then treated with 500 ng/ml α-thrombin (Thr) or 50 ng/ml PDGF for 1, 5, or 15 min. Cells were also treated with 5 U/ml S. chromofuscus PLD (PLD), 4 U/ml B. cereus PC-PLC (PLC), or 160 nM PMA for 15 min. [3H]PA was determined in Materials and Methods. Values are expressed as a percentage of values obtained from control cells. Data are means ± SE from three independent experiments.

Figure 4. Lack of effect of DOG (a) and PC-PLC (b) on F-actin content. II9 cells, grown and serum starved on 6-well plates, were treated with various concentrations of DOG (a) or PC-PLC (b), or 500 ng/ml α-thrombin (Thr) for 15 min. F-actin content was measured as described in Materials and Methods. Data are means of two independent experiments for (a) and means ± SE of three independent experiments for (b).

Figure 5. Effect of PKC inhibitors on actin polymerization. B. cereus PLC (b) or 500 ng/ml α-thrombin (Thr) for 15 min. Data are from two independent experiments for (a) and means of three independent experiments for (b).

1.4 ° 1.3 1.2 1.1 1.0

1.3 1.2 1.1 1.0

0 10 20 30 60

Incubation time (min)

Effects of Phosphatidic Acid and Other Phospholipids

Because the experiments shown in Figs. 4 and 5 indicated that DAG and PKC play little, if any, role in actin polymerization induced by α-thrombin in II9 cells, the possibility that PA, the first product of PC-PLD action, has an important function was explored. To test for PA activation of actin polymerization, cells were incubated with various concentrations of PA micelles. Fig. 6 shows that actin polymerization was activated by PA, with a significant effect at 0.01 μM.
Figure 5. Lack of effect of PKC down-regulation (a) and H-7 (b) on the α-thrombin induced increase in F-actin content. Cells were grown on 6-well plates and serum starved for 1 d. The cells were treated with 0.8 μM PMA (PKC down-regulated) for 1 d (a) or with various concentrations of H-7 for 30 min (b), and then incubated with 500 ng/ml α-thrombin for 15 min. Data are means ± SE from three independent experiments.

μg/ml and a maximal increase at 1 μg/ml (Fig. 6 a). Fig. 6 b shows the time course with 1 μg/ml PA. There was a very rapid increase within the first 5 min, and the maximal increase was similar to that induced by α-thrombin or B. cereus PLD (compare Fig. 6 b with Fig. 2). To test for specificity, IIC9 cells were also incubated with 1 μg/ml of different phospholipid micelles (Fig. 7). None of the other tested phospholipids, including polyphosphoinositides, significantly activated actin polymerization.

Inhibitory Effects of PMA and PDGF

Fig. 3 shows that PMA caused a rise in PA. However, it did not induce stress fiber formation (Fig. 1 f) or activate actin polymerization (data not shown). To test for a possible inhibitory effect of PMA on actin polymerization, cells were incubated with PA or PLD after preincubation with PMA. Fig. 8 shows that PMA completely blocked actin polymerization induced by PA and largely inhibited that induced by PLD. Cytochalasin D, a known inhibitor of F-actin formation (5) had similar effects. These results indicate that PMA has an inhibitory effect on actin polymerization induced by PA.

A possible delayed inhibitory effect of PDGF on actin polymerization was also studied by incubating cells with PDGF for 15 min and then with various concentrations of PA. PDGF completely blocked actin polymerization induced by 0.01 μg/ml PA, and partly inhibited that produced by PA concentrations from 0.05 to 1 μg/ml (data not shown).

Discussion

The present results show that in IIC9 fibroblasts, α-thrombin elevates PA (Fig. 3), induces a morphological change from

Figure 6. Effect of PA on F-actin content. Cells, grown and serum starved, were incubated with various concentrations of PA for 15 min (a), or incubated with 1 μg/ml of PA for the times indicated (b). Data are means ± SE from three independent experiments.

Figure 7. Effect of different phospholipids on F-actin contents. Cells, grown and serum starved, were incubated with 1 μg/ml of the indicated phospholipids for 15 min. Data are means ± SE from three independent experiments.
a semiround to an elongated form with enhanced appearance of stress fibers (Fig. 1), and increases actin polymerization (Fig. 2). Since *S. chromofuscus* PLD induces similar effects (Figs. 1–3), these observations suggest that activation of PC-PLD is responsible for the change in cell morphology and increase in actin polymerization. PC hydrolysis induced by α-thrombin in IIC9 cells increases PA and DAG, which in turn activates PKC ε (12). Thus, these candidates, PA, DAG, and PKC ε, could potentially play a role in the activation mechanism of actin polymerization.

