We provided genetic and biochemical evidence that supported the conclusion that the product of *ppgB* gene of *Escherichia coli* exhibited diacylglycerol pyrophosphate (DGPP) phosphatase activity. DGPP phosphatase activity was absent in *ppgB* mutant cells and was expressed at high levels in cells carrying the wild-type *ppgB* gene on a runaway replication plasmid. The *ppgB* mutant has been primarily characterized by a defect in phosphatidate (PA) phosphatase activity and also exhibits defects in lyso-PA phosphatase and phosphatidylglycerophosphate phosphatase activities. The defective PA phosphatase in the *ppgB* mutant was shown to be a Mg\(^{2+}\)-independent PA phosphatase activity of the DGPP phosphatase enzyme. We characterized DGPP phosphatase activity in membranes from cells overproducing the *ppgB* gene product. DGPP phosphatase catalyzed the dephosphorylation of the \( \beta \) phosphate of DGPP to form PA followed by the dephosphorylation of PA to form diacylglycerol. The specificity constant \( (V_{\text{max}}/K_m) \) for DGPP was 9.3-fold greater than that for PA. The pH optimum for the DGPP phosphatase reaction was 6.5. Activity was independent of a divalent cation requirement, was potently inhibited by Mn\(^{2+}\) ions, and was insensitive to inhibition by N-ethylmaleimide. Pure DGPP phosphatase from *Saccharomyces cerevisiae* was shown to be similar to the *E. coli* DGPP phosphatase in its ability to utilize lyso-PA and phosphatidylglycerophosphate as substrates in vitro.

DGPP\(^1\) is a novel phospholipid that was first identified from the plant *Catharanthus roseus* (1). It contains a pyrophosphate group attached to DG (Fig. 1). DGPP has subsequently been found in a variety of plants (2, 3) and in the yeast *S. cerevisiae* (4). DGPP is synthesized from PA and ATP via the reaction catalyzed by the membrane-associated enzyme PA kinase (1) and is dephosphorylated to PA via the reaction catalyzed by the membrane-associated enzyme DGPP phosphatase (4) (Fig. 1). Recent studies have shown that DGPP and PA levels accumulate in plant tissues upon G protein activation (3, 5, 6). PA accumulation is the result of activated phospholipase C/DG kinase activities and the result of phospholipase D activity (5, 6). DGPP accumulation is the result of activated PA kinase activity (3). PA is a phospholipid intermediate used for the synthesis of phospholipids and triacylglycerols (7–9) and plays a role in cell signaling. PA regulates the activity of several lipid-dependent enzymes (10–13) and has mitogenic effects in mammalian cells (14–17). In addition, PA is the source of the signaling lipids DG (Fig. 1) (18) and lyso-PA (17). It is not yet clear what role DGPP plays in lipid metabolism and cell signaling, but it has been suggested that DGPP may function to attenuate the signaling functions of PA or may serve as a signaling molecule itself (3, 4).

DGPP phosphatase has recently been purified to homogeneity from *S. cerevisiae* (4). The enzyme has a subunit molecular mass of 34 kDa (4). When DGPP is supplied as a substrate in vitro, the enzyme removes the \( \beta \) phosphate of DGPP to generate PA and then removes the \( \alpha \) phosphate to generate DG (4). Although DGPP phosphatase can also utilize PA as a substrate in the absence of DGPP, the enzyme has a 10-fold higher specificity constant for DGPP (4). The PA phosphatase activity of the DGPP phosphatase enzyme is distinct from the conventional PA phosphatase enzyme (8, 19, 20) that is used for the synthesis of phospholipids and triacylglycerols in *S. cerevisiae* (4). The conventional PA phosphatase enzyme has a Mg\(^{2+}\) ion requirement and is sensitive to inhibition by NEM (19, 20). The PA phosphatase activity of the DGPP phosphatase enzyme does not have a Mg\(^{2+}\) ion requirement and is insensitive to NEM (4).

Two forms of PA phosphatase activity exist in mammalian cells. The conventional form of the enzyme associates with the endoplasmic reticulum, has a Mg\(^{2+}\) ion requirement, is inhibited by NEM, and is responsible for the synthesis of phospholipids and triacylglycerols (21). The other form of the enzyme is associated with the plasma membrane, does not have a Mg\(^{2+}\) ion requirement, is insensitive to NEM, and is thought to be involved in lipid signaling pathways (21).

