**Myristylated Alanine-rich C Kinase Substrate (MARCKS) Produces Reversible Inhibition of Phospholipase C by Sequestering Phosphatidylinositol 4,5-Bisphosphate in Lateral Domains**

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The myristoylated alanine-rich protein kinase C substrate (MARCKS) is a major protein kinase C (PKC) substrate in many different cell types. MARCKS is bound to the plasma membrane, and several recent studies suggest that this binding requires both hydrophobic insertion of its myristate chain into the bilayer and electrostatic interaction of its cluster of basic residues with acidic lipids. Phosphorylation of MARCKS by PKC introduces negative charges into the basic cluster, reducing its electrostatic interaction with acidic lipids and producing translocation of MARCKS from membrane to cytoplasm. The present study shows that physiological concentrations of MARCKS (<10 μM) inhibit phospholipase C (PLC)-catalyzed hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) in phospholipid vesicles. A peptide corresponding to the basic cluster, MARCKS(151–175), produces a similar inhibition, which was observed with both PLC-δ1 and -β1. Direct fluorescence microscopy observations demonstrate that the MARCKS peptide forms lateral domains enriched in the acidic lipids phosphatidylycerine and PIP2 but not PLC, which accounts for the observed inhibition of PIP2 hydrolysis. Phosphorylation of MARCKS(151–175) by PKC releases the inhibition and allows PLC to produce a burst of inositol 1,4,5-trisphosphate and diacylglycerol.

Stimulation of the calcium/phospholipid second messenger system activates protein kinase C (PKC)† for reviews see Refs. 1 and 2. The major PKC substrate in many cell types is the myristoylated alanine-rich protein kinase C substrate or MARCKS protein (for reviews see Refs. 3 and 4). MARCKS can cross-link actin, bind calcium-calmodulin, and adsorb to membranes; these three processes can be inhibited by PKC-catalyzed phosphorylation of MARCKS. Although the specific cellular function(s) of MARCKS is not known, several experiments suggest it is involved in phagocytosis, secretion, and/or membrane recycling. For example, MARCKS has a punctate distribution in the plasma membranes of macrophages, where it is localized in nascent phagosomes (5, 6); it is highly concentrated in presynaptic terminals (7); and it cycles between the plasma membrane and lysosomes in fibroblasts (8). An understanding of the interaction of MARCKS with the phospholipid bilayer component of a biological membrane should provide information about how it may function in phagocytosis and secretion.

Binding of MARCKS to the plasma membrane requires both hydrophobic insertion of its myristate chain into the bilayer and electrostatic interaction of the cluster of basic residues in its effector domain with acidic lipids (9–12). Phosphorylation of MARCKS by PKC introduces negative charges into the basic cluster, reducing its electrostatic interaction with acidic lipids; in many cell types this produces translocation of MARCKS from membrane to cytoplasm by a mechanism that has been termed the myristoyl-electrostatic switch (13, 14). Calcium-calmodulin binds with high affinity to the basic effector region of MARCKS, which also produces translocation of the protein from membranes (11, 12).

We report here that physiological concentrations of MARCKS (<10 μM) or a peptide corresponding to its basic domain, MARCKS(151–175), inhibit phospholipase C (PLC)-catalyzed hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) in phospholipid vesicles by sequestering PIP2 but not PLC into lateral domains. The domains were visualized directly by fluorescence digital imaging microscopy. Phosphorylation of MARCKS(151–175) by PKC releases the inhibition and allows PLC to produce a burst of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). The addition of calcium-calmodulin produces a similar burst of IP3. We discuss the potential relevance of these results to phagocytosis in macrophages.

**EXPERIMENTAL PROCEDURES**

Phospholipids—The lipids 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (PC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (PE), and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine (PS) were obtained from Avanti Polar Lipids (Alabaster, AL). These lipids were used for the PIP2 hydrolysis experiments illustrated in Figs. 1–3. The lipids dioleoyl-sn-glycero-3-phosphocholine, dioleoyl-sn-glycero-3-phosphoserine, and NBD-PS, also obtained from Avanti, were used for the fluorescence microscopy experiments illustrated in Fig. 4. The ammonium salt of PIP2 was obtained from Calbiochem (San Diego, CA) or Boehringer Mannheim; tritiated PIP2 was from DuPont NEN (Scheme 1).

