Regulation of Phosphatidate Phosphatase Activity by Inositol in *Saccharomyces cerevisiae*[†]

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Regulation of phosphatidate phosphatase (EC 3.1.34) activity was examined in *Saccharomyces cerevisiae* cells supplemented with phospholipid precursors. Addition of inositol to the growth medium of wild-type cells resulted in a twofold increase in phosphatidate phosphatase activity. The increase in phosphatidate phosphatase activity was not due to soluble effector molecules, and inositol did not have a direct effect on enzyme activity. The phosphatidate phosphatase activity associated with the mitochondrial, microsomal, and cytosolic fractions of the cell was regulated by inositol in the same manner. Cells supplemented with inositol had elevated phospholipid levels and reduced triacylglycerol levels compared with unsupplemented cells. Serine, ethanolamine, and choline did not significantly affect the phosphatidate phosphatase activity of cells grown in the absence or presence of inositol. Enzyme activity was not regulated in inositol biosynthesis regulatory mutants, suggesting that regulation by inositol is coupled to regulation of inositol biosynthesis. Phosphatidate phosphatase activity was pleiotropically expressed in structural gene mutants defective in phospholipid biosynthesis. These results suggested that phosphatidate phosphatase was regulated by inositol at a genetic level.

Phosphatidate (PA) is an important branch point intermediate for the synthesis of phospholipids, diacylglycerols, and triacylglycerols in the unicellular eucaryote *Saccharomyces cerevisiae* (19). In the primary pathway of phospholipid biosynthesis, phosphatidylcholine is derived from PA via the reaction sequence PA → CDP-diacylglycerol → phosphatidylserine → phosphatidylethanolamine → phosphatidylmonomethylethanolamine → phosphatidyl dimethylethanolamine → phosphatidylcholine (19). Phosphatidylinositol, phosphatidylglycerol, and cardiolipin are also derived from CDP-diacylglycerol (19). Diacylglycerols and triacylglycerols are derived from PA via the reaction sequence PA → diacylglycerol → triacylglycerol (19). The diacylglycerol synthesized from PA can also be used for synthesis of phosphatidylethanolamine and phosphatidylcholine by the auxiliary CDP-ethanolamine- and CDP-choline-based pathways, respectively (28), when synthesis of phosphatidylserine is blocked and cells are supplemented with ethanolamine and choline (2, 32, 36).

Addition of ethanolamine or choline to inositol-containing growth medium leads to repression of the enzymes (CDP-diacylglycerol synthase [24], phosphatidylserine synthase [3, 7, 30, 37], phosphatidylserine decarboxylase [8], and the phospholipid N-methyltransferases [8, 30, 42, 43, 45, 46]) in the primary pathway for phosphatidylcholine biosynthesis. Repression of these enzymes by ethanolamine and choline is absolutely dependent on inositol (3, 24, 30, 37, 45, 46). Inositol alone partially represses these enzymes (3, 24, 30, 37, 45, 46). The enzyme responsible for synthesis of phosphatidylinositol from CDP-diacylglycerol (phosphatidylinositol synthase) is not repressed by phospholipid precursors (15, 30). However, the enzyme responsible for inositol synthesis (inositol-1-phosphate synthase) is repressed by inositol (13, 22, 30, 34). The enzymes (3, 24, 30, 37) in the

primary biosynthetic pathway for phosphatidylcholine biosynthesis and inositol biosynthesis (12, 13, 17, 30, 34) are not regulated in inositol biosynthesis regulatory mutants. These findings indicate that phosphatidylcholine biosynthesis is coordinately regulated with the regulation of inositol biosynthesis (21).

S. cerevisiae presumably synthesizes required amounts of phosphatidylethanolamine and phosphatidylcholine by the CDP-ethanolamine- and CDP-choline-based pathways, respectively (28), when the enzymes in the primary biosynthetic pathway of phosphatidylcholine biosynthesis are repressed by ethanolamine and choline in inositol-containing medium. PA phosphatase catalyzes the formation of the diacylglycerol needed for these reactions (27, 28). The aim of the present work was to explore the role of inositol and other phospholipid precursors on the regulation of PA phosphatase activity. We show that, unlike the repressive effect of inositol on the enzymes in the primary phospholipid biosynthetic pathway, addition of inositol to the growth medium resulted in an increase in PA phosphatase activity. The results of studies with various mutants of phospholipid metabolism suggested that PA phosphatase activity was regulated at a genetic level.