A PA phosphatase activity has been identified from the membrane fraction of *Escherichia coli* (22), and mutants defective in this activity have been isolated that are defined by the *ppgB* gene (23–25). The PA phosphatase activity in *ppgB* mutants has been measured using the assay conditions described for the conventional Mg\(^{2+}\)-dependent PA phosphatase enzyme (23–25). However, these assay conditions would not reveal the existence of a Mg\(^{2+}\)-independent PA phosphatase activity such as that displayed by the DGPP phosphatase enzyme. With this in mind, we tested the hypothesis that *E. coli* possessed a Mg\(^{2+}\)-independent PA phosphatase activity of a DGPP phosphatase.
phatase enzyme. In this study we provided both genetic and biochemical evidence that the pgpB gene encoded for a DGPP phosphatase activity that can also utilize PA, lyso-PA, and phosphatidylglycerophosphate as substrates.

**EXPERIMENTAL PROCEDURES**

**Materials**

All chemicals were reagent grade. Growth medium supplies were purchased from Difco. Radiochemicals and ENHANCE were from DuPont NEN. Scintillation counting supplies were from National Diagnostics. Nucleotides, glycerol 3-phosphate, NEM, Triton X-100, isopropyl β-D-thiogalactoside, and bovine serum albumin were purchased from Sigma. Phospholipids were purchased from Avanti Polar Lipids and Sigma. Protein assay reagent was purchased from Bio-Rad. Silica Gel 60 thin-layer chromatography plates were from EM Science. E. coli DG kinase was obtained from Lipidex Inc.

**Methods**

**Strains and Growth Conditions**—The E. coli strains used in this work are listed in Table I. Strains CF10 and CF20 are mutants with disrupted alleles in the pgpA and pgpB genes, respectively (25). Strain CF30 is a mutant with disrupted alleles in both the pgpA and pgpB genes (25). Plasmid pTI5-217 is a runaway replication plasmid that contains the pgpB gene under the control of a tac promoter (24). Cultures were grown in LB medium and maintained on LB agar. Growth media were supplemented with ampicillin (100 μg/ml), kanamycin (50 μg/ml), or chloramphenicol (25 μg/ml) as needed. Cultures were grown to the exponential phase of growth at 30°C and then harvested by centrifugation. Strain JM103 bearing plasmid pTI5-217 was shifted to 37°C and incubated for 1 h to increase the plasmid copy number (24). Isopropyl β-D-thiogalactoside (2 mm) was then added to the growth medium to induce the expression of the pgpB gene product (24). After incubation for 1 h, the induced cells were harvested by centrifugation.

**Preparation of Enzymes**—All steps were performed at 5°C. E. coli cells were suspended in 50 mM Tris-maleate (pH 7.0) buffer containing 0.1 mM CDP-DG, 1.5 mM Triton X-100, 10 mM 2-mercaptoethanol, and enzyme protein in a total volume of 0.1 ml. The chloroform-soluble phospholipid product of the reaction, PA, was analyzed by thin-layer chromatography on potassium oxalate-treated plates using the solvent system chloroform/acetone/methanol/glacial acetic acid/water (50:15:13:12:4) (4). The positions of the labeled phospholipids on the chromatograms were determined by autoradiography. The amount of labeled phospholipids was determined by scintillation counting.

PA phosphatase activity was measured by following the release of water-soluble [32P]P, from chloroform-soluble [32P]P(DGPP (5,000–10,000 cpm/nmol) or by following the formation of [32P]P from [α-32P]P(DGPP (2,000–5,000 cpm/nmol) as described by Wu et al. (4). The reaction mixture contained 50 mM Tris-maleate buffer (pH 7.0), 0.1 mM PA, 1 mM Triton X-100, 2 mM Na2EDTA, 10 mM 2-mercaptoethanol, and enzyme protein in a total volume of 0.1 ml. The chloroform-soluble phospholipid product of the reaction, PA, was analyzed and quantitated using thin-layer chromatography on potassium oxalate-treated plates using the solvent system chloroform/acetone/methanol/glacial acetic acid/water (50:15:13:12:4) (4). The positions of the labeled phospholipids on the chromatograms were determined by autoradiography. The amount of labeled phospholipids was determined by scintillation counting.