NBD-PIP2, shown in Scheme 1, was synthesized by a method de-
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**RESULTS**

**MARCKS Inhibits the PLC-catalyzed Hydrolysis of PIP$_2$—** Fig. 1 shows that MARCKS inhibits the production of IP$_3$ by PLC-$\beta_1$ in a concentration-dependent manner: 3 $\mu$M MARCKS produces about a 5-fold inhibition when the lipid concentration is $0.2$ mM. The concentration of MARCKS in nerve cells is $10$ $\mu$M (7), and the effective concentration of lipids in the plasma membrane of a 10-$\mu$m radius cell is $1$ mM; thus the lipid/protein ratio in these experiments is comparable with that in an intact cell. Parallel experiments under the same conditions show that a peptide corresponding to the MARCKS basic effector region, MARCKS(151–175) = KKKKKRFSFKKSFKLSGFSFKKNNK, produces identical inhibition of PLC-$\beta_1$ (not shown).

**Fig. 2** shows that the MARCKS(151–175) peptide also produces a concentration-dependent inhibition (decrease in the slope of the lines) of PLC-catalyzed hydrolysis of PIP$_2$ in lipid vesicles. The concentration of lipid vesicles is 4-fold higher in these experiments, and about a 4-fold higher concentration of peptide is required to produce equivalent inhibition. The percentage of inhibition produced by a given concentration of MARCKS(151–175) peptide (or MARCKS) depends on the surface concentration (i.e., peptide/lipid ratio) rather than the bulk concentration of peptide because most of the peptide is bound to the vesicles (28, 29). Control experiments (not shown) demonstrate the percentage of inhibition is independent of the concentration of PLC-$\beta_1$ (0.02–0.2 nM), the order of addition of enzyme and peptide, the free concentration of Ca$^{2+}$ (0.1–300 $\mu$M, as determined by a calcium electrode), and the mole fraction of PIP$_2$ in the vesicles (0.2, 0.5, 1, and 2%). Substituting PE for PC in the LUVs or adding 10% cholesterol did not affect the percentage of inhibition significantly (not shown).

**Activation of PKC or Addition of Ca$^{2+}$-Calmodulin Reverses the Inhibition of PLC by MARCKS(151–175)—** The inhibition of lipid monolayers. This apparatus uses a Wilhelmy plate to monitor the surface pressure of the monolayers and has a feedback circuit that increases the area of the monolayer to maintain a constant surface pressure as an adsorbing solute penetrates the monolayer. Dibucaine (23), signal peptides (24), substance P antagonists (25), myristic acid, and short chain phospholipids (26), which all should penetrate the monolayer, produce an increase in area. Lys$_2$, which does not penetrate the polar head group region when it adsorbs to a bilayer vesicle, does not increase the monolayer area (16).

**Scheme 1. Structure of NBD-PIP$_2$ used in the fluorescence microscopy experiments.**

In brief, 4,5-di-O-phosphoryl-o-myoinositol 1-O-(6-aminohexanoyl)-2-stearoyl-sn-glycerol phosphate tetrasodium salt (7 mg) in 1.2 ml of 0.25 mM tetracylammonium bicarbonate (TEAB) buffer was treated with 5 mg of NBD-aminoacaproyl N-hydroxysuccinimide ester (Pierce) in 400 $\mu$l of dimethylformamide, stirred overnight at room temperature, and lyophilized, and the residue was washed with acetone to remove reagent. Crude NBD-PIP$_2$ was dissolved in water and purified on DEAE-cellulose (NaHCO$_3$ form) by elution with a step gradient of 0.1–1.0 $M$ of TEAB followed by 1.3 $M$ TEAB, in which the NBD-PIP$_2$ eluted (78% yield). The molecular weight, determined by matrix-assisted laser desorption ionization mass spectrometry, was $1151$, which agreed well with the value calculated for the free acid, 1148.

**PLC—** PLC-$\beta_2$ was purified from bovine brain using the method of Rhee et al. (20); we substituted chromatography on a Phenyl Superose HR 5/5 column for their final step. We also performed some experiments with recombinant PLC-$\beta_1$, which was obtainable elsewhere.  

The results obtained with recombinant PLC-$\beta_1$ agreed qualitatively with the results obtained with the native protein. Fluorescent PLC-$\beta_1$ was obtained by labeling recombinant PLC-$\beta_1$ nonspecifically with acrylodan to a level of about 1 acrylodan per PLC.  

**PKC—** Protein kinase C-$\beta$II was a generous gift from Alexandra Newton. It was produced using a baculovirus expression system and was purified to homogeneity as described by Orr et al. (21).