A role for PKC ε activation in actin polymerization was excluded by four different experiments, and all the results indicated that this PKC isoform was ineffective. PMA and DOG, which induce PKC ε translocation in IIC9 cells (12), caused little or no actin polymerization (Fig. 4 a). PC-specific PLC from *B. cereus* which increases DAG and causes PKC ε translocation (12), did not increase F-actin (Fig. 4 b). Furthermore, PKC down-regulation caused by incubation with PMA for 24 h did not block the increase of actin polymerization caused by α-thrombin (Fig. 5 a), and H-7, a PKC inhibitor, slightly inhibited actin polymerization only at a high concentration (Fig. 5 b).

There have been divergent results regarding the activation of actin polymerization by PKC in different cell types. In rat basophilic leukemia cells, PMA and DOG activated actin polymerization, and antigen-stimulated actin polymerization was blocked by the PKC inhibitors sphingosine and staurosporine (1). In neutrophils, PMA and DOG activated actin polymerization, but PMA-induced actin polymerization was not inhibited by PKC inhibitors such as H-7, calphostin C, sphingosine, and staurosporine (9). However, in human fibroblasts, PMA was ineffective in inducing membrane ruffling and also inhibited PDGF-induced membrane ruffling (21), suggesting that PKC is an inhibitor rather than an activator of actin polymerization in these cells. In *Dictyostelium discoideum*, DOG activated actin polymerization by inducing de novo synthesis of actin nucleation sites in the absence of PKC (29), but the increase in actin polymerization was not blocked by staurosporine or mimicked by PMA.

In the present studies with IIC9 cells, actin polymerization was not activated by DOG (Fig. 4 a), and although *B. cereus* PC-specific PLC produced a large increase in DAG (12), it did not activate actin polymerization at any concentration tested (Fig. 4 b). The negative result with the PLC was not due to its failure to increase DAG in the inner leaflet of the membrane since it induced membrane translocation of PKC within 30 s in IIC9 cells (unpublished findings). Furthermore, other studies have shown that DAG, produced by exogenous PC-specific PLC, readily crosses the plasma membrane in Chinese hamster V79 fibroblasts (23), indicating a very rapid transbilayer movement of DAG.

The lack of effects of DAG and PKC on actin polymerization in IIC9 cells led us to examine if PA could be an activator by incubating cells with different phospholipid micelles. Phospholipids have been shown to cross the lipid bilayer membrane (reviewed in 36) and there is evidence that exogenous PA is rapidly incorporated into the outer leaflet of lipid bilayer and internalized into cells (24, 32, 36). In IIC9 cells, exogenous PLD rapidly translocates PKC ε and increases DAG (data not shown). These data are consistent with the conclusion that PA produced by PLD acting on the outer leaflet of the plasma membrane moves into the inner leaflet and is then hydrolyzed to DAG which activates PKC ε translocation.

In IIC9 cells, PA induced an activation of actin polymerization that was specific for this phospholipid (Fig. 6), although it must be recognized that some of the other phospholipids may not have produced significant increases in their plasma membrane concentrations due to slow incorporation. The activation induced by PA was dose dependent and rapid, with increases being detectable at 0.01 μg/ml and at 1 min (Fig. 6 a). A recent report has also indirectly implicated PA in actin polymerization in platelets because of the correlated increases of PA and F-actin induced by pervanadate, a protein tyrosine phosphatase inhibitor (25). In these cells the origin of the PA was apparently DAG generated from PIP2 by PLC. This is different from the situation in IIC9 cells where PA is mainly derived from PC-hydrolysis by PLD (for review see reference 12 and Fig. 3). There is another recent indication to support PA activation of actin polymerization. Lysophosphatidic acid (LPA) increased PA by activating PC-hydrolyzing PLD in Rat-1 fibroblasts (35), and activated the formation of focal adhesion and stress fibers in Swiss 3T3 cells (27).