PA phosphatase activity was measured by following the release of water-soluble [32P]P, from chloroform-soluble [32P]P(lyso-PA (20,000 cpm/nmol) (28). The reaction mixture contained 50 mM Tris-maleate buffer (pH 7.0), 0.1 mM PA, 1 mM Triton X-100, 2 mM Na2EDTA, 10 mM 2-mercaptoethanol, and enzyme protein in a total volume of 0.1 ml. The chloroform-soluble phospholipid product of the reaction, PA, was analyzed and quantitated using thin-layer chromatography on potassium oxalate-treated plates using the solvent system chloroform/acetone/methanol/glacial acetic acid/water (50:15:13:12:4) (4). The positions of the labeled phospholipids on the chromatograms were determined by autoradiography. The amount of labeled phospholipids was determined by scintillation counting.

PA phosphatase activity was measured by following the release of water-soluble [32P]P, from chloroform-soluble [32P]P(lyso-PA (20,000 cpm/nmol) (28). The reaction mixture contained 50 mM Tris-maleate buffer (pH 7.0), 0.1 mM PA, 1 mM Triton X-100, 2 mM Na2EDTA, 10 mM 2-mercaptoethanol, and enzyme protein in a total volume of 0.1 ml. The chloroform-soluble phospholipid product of the reaction, PA, was analyzed and quantitated using thin-layer chromatography on potassium oxalate-treated plates using the solvent system chloroform/acetone/methanol/glacial acetic acid/water (50:15:13:12:4) (4). The positions of the labeled phospholipids on the chromatograms were determined by autoradiography. The amount of labeled phospholipids was determined by scintillation counting.

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**RESULTS**

**PA Phosphatase and DGPP Phosphatase Activities in pgpB Mutant Cells and in Cells Overexpressing the pgpB Gene Product**—The original E. coli pgpB mutant was isolated in a biochemical screen that was designed to isolate cells defective in PG phosphatase activity (23). Biochemical analysis has

**Table 1**

| Strain  | Relevant genotype          | Relevant phenotype                      | Ref. |
|---------|--------------------------|----------------------------------------|------|
| TI80A*  | recA+ srh::Tn10          | Wild-type phosphatase activity          | 25   |
| JM103   | lac+                     | Wild-type phosphatase activity          | 24   |
| CF10    | pgpB235::Kan'           | Defective in PGP phosphatase activity   | 25   |
| CF20    | pgpB159::Amp'           | Defective in PA phosphatase, lyso-PA phosphatase, and PGP phosphatase activities | 25   |
| CF30    | pgpB235::Kan'pgpB159::Amp' | Defective in PA phosphatase, lyso-PA phosphatase, and PGP phosphatase activities | 25   |
| JM103/pTI5–217 | pgpBCam' | Elevated PA phosphatase, lyso-PA phosphatase, and PGP phosphatase activities | 24   |

* Isogenic parent strain of CF10, CF20, and CF30.
The E. coli 

The E. coli pgpB Gene Encodes for DGPP Phosphatase

FIG. 2. PA phosphatase and DGPP phosphatase activities in E. coli strains. Cells from the indicated strains were grown to the exponential phase of growth and harvested by centrifugation, and the membrane fraction was prepared. Panel A, PA phosphatase activity was measured in the absence (open bars) and presence (hatched bars) of MgCl₂. The reaction measured in the absence of MgCl₂ contained 2 mM Na₂EDTA. Panel B, DGPP phosphatase activity was measured using [β-³²P]DGPP as the substrate. activities (24). The overexpression of the pgpB gene product also resulted in elevated levels (370-fold) of the PA phosphatase activity that was measured in the absence of Mg²⁺ ions (Fig. 2A).

We next examined the levels of DGPP phosphatase activity. In wild-type cells, the specific activity of DGPP phosphatase (Fig. 2B) was approximately 6-fold greater than that of the PA phosphatase activities measured in the presence or absence of Mg²⁺ ions (Fig. 2A). DGPP phosphatase activity was not detected in pgpB mutant cells (Fig. 2B). Furthermore, the specific activity of DGPP phosphatase from cells that overexpress the pgpB gene product was 310-fold greater than that found in wild-type cells (Fig. 2B).