**Monolayer Experiments—** We used an apparatus designed by Fromherz (22) to study the penetration of MARCKS(151–175) into phospholipid monolayers. This apparatus uses a Wilhelmy plate to monitor the surface pressure of the monolayers and has a feedback circuit that increases the area of the monolayer to maintain a constant surface pressure as an adsorbing solute penetrates the monolayer. Dibucaine (23), signal peptides (24), substance P antagonists (25), myristic acid, and short chain phospholipids (26), which all should penetrate the monolayer, produce an increase in area. Lys$_2$, which does not penetrate the polar head group region when it adsorbs to a bilayer vesicle, does not increase the monolayer area (16).

**Binding Measurements—** We used two different techniques to measure the effect of MARCKS(151–175) on the binding of PLC-$\beta_1$ to phospholipid vesicles. The sucrose-loaded vesicles, described as above, were used. In the first technique, described in detail elsewhere (16, 17, 27), uses LUVs loaded with sucrose to increase their density, which are then centrifuged in a solution containing PLC. The concentrations of PLC in the supernatant and the pellet are determined by measuring the rate at which the PLC hydrolyzes PIP$_2$ in micelles. We also measured the increase in fluorescence that occurs when acrylodan-labeled PLC-$\beta_1$ binds to phospholipid vesicles. The addition of MARCKS(151–175) should decrease the fluorescence if it displaces PLC from the vesicles.

**Measurement of PIP$_2$ Hydrolysis—** PIP$_2$ hydrolysis in vesicles containing [3H]PIP$_2$ was initiated by addition of PLC and terminated by addition of 250 $\mu$l of ice-cold 10% trichloroacetic acid and 25 $\mu$l of 10% Triton X-100. The samples, which were incubated on ice until a white precipitate formed, were then centrifuged at 12,000 $\times$ g for 60 s. The supernatant was removed, mixed with 1 ml of chloroform/methanol (2:1), and the upper phase, which contained the [3H]IP$_3$ product, was transferred to a scintillation vial for counting.

2 J. M. Jenco, L. W. Runnels, A. J. Morris, and S. F. Scarlata, submitted for publication.

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**Fig. 1.** MARCKS purified from murine brain inhibits the hydrolysis of PIP\textsubscript{2} in phospholipid vesicles catalyzed by PLC-\(\beta\). LUVs were formed by the extrusion method (17, 27) from a 1:1:1 mixture of PC/PE/PS + 1% PIP\textsubscript{2} in a solution containing 60 mM KCl, 40 mM NaCl, 25 mM HEPES, 3 mM EGTA, 0.3 mM EDTA. At \(t = 0\), PLC-\(\beta_1\) (\(-0.1 \text{nM final concentration}\)) obtained from bovine brain was added to 150 \(\mu\text{M}\) of solution containing LUVs (total lipid = 0.2 mM, accessible lipid = 0.1 mM) and the concentration of MARCKS indicated; the final solution also contained 2 mM dithiothreitol, 0.1 mM bovine serum albumen, 0.8 mM MgCl\textsubscript{2}, 2 mM CaCl\textsubscript{2}, \(-10 \mu\text{M free Ca}^{2+}\) from electrode measurements. Production of radioactive IP\textsubscript{3} due to hydrolysis of tritiated PIP\textsubscript{2} was monitored. The ordinates in Figs. 1, 2, and 3 represent the percentage of accessible PIP\textsubscript{2} (\(1/2\) total) hydrolyzed at the time indicated.

**Fig. 2.** MARCKS(151–175) inhibits the PLC-\(\beta_1\) catalyzed hydrolysis of PIP\textsubscript{2} in phospholipid vesicles. The experimental conditions are as in Fig. 1, except the total lipid concentration is 0.8 mM rather than 0.2 mM. The vertical lines represent the S.D. of \(n \geq 3\) experiments.