There is much evidence to support the idea that LPA functions as an agonist in transmembrane signaling. This includes the existence of a putative receptor for LPA, activation of a pertussis toxin-insensitive G-protein with PIP2 hydrolysis and Ca2+ mobilization, activation of a PC-hydrolyzing PLD, and activation of actin polymerization (27, 30, 33–35). These findings raise the possibility that PA might activate actin polymerization by a similar mechanism. However, PA can rapidly cross the lipid bilayer (24, 32, 36), and it is difficult to distinguish a direct effect of PA after crossing the bilayer from one involving a putative receptor.

Interestingly, PDGF induced a rapid, but transient increase in F-actin content (Fig. 2 c), which was accompanied by cell rounding and a loss of stress fibers (Fig. 1). Since there was an increase of PA that was maximal at 5 min (Fig. 3), it is clear that other changes induced by the growth factor caused a reversal of the effects of PA. In *Dictyostelium* mutants in which the gene for the phosphotyrosine phosphatase was disrupted, tyrosine phosphorylation of actin was rapid and prolonged, and the cells became round (14), suggesting that tyrosine phosphorylation was involved in the cell shape change. In the present study, PDGF was found to inhibit actin polymerization induced by PA. These findings support the idea that tyrosine phosphorylation is responsible for the differences in morphological and actin responses produced by the growth factor in IIC9 cells compared with those induced by α-thrombin.
PMA did not increase F-actin by itself, even though it increased PA (Fig. 3). The discrepancy can be explained by the findings that PMA inhibited PA-induced actin polymerization (Fig. 8) and caused the disappearance of stress fibers and cell rounding (Fig. 1f). Interestingly, the changes in cell morphology and stress fibers induced by PMA were similar to those induced by PDGF (Fig. 1e) or cytochalasin D (not shown). PDGF and PMA may utilize a common mechanism to inhibit actin polymerization induced by PA.

PA has been reported to increase cytosolic Ca²⁺ in some cells by activating Ca²⁺ uptake (26, 28) or Ca²⁺ release from intracellular stores (22). Thus, it is possible that PA might activate actin polymerization by regulating Ca²⁺-dependent actin binding proteins such as gelsolin (16, 37) or villin (11). In IIC9 cells, the activation of actin polymerization induced by exogenous PLD or α-thrombin was largely or completely blocked by preincubating cells with EGF or bis (2-amino-phenoxy)ethane-N,N,N',N'-tetraacetate acetoxymethyl ester to deplete or buffer intracellular Ca²⁺ (data not shown). However, ionomycin induced a large Ca²⁺ increase, but did not activate actin polymerization (data not shown), in agreement with findings in other cells (1, 21, 29). These results suggest that PA does not activate actin polymerization by increasing cytosolic Ca²⁺ over basal. However, a basal level of Ca²⁺ is required for actin polymerization.

What are the possible mechanisms by which PA activates actin polymerization? The first mechanism is that PA might activate a PA-dependent protein kinase(s) (3), which might affect actin polymerization or regulate actin-binding proteins (6). A second possible mechanism is that PA is deacylated into LPA by phospholipase A₂, and LPA activates actin polymerization. There is a report that LPA induces stress fiber formation, perhaps by regulating the activity of the small molecular weight GTP-binding protein rho (27). In IIC9 cells, LPA also induced actin polymerization, but there was no significant increase of LPA in cells incubated with α-thrombin or PLD (unpublished data). Furthermore, although pertussis toxin markedly inhibited the effect of LPA on actin polymerization, it did not alter the effect of PLD and had a minimal effect on the action of PA (unpublished data). Thus, the possibility that PA activates actin polymerization by LPA may not be the case in IIC9 cells. A third possible mechanism is that PA interacts directly with actin-binding proteins as has been proposed for polyphosphoinositides (6). These phospholipids have been proposed to regulate actin polymerization through their ability to bind to actin-binding proteins such as profilin, villin and gelsolin (4, 6, 16). The binding of the phosphoinositides has been proposed to release actin monomers or free barbed ends of actin filaments from actin-binding proteins–actin complexes or actin-binding proteins–actin filament complexes. According to this model, the hydrolysis of polyphosphoinositides by agonists would decrease F-actin content, and their subsequent rise due to resynthesis would allow actin polymerization. However, in IIC9 cells, none of polyphosphoinositides (PIP, PIP₂, and PIP₃) significantly activated actin polymerization (Fig. 8), and there is no evidence that growth factors increase PIP₂ or PIP above basal levels in any cell. Furthermore, in a detailed study using A431 cells incubated with EGF, bradykinin, and cholera toxin, discrepancies between the phosphoinositide changes and the levels of gelsolin–actin complex were observed (8). Thus, the present findings suggest that PA might be an alternative to phosphoinositides in regulating actin polymerization. There is a report that PA has a much lower effect in binding with profilin to induce actin polymerization than PIP and PIP₃ (19), but other actin binding proteins have not been studied.