A pgpA mutant was isolated in the same biochemical screen that identified the pgpB mutant (23). This mutant exhibits a defect in PGP phosphatase activity, while PA phosphatase and lyso-PA phosphatase activities are present at wild-type levels (23). We examined PA phosphatase (measured in the presence or absence of Mg²⁺ ions) and DGPP phosphatase activities in a mutant in which the pgpA gene was disrupted (25). This mutant has the same properties as the original pgpA mutant (23, 25). The levels of PA phosphatase (Fig. 2A) and DGPP phosphatase (Fig. 2B) activities from pgpA mutant cells were the same as that from its isogenic wild-type parent (Fig. 2B).

Properties DGPP Phosphatase and Mg²⁺-independent PA Phosphatase Activities—The membrane fraction of strain JM103/pT15-217 was used for the characterization of DGPP phosphatase and Mg²⁺-independent PA phosphatase activities. The elevated expression of the pgpB gene product from these cells represents a considerable enrichment of these activities that is equivalent to a nearly 600-fold purification over that expressed in the cell extract of wild-type E. coli.

We examined the thermostability of DGPP phosphatase and PA phosphatase activities. Membranes were heated at 50 °C, and samples were removed at various time intervals. DGPP phosphatase and PA phosphatase activities were then measured at 30 °C under standard assay conditions. In this experiment, DGPP phosphatase and PA phosphatase activities were inactivated with identical kinetics (Fig. 3). A t½ of 55 s for the inactivation of both phosphatase activities was calculated from a replot of the log activity versus time of incubation (Fig. 3).

The time dependence of the DGPP phosphatase reaction was examined using [α-³²P]DGPP as the substrate (Fig. 4). By using the α-labeled substrate we could distinguish the removal of the β phosphate of DGPP from the removal of the pyrophosphate moiety of DGPP. The water-soluble fraction of the reaction was also analyzed. DGPP phosphatase catalyzed the dephosphorylation of the β phosphate of DGPP, and after an initial lag period, catalyzed the dephosphorylation of the α

shown that pgpB mutant cells are also defective in PA phosphatase and lyso-PA phosphatase activities (23). These mutant cells exhibit the greatest reduction in PA phosphatase activity (23). We examined the levels of PA phosphatase activity in membranes derived from a well characterized mutant with a pgpB allele that has been disrupted by the insertion of an Amp' gene fragment (25). This mutant has the same properties as the original pgpB mutant (23, 25). We measured PA phosphatase activity in the presence of Mg²⁺ ions as described previously (23–25). The level ofPA phosphatase activity in the pgpB mutant was 75% lower than the activity found in its isogenic wild-type parent (Fig. 2A). We next examined if E. coli possessed a Mg²⁺-independent PA phosphatase activity. In these experiments, the reaction mixture also contained Na₂EDTA, and the membranes used for the enzyme assays were dialyzed to remove divalent cations. Indeed, a Mg²⁺-independent activity was measured in E. coli. Whether Mg²⁺ ions were present or not, the levels of PA phosphatase activity were similar in wild type cells and were reduced to the same extent in pgpB mutant cells (Fig. 2A).

The pgpB gene has been cloned, sequenced, and shown to be the structural gene for the PA phosphatase activity defined by the pgpB mutation (24). The protein product encoded by the pgpB gene is expressed at elevated levels in cells bearing the wild-type pgpB gene under the control of a tac promoter on a runaway replication plasmid (24). These cells also overexpress PA phosphatase, lyso-PA phosphatase, and PGP phosphatase activities (24). The overexpression of the pgpB gene product also resulted in elevated levels (370-fold) of the PA phosphatase activity that was measured in the absence of Mg²⁺ ions (Fig. 2A).

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2 GenBank™ accession no. M23628.
phosphate of DGPP (Fig. 4B). Thus, DGPP phosphatase dephosphorylated DGPP to PA and subsequently dephosphorylated PA to DG.

The kinetics of activity toward DGPP and PA were examined using Triton X-100/phospholipid-mixed micelles. This mixed micellar system allows the analysis of lipid-dependent enzymes using surface dilution kinetics, a model system which mimics the physiological surface of the membrane (30). Positive cooperative kinetics were exhibited with respect to the surface concentration of DGPP (Fig. 5). The data were analyzed according to the Hill equation using the EZ-FIT Enzyme Kinetic Model Fitting Program (31). This analysis yielded a Hill number of 1.45, a $K_m$ value for DGPP of 2.3 mol%, and a $V_{max}$ value of 2,167 nmol/min/mg. Positive cooperative kinetics were also exhibited with respect to the surface concentration of PA (Fig. 5). Analysis of this data according to the Hill equation gave a Hill number of 3.9, a $K_m$ value for PA of 3.1 mol%, and a $V_{max}$ value of 513 nmol/min/mg. The specificity constants ($V_{max}/K_m$) for DGPP and PA were 942 and 101, respectively.