MATERIALS AND METHODS

Materials. All chemicals used were reagent grade. Growth medium supplies were purchased from Difco Laboratories. *myo*-Inositol, choline, ethanolamine, L-serine, *sn*-glycerol 3-phosphate, bovine serum albumin, phospholipids, and neutral lipids were purchased from Sigma Chemical Co. Triton X-100 was a gift from Rohm & Haas Co. Radiochemicals were purchased from Du Pont-New England Nuclear and ICN Pharmaceuticals, Inc. Scintillation supplies were purchased from National Diagnostics. Dioleoyl-PA and dioleoyldiacylglycerol were purchased from Avanti Polar Lipids. Palmitoyl coenzyme A was purchased from Pharmacia Biotechnology, Inc. *Escherichia coli* diacylglycerol kinase was obtained from Lipidex Inc. Silica gel-loaded SG81

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TABLE 1. *S. cerevisiae* strains used in this work

Strain	Genotype	Phenotype and biochemical defect	Source or reference
Wild type (<i>ade5</i>)	<i>MATa ade5</i>		11, 18, 30
<i>opil</i> mutant	<i>MATa ade5 opil-1</i>	Excretes inositol, overproduces inositol 1-phosphate synthase	16, 17
<i>ino2</i> mutant	<i>MATα ino2-21 lys2</i>	Inositol auxotroph, lacks inositol 1-phosphate synthase	10-13, 29
<i>ino4</i> mutant	<i>MATα ino4-39 lys2</i>	Inositol auxotroph, lacks inositol 1-phosphate synthase	10-13, 29
<i>cdg1</i> mutant	<i>MATa cdg1 ade5</i>	Excretes inositol, defective in CDP-diacylglycerol synthase activity	31
<i>cho1</i> mutant	<i>MATα cho1-1 ino1-13 lys2 can1</i>	Ethanolamine-choline auxotroph, defective in phosphatidylserine synthase activity	1, 2
<i>cho2</i> mutant	<i>MATα cho2-1 lys2</i>	No phenotype, defective in phosphatidylethanolamine methyltransferase activity	E. Summers; 21
<i>opi3</i> mutant	<i>MATα opi3-3 lys1</i>	No phenotype, defective in phospholipid methyltransferase activity	11, 18
<i>ino1</i> mutant	<i>MATα ino1-13 lys2 can1</i>	Inositol auxotroph, defective in inositol 1-phosphate synthase activity	11, 13

chromatography paper was purchased from Whatman Inc. Precoated Silica Gel 60 analytical thin-layer chromatography plates were purchased from E. Merck AG.

Preparation of substrates. [γ - 32 P]PA was synthesized enzymatically from [γ - 32 P]ATP and diacylglycerol by using *E. coli* diacylglycerol kinase under the assay conditions described by Walsh and Bell (44). [2 - 3 H]PA was prepared from [2 - 3 H]glycerol 3-phosphate and palmitoyl coenzyme A by using yeast microsome-associated glycerol 3-phosphate acyltransferase under the assay conditions described by Tillman and Bell (41). Labeled PA was purified by thin-layer chromatography by using the solvent system chloroform-methanol-water (65:25:4, vol/vol/vol).

Yeast strains and growth conditions. Descriptions and sources of the strains used in this study are shown in Table 1. Cultures were maintained on YEPD (1% yeast extract, 2% peptone, 2% glucose, 2% agar) medium plates. The composition of complete synthetic medium is described elsewhere (11, 20). Cells were grown in 100-ml batches of complete synthetic medium containing inositol (50 μ M), serine (1 mM), ethanolamine (1 mM), and choline (1 mM) where indicated. Cultures were incubated at 28°C on a rotary shaker (New Brunswick Scientific Co., Inc.) at 200 rpm. Cells were grown to the exponential (2×10^7 to 4×10^7 CFU/ml) and stationary (7×10^7 to 2×10^8 CFU/ml) phases of growth and harvested by centrifugation. Cell numbers were determined by microscopic examination with a hemacytometer.