To determine if PA has a more general role in inducing actin polymerization, the present studies need to be extended to other cells, particularly those in which agonists elevate PA. More interestingly, the mechanism(s) by which PA alters actin assembly/disassembly needs to be elucidated. An attractive possibility, suggested by previous work, is that the phospholipid interacts with actin-binding proteins. However, a role for a PA-activated protein kinase also needs to be explored, as does the apparent negative effects of PKC and tyrosine kinase. Although the phenomenon of agonist-induced PC hydrolysis by PLD is widespread (10), the physiological role of the PA generated remains obscure. The present findings suggest an important function for this lipid.

We thank Dr. Thomas Jetton for help with fluorescence microscopy. We also thank Drs. Linda J. Van Eldik and Sally H. Zigmond for careful review of this manuscript.

Received for publication 20 July 1993 and in revised form 18 August 1993.

References

1. Aggar, J. R. 1991. Regulation of the antigen-induced F-actin response in rat basophilic leukemia cells by protein kinase C. J. Cell Biol. 112:1157–1163.
2. Bligh, K. M., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911–917.
3. Bocckino, S. B., P. B. Wilson, and J. H. Exton. 1991. Phosphatidate-dependent protein phosphorylation. Proc. Natl. Acad. Sci. U.S.A. 86:6210–6213.
4. Cao, L.-G., G. G. Babcock, P. A. Rubenstein, and Y.-L. Wang. 1992. Effects of profilin and profilactin on actin structure and function in living cells. J. Biol. Chem. 267:1023–1029.
5. Cooper, J. A. 1987. Effects of cytochalasin and phalloidin on actin. J. Cell Biol. 105:1473–1478.
6. Cooper, J. A. 1991. The roles of actin polymerization in cell motility. Annu. Rev. Physiol. 53:585–605.
7. Cunningham, C. C., T. P. Stossel, and D. J. Kwiatkowski. 1991. Enhanced motility in NIH 3T3 fibroblasts that overexpress gelsolin. Science (Wash. DC). 251:1233–1236.
8. Dadbury, C. Y., E. Patton, J. A. Cooper, and L. J. Pike. 1991. Lack of correlation between changes in polyphosphoinositide levels and actin/gelsolin complexes in A431 cells treated with epidermal growth factor. J. Cell Biol. 112:1151–1156.
9. Downey, G. P., C. K. Chan, P. Lea, A. Takai, and S. Grinstein. 1992. Phorbol ester-induced actin assembly in neutrophils: role of protein kinase C. J. Cell Biol. 116:695–706.
10. Exton, J. H. 1990. Signaling through phosphatidylincholine breakdown. J. Biol. Chem. 265:1–4.
11. Friederich, E., K. Vancomperoolle, C. Huet, M. Goethals, J. Finidori, J. Vandekerckhove, and D. Louvard. 1992. An actin-binding site containing a conserved motif of charged amino acid residues is essential for the morphogenetic effect of villin. Cell. 70:81–92.
12. Ha, K-S., and J. H. Exton. 1993. Differential translocation of protein kinase C isoforms by thrombin and platelet-derived growth factor: a possible function for phosphatidylinositol-derived diacylglycerol. J. Biol. Chem. 268:10534–10539.
13. Hennessey, E. S., D. R. Drummond, and J. C. Sparrow. 1993. Molecular genetics of actin function. Biochem. J. 282:657–671.
14. Howard, P. K., B. M. Setton, and R. A. Firtel. 1993. Tyrosine phosphorylation of actin in Dictyostelium associated with cell shape changes. Science (Wash. DC). 259:241–244.
15. Howard, T. H., and C. O. Oresajo. 1985. The kinetics of chemotactic peptide-induced change in F-actin content, F-actin distribution, and the shape of neutrophils. J. Biol. Chem. 101:1078–1085.
16. Janney, P. A., J. Lamb, P. G. Allen, and P. T. Matsudaira. 1992. Phospholipase A₂-binding peptides derived from the sequences of gelsolin and villin. J. Biol. Chem. 267:11818–11823.
17. Kamoh, H., S. Imai, K. Yamao, and F. Sakane. 1992. Purification and partial characterization of actin-binding proteins from cultured mesangial cells. J. Biol. Chem. 265:14457–14462.
19. Lassing, I., and U. Lindberg. 1985. Specific interaction between phosphatidylinositol 4,5-bisphosphate and profilactin. Nature (Lond.). 314:472-474.