Maximum DGPP phosphatase activity was observed at pH 6.5 (Fig. 6). DGPP phosphatase activity was independent of any divalent cation requirement and was stimulated (35%) by 2 mM Na$_2$EDTA. DGPP phosphatase activity was not significantly affected by the addition of 5 mM NEM to the assay system. The addition of Mn$^{2+}$ ions to the assay resulted in a dose-dependent inactivation of DGPP phosphatase activity (Fig. 7). This inactivation followed positive cooperative kinetics (Hill number of 3) and the IC$_{50}$ value for MnCl$_2$ was calculated to be 34 mM. PA phosphatase activity was also inhibited by Mn$^{2+}$ ions (data not shown). Furthermore, the Mg$^{2+}$-independent PA phosphatase activity was stimulated (25%) by 2 mM Na$_2$EDTA and was not significantly affected by 5 mM NEM. The addition of 1 mM MnCl$_2$ to the assay systems for DGPP phosphatase and PA phosphatase (measured in the presence or absence of Mg$^{2+}$ ions) resulted in the total inhibition of all three activities in wild-type cells, in cells that overproduced the pgpB gene product, and in pgpA mutant cells (data not shown).

The E. coli pgpB Gene Encodes for DGPP Phosphatase—Based on the ability of the pgpB gene product to utilize DGPP, PA, lyso-PA, and PGP as substrates, we examined the ability of pure DGPP phosphatase from S. cerevisiae to utilize lyso-PA and PGP as substrates. The yeast DGPP phosphatase enzyme catalyzed the dephosphorylation of [32P]lyso-PA to PA in a dose-dependent manner (Fig. 8). The enzyme exhibited positive cooperative kinetic behavior toward the surface concentration of lyso-PA (Hill number of 1.5). The $K_m$ and $V_{max}$ values for the reaction were calculated according to the Hill equation to be 0.74 mol% and 167 nmol/min/mg.

The PGF phosphatase activity of yeast DGPP phosphatase
was measured by a coupled enzyme assay using PGP synthase activity to generate the PGP substrate for the reaction. Membranes from E. coli strain CF30 were used as the source of PGP synthase. This strain contains disrupted alleles in both the pgpA and pgpB genes and is partially defective in PGP phosphatase activity (25). Thus, the PGP synthesized via the PGP synthase reaction accumulates and can be used for the reaction catalyzed by the yeast DGPP phosphatase. The yeast DGPP phosphatase was incubated with CDP-DG,[3H]glycerol-3-phosphate, and the E. coli membranes. Following incubation, the chloroform-soluble products of the reaction were analyzed by thin-layer chromatography. In this experiment, yeast DGPP phosphatase catalyzed a time-dependent reaction utilizing the PGP generated in the PGP synthase reaction for the synthesis of PG (Fig. 9).

**DISCUSSION**

In this study we tested the hypothesis that the E. coli pgpB gene encoded a DGPP phosphatase activity. The product of the pgpB gene is a 28-kDa membrane-associated enzyme which has the ability to dephosphorylate PA, lyso-PA, and PGP in vitro (23–25). We questioned whether the PA phosphatase activity defined by the pgpB gene (23) was in fact a Mg2+-independent activity of the DGPP phosphatase enzyme similar to the activity characterized in S. cerevisiae (4). We showed that E. coli possessed a PA phosphatase activity that was independent of any Mg2+ ion requirement and was insensitive to inhibition by NEM. The expression of the Mg2+-independent PA phosphatase activity was reduced in pgpB mutant cells and elevated in cells overexpressing the wild-type pgpB gene. A residual amount of PA phosphatase activity was present in the pgpB mutant indicating that the pgpB gene product was not the only source of PA phosphatase activity in E. coli. DGPP phosphatase activity was present in E. coli and its expression was not detected in the pgpB mutant. Thus, the lack of DGPP phosphatase activity was the most pronounced phenotype of the pgpB gene.