Preparation of cellular fractions. All cellular fraction preparation steps were performed at 8°C. Cell extracts were prepared by disrupting cells with glass beads in 50 mM Tris hydrochloride buffer (pH 7.5) containing 1 mM disodium EDTA, 0.3 M sucrose, and 10 mM 2-mercaptoethanol as described previously (30). Mitochondrial (4) and microsomal (14) fractions of cells were prepared by differential centrifugation. Membrane fractions were suspended in 50 mM Tris hydrochloride buffer (pH 7.5) containing 10 mM 2-mercaptoethanol, 10 mM MgCl₂, and 20% glycerol. The postmicrosomal supernatant was used as the cytosolic fraction.

Enzyme assays. The optimal conditions for assay of PA phosphatase activity were determined in detail with membrane-associated enzyme. The substrate PA was delivered to

the assay as a uniform mixed micelle with Triton X-100 at a molar ratio of Triton X-100 to PA of 10:1. About 10-fold greater activity was observed when PA was part of a mixed micelle than when PA was added to the assay in the absence of detergent. Assays were conducted at 30°C. PA phosphatase activity was routinely measured for 20 min by monitoring the release of water-soluble 32 P_i from 2 mM [γ - 32 P]PA (1,000 to 2,000 cpm/nmol) in 50 mM Tris-maleate buffer (pH 7.0) containing 20 mM Triton X-100, 10 mM 2-mercaptoethanol, 2 mM MgCl₂, 0.2 mg of bovine serum albumin per ml, and enzyme protein in a total volume of 0.1 ml. The reaction was terminated by addition of 0.5 ml of 0.1 N HCl in methanol. Chloroform (1 ml) and 1 M MgCl₂ (1 ml) were added, the system was mixed, and the phases were separated by 2 min of centrifugation at $100 \times g$. Liquiscint (4 ml) was added to a 0.5-ml sample of the aqueous phase, and radioactivity was determined by scintillation counting. Alternatively, PA phosphatase activity was measured by monitoring the formation of [2 - 3 H]diacylglycerol from [2 - 3 H]PA (1,000 cpm/nmol) under the assay conditions described above. The chloroform-soluble lipid product of the reaction, diacylglycerol, was analyzed with standard diacylglycerol by one-dimensional paper chromatography on EDTA-treated SG81 paper (39) by using the solvent system hexane-diethyl ether-glacial acetic acid (30:70:1). Carrier standard diacylglycerol was added to the chloroform phase before separation, and the position of the labeled diacylglycerol on the chromatograms was determined after exposure to iodine vapor. The amount of labeled diacylglycerol was determined by liquid scintillation counting. All assays were linear with time and protein concentration. One unit of enzymatic activity was defined as the amount of enzyme that catalyzed the formation of 1 nmol of product per min. Specific activity was defined as units per milligram of protein.

Protein determination. Protein was determined by the method of Bradford (6), with bovine serum albumin as the standard.

Lipid composition analysis. Cultures (5 ml) were grown in the indicated medium in the presence of 5 μ Ci of [2 - 14 C] acetate. Cells were harvested by centrifugation at exponential and stationary phases of growth, washed with distilled

FIG. 1. PA phosphatase activity in wild-type cells grown in the absence or presence of phospholipid precursors. Cells were grown in complete synthetic medium with or without inositol in the absence (control) or presence of serine, ethanolamine, and choline. Cells were harvested in the exponential phase of growth, cell extracts were prepared, and PA phosphatase activity was measured as described in the text. The specific activities (units per milligram) of PA phosphatase were calculated from triplicate determinations from a minimum of three independent growth studies.

water, and suspended in 3 ml of chloroform-methanol-water (1:2:0.8, vol/vol/vol). The cell suspension was mixed with 0.05 g of glass beads and vortexed intermittently for 1 h at room temperature. Lipids were extracted from the broken-cell suspension as described by Bligh and Dyer (5). The chloroform phase was dried, and the residue was dissolved in chloroform-methanol (1:1, vol/vol). The ^{14}C -labeled lipids were separated by one-dimensional paper chromatography on EDTA-treated SG81 paper (39) by using the solvent system hexane-diethyl ether-glacial acetic acid (30:70:1) as previously described (38). Carrier standard lipids were added to the extracts before separation, and the positions of the labeled lipids on the chromatograms were determined after exposure to iodine vapor. The amounts of labeled lipids on the chromatograms were determined by liquid scintillation counting.