20. Melamed, I., G. P. Downey, and C. M. Roifman. 1991. Tyrosine phosphorylation is essential for microfilament assembly in B lymphocytes. Biochem. Biophys. Res. Commun. 176:1424-1429.

21. Mellström, K., C.-H. Heldin, and B. Westermark. 1988. Induction of circular membrane ruffling on human fibroblasts by platelet-derived growth factor. Exp. Cell Res. 177:347-359.

22. Moolenaar, W. H., W. Kruijer, B. C. Tilly, I. Verlaan, A. J. Bierman, and S. W. de Laat. 1986. Growth factor-like action of phosphatidic acid. Nature (Lond.). 323:171-173.

23. Pagano, R. E., and K. J. Longmuir. 1985. Phosphorylation, transbilayer movement, and facilitated intracellular transport of diacylglycerol are involved in the uptake of a fluorescent analog of phosphatidic acid by cultured fibroblasts. J. Biol. Chem. 260:1086-1094.

24. Putney, J. W., J. M. van De Walle, and R. A. Haddas. 1980. Is phosphatidic acid a calcium ionophore under neurohumoral control? Nature (Lond.). 284:345-347.

25. Ridley, A. L., and A. Hall. 1992. The small GTP binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. Cell. 70:389-399.

26. Salmon, D. M., and T. W. Honeyman. 1980. Proposed mechanism of cholinergic action in smooth muscle. Nature (Lond.). 284:344-345.

27. Shariff, A., and E. J. Luna. 1992. Diacylglycerol-stimulated formation of actin nucleation sites at plasma membranes. Science (Wash. DC). 256:245-247.

28. Shiono, S., K. Kawamoto, N. Yoshida, T. Kondo, and T. Inagami. 1993. Neutransmitter release from lysophosphatidic acid stimulated PC12 cells: involvement of lysophosphatidic acid receptors. Biochem. Biophys. Res. Commun. 193:667-673.

29. Stossel, T. P. 1993. On the crawling of animal cells. Science (Wash. DC). 260:1086-1094.

30. Tokumura, A., T. Tsuchumi, and H. Tsubakatani. 1992. Transbilayer movement and metabolic fate of ether-linked phosphatidic acid (1-O-octadecyl-2-acetyl-sn-glycerol 3-phosphate) in guinea pig peritoneal polymorphonuclear leukocytes. J. Biol. Chem. 267:7275-7283.

31. Van Corven E. J., A. Groenink, K. Jalink, T. Eichholzt, and W. H. Moolenaar. 1989. Lysophosphatidate-induced cell proliferation: identification and dissection of signaling pathways mediated by G proteins. Cell. 59:45-54.

32. Van der Bend, R. L., J. Brunner, K. Jalink, E. J. van Corven, W. H. Moolenaar, and W. J. van Blitterswijk. 1992. Identification of a putative membrane receptor for the bioactive phospholipid, lysophosphatidic acid. EMBO (Eur. Mol. Biol. Organ.) J. 11:2495-2501.

33. Van der Bend, R. L., J. de Widt, E. J. van Corven, W. H. Moolenaar, and W. J. van Blitterswijk. 1992. The biologically active lipid, lysophosphatidic acid, induces phosphatidylcholine breakdown in fibroblasts via activation of phospholipase D. Biochem. J. 283:235-240.

34. Voelker, D. R. 1990. Lipid transport pathways in mammalian cells. Experientia (Basel). 46:569-579.

35. Yu, F-X., H-Q. Sun, P. A. Janmey, and H. L. Yin. 1992. Identification of polyphosphoinositide-binding sequence in an actin monomer-binding domain of gelsolin. J. Biol. Chem. 267:14616-14621.