PLC-catalyzed hydrolysis of PIP\textsubscript{2} by MARCKS(151–175) can be reversed rapidly by procedures that desorb this peptide or the native MARCKS protein from membranes (Fig. 3). MARCKS(151–175) contains the only serines in MARCKS that are phosphorylated by PKC (30); phosphorylation introduces negative charges (3 phosphates = 6 negative charges) into the peptide, reducing its electrostatic interaction with acidic lipids and producing translocation of the peptide from membrane to solution (13, 28). Fig. 3A shows activation of PKC by adding ATP at time \(t = 2\) min (arrow) reverses the inhibition produced by MARCKS(151–175); similar results were obtained when ATP was present in the solution and PKC was added at \(t = 2\) min (not shown). Three control experiments not illustrated here support this interpretation. First, when PKC is not present, 100 \(\mu\text{M}\) ATP affects neither the ability of PLC to hydrolyze PIP\textsubscript{2} in the absence of peptide nor the ability of MARCKS(151–175) to inhibit PLC (i.e. results were equivalent to the filled and open circles, respectively, in Fig. 3A). Second, when ATP is not present, PKC affects neither the ability of PLC to hydrolyze PIP\textsubscript{2} in the absence of peptide nor the inhibition produced by the peptide. Third, addition of this sample of PKC-\(\beta_1\) alone does not produce significant hydrolysis of PIP\textsubscript{2} (unlike many commercial preparations of PKC, which are contaminated with PLCs).

Calmodulin binds with high (nM) affinity to either MARCKS(151–175) or MARCKS in the presence of calcium (31), displacing both the peptide (28) and the protein (11) from phospholipid vesicles or cell membranes (12). Fig. 3B shows that increasing the level of Ca\textsuperscript{2+}-calmodulin reverses the inhibition of PLC-catalyzed PIP\textsubscript{2} hydrolysis.

A MARCKS Effector Region Peptide Sequesters PIP\textsubscript{2} in Lateral Domains—Our working hypothesis is that MARCKS inhibits the enzyme-catalyzed hydrolysis of PIP\textsubscript{2} by sequestering PIP\textsubscript{2} but not PLC in lateral domains (see Fig. 5B). Earlier fluorescence microscopy observations (19) on phospholipid vesicles (see also Fig. 4D) showed that a peptide corresponding to the basic effector region of MARCKS produces lateral domains enriched in the monovalent acidic lipid PS. Our new measurements show a polyvalent acidic lipid, NBD-labeled PIP\textsubscript{2}, also is sequestered in these domains (Fig. 4B), but acrylodan-labeled PLC-\(\beta_1\) is not (Fig. 4F). Thus domain formation isolates PLC-\(\beta_1\) from its substrate PIP\textsubscript{2}.

In the absence of peptides or proteins, the acidic lipids PS (Fig. 4C) and PIP\textsubscript{2} (Fig. 4A) are distributed uniformly in a phospholipid vesicle; both mixing entropy and electrostatic free energy terms contribute to this random distribution. The energetic factors that allow basic peptides to produce domains enriched in these lipids (e.g. Fig. 4, B and D) have not been fully identified. These factors will be difficult to unravel with MARCKS(151–175), because it adsors to membranes in a complicated manner that involves both electrostatic and hydrophobic interactions, as discussed below. Lys\textsubscript{5}, however, a peptide corresponding to the first five residues of MARCKS(151–175), also forms domains with acidic lipids (not shown). Lys\textsubscript{5} adsors to phospholipid vesicles mainly through electrostatic interactions (16), which suggests that electrostatic interactions alone are sufficient to induce domain formation. As predicted by simple electrostatic models, other polyvalent basic ligands (e.g. tetravalent spermine) produce lateral domains enriched in PS in phospholipid vesicles (not shown). A corollary of our working hypothesis is that Lys\textsubscript{5} and spermine also should inhibit PLCs. They do; both Lys\textsubscript{5} and spermine inhibit the activity of PLC-\(\beta_1\) and -\(\delta_1\), significantly (\(-10\)-fold) at concentrations (\(-1\) mM) that induce domains enriched in NBD-PS (data not shown).

Although hydrophobic interactions are not required for domain formation, two experiments show that these interactions contribute to the membrane binding of MARCKS(151–175). First, elegant spin label experiments suggest the five Phe...
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icles and sequesters the acidic lipids PS and PIP2 in lateral domains are gradually becoming clear. We know the binding of MARCKS(151-175) to phospholipid vesicles depends on at least three factors. First, there is a long range Coulomb attraction between the basic residues in the peptide and the acidic lipids in the membrane. Second, there is a short range electrostatic “Born” or “image charge” repulsion of the positively charged residues on a basic peptide from the low dielectric membrane (16). Third, there is a short range hydrophobic interaction of the five Phe groups in MARCKS(151-175) with the membrane, as suggested by both spin label experiments (29) and the monolayer results reported here. Although each Phe residue could contribute 2.5 kcal/mol of hydrophobic energy to the membrane interaction (38), the observation that MARCKS(151-175) does not bind to electrically neutral PC membranes suggests that the Born repulsion is even stronger (28). The steep sigmoidal dependence of peptide binding on the mole fraction of acidic lipids in the membrane (28) illustrates the primary importance of Coulomb interactions in the membrane binding of MARCKS(151-175).