RESULTS

Effect of inositol on PA phosphatase activity in wild-type cells. The *ade5* strain shows normal regulation of phospholipid biosynthesis (18, 30) and was used as a wild-type strain. Addition of inositol to the growth medium of wild-type cells resulted in a twofold increase in PA phosphatase activity (Fig. 1). Addition of serine, ethanolamine, and choline to the growth medium did not significantly affect PA phosphatase activity in the absence or presence of inositol (Fig. 1).

We examined whether the elevation in PA phosphatase activity in cells grown in the presence of inositol was due to the presence of a soluble activator or loss of a soluble inhibitor. The cell extracts for each growth condition described in Fig. 1 were mixed and incubated for 20 min at 30°C. After incubation, PA phosphatase activity was measured. The activity in the mixed-cell extracts was the average specific activity of PA phosphatase in each cell extract assayed separately. These results suggest that the increase in PA phosphatase activity was not due to soluble effector molecules.

To examine whether inositol had a direct effect on PA phosphatase activity, the cell extract from cells grown in the

FIG. 2. PA phosphatase activity in subcellular fractions of wild-type cells grown in the absence or presence of inositol. Cells were grown in complete synthetic medium in the absence or presence of inositol. Cells were harvested in the exponential phase of growth; the cell extract, mitochondrial, microsomal, and cytosolic fractions were isolated; and activity was measured as described in the text. The specific activities (units per milligram) of PA phosphatase were calculated from triplicate determinations from a minimum of three independent growth studies.

absence of inositol was assayed for activity in the presence of inositol. Addition of 10, 50, and 100 μM inositol did not affect PA phosphatase activity under standard assay conditions.

PA phosphatase activity was associated with the mitochondrial, microsomal, and cytosolic fractions of the cell (Fig. 2). About 25 to 30% of the total PA phosphatase activity was associated with the soluble fraction. The remainder of the PA phosphatase activity was about equally distributed between the mitochondrial and microsomal fractions. PA phosphatase activity was elevated about twofold in each of the subcellular fractions isolated from wild-type cells grown in the presence of inositol compared with those fractions isolated from cells grown in the absence of inositol. These results further indicate that the increase in PA phosphatase activity due to inositol supplementation was not due to soluble effectors.

In each of the subcellular fractions, PA phosphatase activity was twofold higher in stationary-phase cells than in exponential-phase cells when grown in the absence of inositol. There was also a twofold increase in PA phosphatase activity in stationary-phase cells compared with exponential-phase cells grown in the presence of inositol. Overall, PA phosphatase activity was about fourfold higher in stationary-phase cells grown with inositol than in exponential-phase cells grown without inositol.

Effect of inositol on the lipid composition of wild-type cells. Since PA phosphatase activity was elevated in exponential-phase cells grown in the presence of inositol, we examined the effect of inositol on the total lipid composition. The steady-state lipid composition of wild-type exponential-phase cells grown in the absence or presence of inositol is presented in Table 2. Addition of inositol to the growth medium resulted in a 7% increase in phospholipids and an 11% decrease in triacylglycerols. Sterols increased slightly (3%), and the diacylglycerol content did not change significantly in inositol-supplemented cells. In the absence or presence of inositol, stationary-phase cells had elevated

TABLE 2. Lipid composition of wild-type *ade5* cells grown in the absence or presence of inositol^a

Growth condition	Mean $\pm$ SEM % of total lipid of:			
	Triacyl-glycerol	Diacyl-glycerol	Phospho-lipid	Sterol
Without inositol	49.2 $\pm$ 1.5	4.0 $\pm$ 0.1	35.2 $\pm$ 0.4	11.6 $\pm$ 1.5
With inositol	38.2 $\pm$ 1.7	4.6 $\pm$ 0.3	42.3 $\pm$ 3.0	14.9 $\pm$ 0.2

^a Cells were grown in complete synthetic medium in the absence or presence of 50 μ M inositol. Cells were harvested in the exponential phase of growth (2×10^7 to 4×10^7 CFU/ml). Lipid composition was determined by [14 C]acetate labeling as described in the text. The data are averages of three determinations.

triacylglycerol and reduced levels of phospholipids, as previously described (26, 40).