Our data indicated that the DGPP phosphatase and PA phosphatase activities were due to a single enzyme. DGPP phosphatase activity was overproduced in cells overexpressing the pgpB gene product and the extent of its overproduction paralleled that of the Mg2+-independent PA phosphatase activity. Both activities were inhibited by Mn2+ ions and were insensitive to inhibition by NEM. In addition, DGPP phosphatase and PA phosphatase activities exhibited the same kinetics of temperature inactivation. The substrate dependence experiments demonstrated that DGPP was a better substrate for the enzyme when compared with PA. The specificity constant for DGPP was 9.3-fold higher than that for PA. In addition, the enzyme exhibited greater cooperative kinetic behavior toward PA when compared with DGPP. This substrate preference was consistent with the elevated DGPP phosphatase activity relative to the PA phosphatase activity found in the various E. coli strains.

The enzymological properties (e.g. dephosphorylation of the β phosphate of DGPP followed by the dephosphorylation of the α phosphate of DGPP, pH optimum, stimulation by Na+,EDTA, inhibition by Mn2+ ions, and insensitivity to NEM) of the E. coli DGPP phosphatase were similar to those described for the enzyme from S. cerevisiae (4). Moreover, the yeast DGPP phosphatase was similar to the E. coli enzyme with respect to the utilization of lyso-PA and PGP as substrates in vitro.

PA phosphatase enzymes have been purified from rat liver (32, 33) and porcine thymus (34) membranes. Interestingly, these mammalian enzymes (32–34) share properties that are strikingly similar to those of the PA phosphatase activity of the DGPP phosphatase enzymes from S. cerevisiae (4) and E. coli. For example, the mammalian enzymes do not have a Mg2+ ion requirement, they are insensitive to inhibition by NEM, and they are inhibited by Mn2+ ions (32–34). In addition, the rat liver Mg2+-independent PA phosphatase, like the DGPP phosphatase enzymes from S. cerevisiae and E. coli, can utilize lyso-PA as a substrate in vitro (35). It will be interesting to examine whether this rat liver Mg2+-independent PA phosphatase can utilize DGPP as a substrate.

The rat liver Mg2+-independent PA phosphatase also utilizes ceramide 1-phosphate and sphingosine 1-phosphate as substrates in vitro (35). These lipid phosphate compounds as well as PA and lyso-PA have been shown to be mediators of cell activation and signal transduction in mammalian cells (17, 36–39). It has been suggested that the rat liver Mg2+-independent PA phosphatase may play a role in regulating the balance of these lipid mediators (35). However, it is unclear whether the rat liver Mg2+-independent PA phosphatase uti-
lizes all of these substrates in vivo. Similarly, it is unclear whether the DGPP phosphatase enzymes from S. cerevisiae and E. coli utilize all of their respective substrates in vivo. It is known that the PGP phosphatase activity encoded by the E. coli pgpB gene product is not essential for the synthesis of PG in vivo (25).

An understanding of the function of DGPP in phospholipid metabolism and cell signaling will require a combination of genetic, molecular, and biochemical approaches. This will require the cloning of those genes that encode enzymes involved in DGPP metabolism and the purification and characterization of the products of these genes. Our laboratories have initiated these studies by purifying a PA kinase from plants (2) and purifying a DGPP phosphatase from S. cerevisiae (4). In preliminary studies, we have identified PA kinase activities in S. cerevisiae (4) and in E. coli (3) and are developing methods for their purification. The finding of DGPP phosphatase and PA kinase activities in S. cerevisiae (4) and E. coli suggests a potential role of DGPP metabolism in these organisms that may be similar to that described in plants (3). The identification of the pgpB gene as the gene encoding for a DGPP phosphatase in E. coli should facilitate genetic and molecular studies aimed at gaining an understanding of the role(s) of DGPP metabolism in E. coli and may also facilitate the isolation of genes encoding for DGPP phosphatase from eukaryotic organisms.