Our results show both MARCKS(151-175) and the simpler peptide Lys5, which does not penetrate the membrane when it binds (16), sequester acidic lipids in domains and inhibit PLCs. This suggests that electrostatic interactions alone are sufficient to produce domains. A simple model for domain formation is based on the observation that MARCKS(151-175) and Lys5 bind much more strongly to membranes (and presumably to domains) that contain a high fraction of acidic lipids (16, 28, 39, 40). Thus formation of a domain enriched in acidic lipids will enhance the binding of these basic peptides, which in turn could stabilize the domains by reducing the net charge of the membrane and decreasing the free energy stored in the electrical double layer.

Can our results with phospholipid vesicles (Figs. 1-5) be extrapolated to biological membranes? If the positive feedback mechanism shown in Fig. 5 operates in cells, MARCKS must form lateral domains in the plasma membranes of these cells. MARCKS does form lateral domains in the plasma membrane of macrophages (5), and recent experiments show these domains are associated with nascent phagosomes (6). The results reported here suggest that the domains formed by MARCKS in biological membranes should be enriched in PS and PIP2 and may also sequester a number of other important lipids and proteins. For example, PKC binds to membranes through both an unspecified interaction with DAG and an electrostatic interaction with acidic lipids (41, 42); the latter may involve the cluster of basic residues in the C2 domain (43, 44). As expected from this electrostatic interaction, Ca2+-dependent isoforms of PKC are spontaneously sequestered in the domains formed by the
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Specifically, phosphorylation of MARCKS in the nascent phagosome membranes (6, 45). Several studies have demonstrated that phorbol ester activation of PKC promotes phagocytosis of PIP₂ (Figs. 1, 2, and 3). PLC (Fig. 4) produces rapid hydrolysis of PIP₂ (Figs. 1, 2, and 3). Addition of MARCKS peptide (blue rectangles) produces domains enriched in PS, PIP₂, and peptide but not PLC (Fig. 4); domain formation can account for the decrease in the PLC-catalyzed hydrolysis of PIP₂ (Fig. 3). C. phosphorylation by PKC or binding to Ca²⁺-calmodulin produces desorption of MARCKS peptide from membranes, which breaks up the domains and restores the PLC activity, increasing IP₃ production (Fig. 3). In the cell (lower right-hand side of figure), PIP₂ release Ca²⁺, which acts with DAG to stimulate PKC (1); this positive feedback loop is indicated by the plus sign. Several other positive and negative feedback loops involving PLCs exist in cells (66–68), and the biological significance of this putative feedback loop is unknown; we speculate that a spatially localized feedback loop may play a role in macrophage phagocytosis. Cellular phosphatases remove the phosphates from the MARCKS effector region, allowing it to associate with the plasma membrane, as indicated by the arrow to the far right of the figure.

MARCKS peptide and acidic lipids in phospholipid vesicles. PKC-α also colocalizes with MARCKS in the forming phagosomes of macrophages (6, 45). Several studies have demonstrated that phorbol ester activation of PKC promotes phagocytosis (45, 46). Inhibitors of PKC block phagocytosis (45, 46), and PKC phosphorylation of MARCKS appears to be important for the final fission step in macrophage phagocytosis (6).

Taken together, these observations suggest that the phenomena we have investigated may be relevant to phagocytosis (for review see Ref. 47). For example, the positive feedback loop illustrated in Fig. 5 could act in the spatially restricted "neck" region joining a nascent phagosome to the plasma membrane. Specifically, phosphorylation of MARCKS in the nascent phagosome should cause its effector region to desorb from the plasma membrane (10–12, 28). Desorption of the effector region will produce two effects. First, desorption will release any PIP₂ sequestered in the forming phagosome, as we have observed with phospholipid vesicles. Second, desorption of the effector region will cause the five Phε residues to move out of the membrane and will transiently decrease the surface pressure in the cytoplasmic monolayer of the phagosome. A small reduction in the surface pressure of a monolayer (e.g. from 25 to 20 mN/m) increases significantly (e.g. 10-fold) the activity of PLC-β1 and other PLCs (26). Thus, in the neck region PLC activity will be stimulated by both increased availability of substrate and decreased local surface pressure; the consequent increase in hydrolysis of PIP₂ will produce a high local concentration of DAG, a known membrane destabilizing agent, and fusogen (48–52). Both the decrease in the surface pressure (53–55) and the increase in local DAG concentration could contribute to fission of the neck region.