PA phosphatase activity in inositol biosynthesis regulatory mutants. Since inositol regulated the levels of PA phosphatase activity in wild-type cells, we examined the levels of this enzyme activity in the *opi1*, *ino2*, and *ino4* inositol biosynthesis regulatory mutant strains. The *opi1* mutant excretes inositol (17) and is constitutively elevated for inositol-1-phosphate synthase (16). PA phosphatase activity in the *opi1* mutant was elevated 1.5-fold compared with wild-type cells grown in the absence of inositol (Fig. 3). Addition of inositol to the growth medium of *opi1* cells did not result in the increase in PA phosphatase activity observed in wild-type cells (Fig. 3). Instead, inositol caused a slight decrease in PA phosphatase activity. The *opi1* mutant also shows pleiotropic effects on CDP-diacylglycerol synthase (23, 24), phosphatidylserine synthase (23, 30, 37), and phospholipid N-methyltransferase (30, 34) activities.

The *ino2* and *ino4* mutants are inositol auxotrophs (11, 13) because they are unable to derepress inositol-1-phosphate synthase (10, 12, 29) and exhibit pleiotropic effects on CDP-diacylglycerol synthase (23), phosphatidylserine synthase (3, 23), and phospholipid N-methyltransferase (34) activities. PA phosphatase activity was reduced in *ino2* mutant cells compared with wild-type cells grown with inositol (Fig. 3). On the other hand, the level of enzyme activity in *ino4* mutant cells was elevated like that of wild-type cells grown with inositol (Fig. 3).

FIG. 3. PA phosphatase activity in inositol biosynthesis regulatory mutants. The wild type (*ade5*) and the indicated inositol biosynthesis regulatory mutants were grown in complete synthetic medium with or without inositol as indicated. Cells were harvested in the exponential phase of growth, cell extracts were prepared, and PA phosphatase activity was measured as described in the text. The specific activities (units per milligram) of PA phosphatase were calculated from triplicate determinations from a minimum of three independent growth studies.

FIG. 4. PA phosphatase activity in structural gene mutants. The wild type (*ade5*) and the indicated structural gene mutants were grown in complete synthetic medium with or without inositol as indicated. The *chol* mutant used in this study was also an inositol auxotroph (2). Cells were harvested in the exponential phase of growth, cell extracts were prepared, and PA phosphatase activity was measured as described in the text. The specific activities (units per milligram) of PA phosphatase were calculated from triplicate determinations from a minimum of three independent growth studies.

PA phosphatase activity in structural gene mutants. PA phosphatase activity was also examined in mutants with defects which are believed to be in the structural genes which encode for primary phosphatidylcholine biosynthetic pathway enzymes. The *cdg1* mutant is defective in CDP-diacylglycerol synthase activity (31). CDP-diacylglycerol synthase, like PA phosphatase, uses PA as a substrate (9). The *cdg1* mutant shows pleiotropic defects in phospholipid biosynthesis, including constitutive overproduction of inositol-1-phosphate synthase and overproduction of phosphatidylserine synthase (31). PA phosphatase activity in the *cdg1* mutant was elevated compared with wild-type cells grown in the absence of inositol (Fig. 4). Activity was not affected by addition of inositol to the growth medium (Fig. 4).

The *chol* mutants are ethanolamine-choline auxotrophs (2) because of a defect in the structural gene for phosphatidylserine synthase (3, 33). Since *chol* mutants have little or no phosphatidylserine synthase activity (1, 32), they synthesize phosphatidylethanolamine and phosphatidylcholine by the auxiliary CDP-ethanolamine- and CDP-choline-based pathways (1), respectively. PA phosphatase activity was reduced in the *chol* mutant compared with wild-type cells grown with inositol (Fig. 4). The *chol* mutant used in this study is also auxotrophic for inositol (2).

The *cho2* (21) and *opi3* (18) mutants are defective in the enzymes responsible for the first and last two phosphatidylethanolamine methylation reactions, respectively, leading to formation of phosphatidylcholine. PA phosphatase activity in the *cho2* and *opi3* mutants was similar to that found in wild-type cells (Fig. 4). Although both enzymes were regulated in response to inositol, the increase in activity was not as great as that observed in wild-type cells (Fig. 4). Addition of choline to inositol-containing medium did not affect expression of PA phosphatase activity in the *cho2* and *opi3* mutants.

The *ino1* mutant is an inositol auxotroph (11) because of a defect in the structural gene for inositol-1-phosphate synthase (13). The level of PA phosphatase activity in the *ino1* mutant was intermediate between those found in wild-type cells grown in the absence or presence of inositol.