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REFERENCES
1. Wissing, J. B., and Behrbohm, H. (1993) FEBS Lett. 315, 95–99
2. Wissing, J. B., and Behrbohm, H. (1993) Plant Physiol. 102, 1243–1249
3. Munnik, T., de Vrije, T., Irvine, R. F., and Musgrave, A. (1996) J. Biol. Chem. 271, 15708–15715
4. Wu, W.-I., Liu, Y., Riedel, B., Wissing, J. B., Fischl, A. S., and Carman, G. M. (1996) J. Biol. Chem. 271, 1868–1876
5. Quarshy, L. M., Yueh, Y. G., Cheshire, J. L., Keller, L. R., Snell, W. J., and Crain, R. C. (1992) J. Cell Biol. 116, 717–744
6. Munnik, T., Arisz, S. A., de Vrije, T., and Musgrave, A. (1995) Plant Cell 7, 2197–2210
7. Carman, G. M., and Henry, S. A. (1989) Annu. Rev. Biochem. 58, 635–669
8. Carman, G. M., and Zeimetz, G. M. (1996) J. Biol. Chem. 271, 13293–13296
9. Kennedy, E. P. (1986) in Lipids and Membranes: Past, Present and Future (Op den Kamp, J. A. F., Roelofsen, B., and Wirtz, K. W. A. eds) pp. 171-206, Elsevier Science Publishers B. V., Amsterdam
10. Bae-Lee, M., and Carman, G. M. (1990) J. Biol. Chem. 265, 7221–7226
11. Moritz, A., DeGraan, P. N. E., Gispen, W. H., and Wirtz, K. W. A. (1992) J. Biol. Chem. 267, 7207–7210
12. Jones, G. A., and Carpenter, G. (1993) J. Cell Biol. 124, 1–4
13. Bhat, B. G., Wang, P., and Coleman, R. A. (1994) J. Biol. Chem. 269, 13172–13178
14. Moolenaar, W. H., Kruijer, W., Tilly, B. C., Verlaan, I., Bierman, A. J., and de Laat, S. W. (1996) Nature 381, 171–173
15. Yu, C.-L., Tsai, M.-H., and Stacey, D. W. (1988) Cell 52, 63–71
16. Gomez-Munoz, A., Martin, A., O’Brien, L., and Brindley, D. N. (1994) J. Biol. Chem. 269, 8937–8943
17. Moolenaar, W. H. (1995) J. Biol. Chem. 270, 12949–12952
18. Exton, J. H. (1990) J. Biol. Chem. 265, 1–4
19. Lin, Y.-P., and Carman, G. M. (1989) J. Biol. Chem. 264, 8641–8645
20. Morlock, K. R., McLaughlin, J. J., Lin, Y.-P., and Carman, G. M. (1991) J. Biol. Chem. 266, 3586–3593
21. Janal, Z., Martin, A., Gomez-Munoz, A., and Brindley, D. N. (1991) J. Biol. Chem. 266, 2988–2996
22. Van den Bosch, H., and Vagelos, P. R. (1970) Biochim. Biophys. Acta 218, 233–248
23. Icho, T., and Raetz, C. R. H. (1983) J. Bacteriol. 153, 722–730
24. Funk, C. R., Zimniak, L., and Dowhan, W. (1992) J. Bacteriol. 174, 205–213
25. Walsh, J. P., and Bell, R. M. (1986) J. Bacteriol. 161, 6239–6247
26. Carman, G. M., and Fischl, A. S. (1992) Methods Enzymol. 209, 305–312
27. Carman, G. M., and Le, L.-Y. (1991) Methods Enzymol. 197, 548–553
28. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
29. Carman, G. M., Deems, R. A., and Dennis, E. A. (1995) J. Biol. Chem. 270, 18711–18714
30. Perrella, F. (1988) Anal. Biochem. 174, 437–447
31. Waggoner, D. W., Martin, A., Dewald, J., Gomez-Munoz, A., and Brindley, D. N. (1995) J. Biol. Chem. 270, 18423–18429
32. Fleming, I. N., and Yeaman, S. J. (1995) Biochem. J. 308, 983–989
33. Carman, G. M., and Zeimetz, G. M. (1996) J. Biol. Chem. 271, 16506–16509
34. Devecha, N., and Irvine, R. F. (1995) Cell 80, 269–278
35. Kolesnick, R., and Golde, D. W. (1994) Cell 77, 325–328
36. Hannun, Y. A. (1994) J. Biol. Chem. 269, 3125–3128
37. Spiegel, S., and Milstien, S. (1995) J. Membr. Biol. 146, 225–237