Finally, our demonstration that basic regions on proteins can sequester PIP₂ reversibly in domains may be relevant to several other biologically important phenomena. For example, recent studies have implicated PIP₂ in calcium-mediated exocytosis (56–59) and in the interaction of cytoskeletal proteins such as profilin with membranes (60–64).
43. Sutton, R. B., Davletov, B. A., Berghuis, A. M., Sudhof, T. C., and Sprang, S. R. (1995) Cell \textbf{80}, 929–938
44. Ponting, C. P., and Parker, P. J. (1996) \textit{Protein Sci.} \textbf{5}, 162–166
45. Zheleznyak, A., and Brown, E. J. (1992) \textit{J. Biol. Chem.} \textbf{267}, 12042–12046
46. Wright, S. D., and Silverstein, S. C. (1982) \textit{J. Exp. Med.} \textbf{156}, 1149–1164
47. Allen, L. A., and Aderem, A. (1996) \textit{Current Opin. Immunol.} \textbf{8}, 36–40
48. Das, S., and Rand, R. P. (1986) \textit{Biochemistry} \textbf{25}, 2882–2889
49. Sanchez-Migallon, M. P., Aranda, F. J., and Gomez-Fernandez, J. C. (1994) \textit{Arch. Biochem. Biophys.} \textbf{314}, 205–216
50. Goni, F. M., Nieva, J. L., Basanez, G., Fidelio, G., and Alonso, A. (1996) \textit{Biochem. Soc. Trans.} \textbf{22}, 839–844
51. Werner, M. H., Bielawska, A. E., and Hannun, Y. A. (1992) \textit{Mol. Pharmacol.} \textbf{41}, 382–386
52. Rand, R. P., and Parsegian, V. A. (1996) \textit{Annu. Rev. Physiol.} \textbf{48}, 201–212
53. Helm, C. A., Israelschvili, J. N., and McGuigan, P. M. (1992) \textit{Biochemistry} \textbf{31}, 1794–1805
54. Evans, E. A., and Skalak, R. (1980) \textit{Mechanics and Thermodynamics of Biomembranes}, pp. 85–91, CRC Press, Boca Raton, FL
55. Cev, G., and Marsh, D. (1987) \textit{Phospholipid Bilayers}, pp. 348–357, Wiley-Interscience, New York
56. Eberhard, D. A., Cooper, C. L., Low, M. G., and Holz, R. W. (1990) \textit{Biochem. J.} \textbf{268}, 15–25
57. Hay, J. C., and Martin, T. F. (1993) \textit{Nature} \textbf{366}, 572–575
58. Hay, J. C., Fisette, P. L., Jenkins, G. H., Fukami, K., Takenawa, T., Anderson, R. A., and Martin, T. F. (1995) \textit{Nature} \textbf{374}, 173–177
59. De Camilli, P., Emr, S. D., McPherson, P. S., and Novick, P. (1996) \textit{Science} \textbf{271}, 1533–1539
60. Lassing, I., and Lindberg, U. (1989) \textit{Nature} \textbf{341}, 472–474
61. Goldschmidt-Clermont, P. J., Machesy, L. M., Balldassare, J. J., and Pollard, T. D. (1990) \textit{Science} \textbf{247}, 1575–1578
62. Goldschmidt-Clermont, P. J., Kim, J. W., Machesy, L. M., Rhee, S. G., and Pollard, T. D. (1991) \textit{Science} \textbf{251}, 1231–1233
63. Ostrander, D. R., Gorman, J. A., and Carman, G. M. (1995) \textit{J. Biol. Chem.} \textbf{270}, 27945–27950
64. Janmey, P. A. (1994) \textit{Annu. Rev. Physiol.} \textbf{56}, 169–191
65. Pap, E. H., Hanicak, A., van Hoek, A., Wirtz, K. W., and Visser, A. J. (1995) \textit{Biochemistry} \textbf{34}, 9118–9125
66. Ryu, S. H., Kim, U.-H., Wahl, M. I., Brown, A. B., Carpenter, G., Huang, K.-P., and Rhee, S. G. (1990) \textit{J. Biol. Chem.} \textbf{265}, 17941–17945
67. Running Deer, J. L., Hurley, J. B., and Yarfitz, S. L. (1995) \textit{J. Biol. Chem.} \textbf{270}, 12623–12628
68. Exton, J. H. (1990) \textit{J. Biol. Chem.} \textbf{265}, 1–4
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