DISCUSSION

In this study, we have shown that addition of inositol to the growth medium of wild-type cells resulted in twofold elevation of PA phosphatase activity. PA phosphatase catalyzes the formation of diacylglycerol, the lipid substrate for the auxiliary CDP-ethanolamine- and CDP-choline-based pathways (28). Although we expected that inositol supplementation would lead to an increase in PA phosphatase activity, we were surprised that ethanolamine and choline did not affect the levels of the enzyme. In combination with inositol, addition of ethanolamine and choline to the growth medium leads to further repression of the primary phosphatidylcholine pathway enzymes compared with inositol alone (3, 24, 30, 37, 45, 46). Furthermore, ethanolamine and choline are the precursors to CDP-ethanolamine and CDP-choline, respectively, for the auxiliary pathways (28). Although the magnitude of the regulation of PA phosphatase activity was not massive, the twofold increase in activity by inositol supplementation was consistent with the inverse regulation of the primary pathway enzymes by phospholipid precursors.

The elevated PA phosphatase activity in response to inositol supplementation was not due to the presence of soluble effectors, and inositol did not have a direct effect on enzyme activity in cell extracts. Like the enzymes (23, 24, 30, 34, 37) in the primary pathway of phosphatidylcholine biosynthesis, PA phosphatase activity was not regulated in the *opil* and *ino2* inositol biosynthesis regulatory mutants. This suggests that, like the phosphatidylcholine primary pathway enzymes, inositol is involved in the regulation of PA phosphatase. PA phosphatase was not affected in the *ino4* mutant. PA phosphatase is the first example of a phospholipid biosynthetic enzyme to be differentially regulated in the *ino2* and *ino4* mutants. This suggests that the products of the *INO2* and *INO4* genes have different roles in the hierarchy of the regulation of phospholipid metabolism in *S. cerevisiae*. In addition, structural gene mutants of the primary phosphatidylcholine pathway exhibited pleiotropic effects on PA phosphatase activity.

PA phosphatase activity is associated with the total membrane and cytosolic fractions of *S. cerevisiae* (26). In this study, we have shown that membrane-associated PA phosphatase activity was about equally distributed between the mitochondrial and microsomal fractions of the cell. The enzyme activity associated with each of the cellular fractions was regulated by inositol in the same manner. As previously shown by Hosaka and Yamashita (26), membrane- and cytosol-associated PA phosphatase activity increased about twofold as cells entered the stationary phase of growth. The increase in PA phosphatase activities is believed to be responsible for the increase in triacylglycerol synthesis in stationary-phase cells (26). Whereas an increase in PA phosphatase activity in the stationary phase of growth correlates with an increase in triacylglycerol content at the expense of phospholipid (26, 40), the increase in PA phosphatase activity in exponential-phase cells in response to inositol supplementation correlated with an increase in phospholipid content at the expense of triacylglycerol. Conversely, when the *ino1* inositol-1-phosphate synthase structural gene mutant is starved for inositol the triacylglycerol content increases at the expense of phospholipids (35). The major change in the phospholipid composition of wild-type cells supplemented with inositol is a two- to threefold increase in phosphatidylinositol and a twofold decrease in phosphatidylserine (18, 30). Only minor changes in the

proportions of phosphatidylcholine and phosphatidylethanolamine result when wild-type cells are grown in the presence of inositol (18, 30).

Little is known about the regulation of the enzymes of the CDP-ethanolamine- and CDP-choline-based pathways in response to inositol supplementation.

In summary, the water-soluble phospholipid precursor inositol regulated the levels of PA phosphatase activity. Regulation of PA phosphatase by inositol is likely to influence the regulation of the primary and auxiliary pathways for the synthesis of phosphatidylethanolamine and phosphatidylcholine and the overall regulation controlling the proportional synthesis of phospholipids and triacylglycerols. The fact that this enzyme was affected in inositol biosynthesis regulatory mutants and structural gene mutants suggests that this regulation occurred on a genetic level. Unequivocal evidence that PA phosphatase is regulated by inositol at a genetic level awaits *in vivo* analysis of the PA phosphatase mRNA and protein levels. The availability of a PA phosphatase mutant and a purified preparation of the enzyme would facilitate the study of this important enzyme in lipid metabolism. Toward this goal, Hosaka and Yamashita (25) have partially purified the soluble form of PA phosphatase and we are developing a purification procedure for the membrane-associated form of the enzyme.

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