Regulation of Phosphatidylinositol Kinase Activity in *Saccharomyces cerevisiae*[†]

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The effects of growth phase and carbon source on membrane-associated phosphatidylinositol kinase in cell extracts of *Saccharomyces cerevisiae* were examined. Phosphatidylinositol kinase activity increased 2- and 2.5-fold in glucose- and glycerol-grown cells, respectively, in the stationary phase as compared with the exponential phase of growth. The increase in phosphatidylinositol kinase activity in the stationary phase of growth correlated with an increase in the relative amounts of phosphatidylinositol 4-phosphate, the product of the reaction. The increase in phosphatidylinositol kinase activity was not due to the presence of water-soluble effector molecules in cell extracts as indicated by mixing experiments. Phosphatidylinositol kinase activity decreased in cell extracts of exponential-phase cells preincubated under phosphorylation conditions which favor cyclic AMP-dependent protein kinase activity. Phosphatidylinositol kinase activity was not affected in cell extracts of stationary-phase cells preincubated under phosphorylation conditions.

In the yeast *Saccharomyces cerevisiae*, phosphatidylinositol (PI) is the third-most-common membrane phospholipid (18). PI is an essential phospholipid for the growth and metabolism of *S. cerevisiae*. Cells which carry a disrupted copy of the gene encoding the enzyme responsible for PI synthesis (i.e., PI synthase) do not synthesize PI and fail to grow (32). When inositol-requiring mutants of *S. cerevisiae* are deprived of inositol, cells undergo changes in the metabolism of lipids, carbohydrates, proteins, and nucleic acids, resulting in a loss of viability (1, 19). The requirement for inositol arises because eucaryotic membranes do not function correctly if they are deficient in inositol-containing lipids (31). In *S. cerevisiae*, PI is the precursor of the polyphosphoinositides PI 4-phosphate (PIP) and PI 4,5-bisphosphate (PIP₂) (24). PI is also the precursor of the phosphoinositol-containing sphingolipids (2) and is required for the synthesis of cell wall glycans (16).

In higher eucaryotic cells, the phosphorylation of PI to the polyphosphoinositides and their subsequent phospholipase C-mediated hydrolysis are involved with various cellular responses to hormones, growth factors, and neurotransmitters (5). The hydrolysis products of PIP₂, namely 1,2-diacylglycerol and inositol trisphosphate, activate protein kinase C and increase cytoplasmic calcium levels, respectively (5). These responses have not been established for *S. cerevisiae*. However, the synthesis and degradation of the polyphosphoinositides in *S. cerevisiae* are important for cell growth (22) and have been implicated in the regulation of ATP levels in respiratory-deficient strains (37).

To gain insight into PI metabolism in *S. cerevisiae*, we examined the effects of the growth phase and carbon source on membrane-associated (30) PI kinase activity. PI kinase is the first enzyme in the phosphorylation sequence of PI and catalyzes the formation PIP and ADP from PI and ATP (11). We show that PI kinase activity is most active in the stationary phase of growth. We also present evidence sug-

gesting that PI kinase activity is modulated by conditions which favor protein phosphorylation.

MATERIALS AND METHODS

Materials. All chemicals used were reagent grade. Growth medium supplies were purchased from Difco Laboratories. ATP, cyclic AMP (cAMP), protein kinase inhibitor, alkaline phosphatase, phospholipids, luciferin-luciferase reagent, *myo*-inositol, and bovine serum albumin were purchased from Sigma Chemical Co. Radiochemicals were purchased from ICN Pharmaceuticals Inc. and Amersham Corp. Scintillation-counting supplies were purchased from National Diagnostics. En³Hance autoradiography enhancer spray was purchased from New England Nuclear Corp. SG81 chromatography paper was purchased from Whatman, Inc. Triton X-100 was a gift from Rohm & Haas Co. CDPdiacylglycerol was prepared from soybean lecithin as described previously (8).

Growth conditions. The representative wild-type *S. cerevisiae* strain was *ade5 MATa* (15, 23). The organism was maintained on YEPD (1% yeast extract, 2% peptone, 2% dextrose) medium plates containing 2% Bacto-Agar. Cells were precultured overnight to late exponential phase in complete synthetic medium (23) with either 2% glucose or 2% glycerol as the carbon source. From this preculture, 4×10^8 CFU was inoculated into 100-ml batches of complete synthetic medium with either 2% glucose or 2% glycerol as the carbon source and 50 μ M inositol where indicated. Cultures were incubated at 28°C on a rotary shaker at 200 rpm. Cells were harvested by centrifugation at the indicated time intervals. Glucose-grown cells were arrested in the G₁ phase of the cell cycle by glucose starvation as previously described (22). Growth of cultures was monitored by plate counts on YEPD medium plates. Cell numbers were determined by microscopic examination with a hemacytometer.

Preparation of cell extracts. 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 (23).

Enzyme assays. All assays were conducted at 30°C. PI

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kinase(ATP:1-phosphatidyl-1D-*myo*-inositol4-phosphotransferase; EC 2.7.1.67) activity was measured for 10 min by monitoring the phosphorylation of 0.4 mM PI with 5 mM [γ - 32 P]ATP (10,000 to 20,000 cpm/nmol) in the presence of 50 mM Tris-maleate buffer (pH 8.5)–14 mM sodium cholate–10 mM MgCl_2 –enzyme protein in a total volume of 0.1 ml (29). PI synthase (CDPdiacylglycerol:*myo*-inositol 3-phosphatidyltransferase; EC 2.7.8.11) activity was measured for 20 min by monitoring the incorporation of 0.5 mM *myo*-[2- ^3H]inositol (10,000 cpm/nmol) into PI in the presence of 50 mM Tris hydrochloride (pH 8.0)–0.2 mM CDPdiacylglycerol–2.4 mM Triton X-100–2 mM MnCl_2 –enzyme protein in a total volume of 0.1 ml (9). The phospholipid products of each reaction (9, 29) were analyzed by thin-layer chromatography with standards. For the PI kinase reaction, the products were a mixture of PIP and PIP_2 , indicating the presence of PI kinase activity (29). The ratios of PIP and PIP_2 for the PI kinase assays from cells grown under the various conditions were approximately 1:2, suggesting that both kinase activities were present at the same levels. 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 was determined by the method of Bradford (7), with bovine serum albumin as the standard.

Phosphorylation-dephosphorylation conditions. Cell extracts were preincubated for 10 min at 30°C under the assay conditions for *S. cerevisiae* cAMP-dependent protein kinase activity (39). The reaction mixture (20 μl) contained 25 mM Tris hydrochloride buffer (pH 7.4), 10 μM cAMP, 5 μM ATP, and 5 mM MgCl_2 . Following the preincubation, the PI kinase assay components were added, and activity was measured in a final volume of 0.1 ml as described above. Cell extracts were also preincubated with 13 μg of protein kinase inhibitor, 10 mM NaF, and 0.02 U ($\mu\text{mol}/\text{min}$) of alkaline phosphatase (pH 10.4) where indicated.

Phospholipid composition analysis. Cultures (5 ml) were grown in the indicated medium in the presence of either 50 μCi of ^{32}P i or 10 μCi of *myo*-[2- ^3H]inositol. Cells were harvested by centrifugation at exponential or stationary phase of growth, washed with distilled 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. Phospholipids were extracted from the broken-cell suspension by the method described by Bligh and Dyer (6). The chloroform phase was dried, and the residue was dissolved in chloroform-methanol (1:1). A portion of the extract was counted by liquid scintillation to determine the total lipid-extractable counts. The ^{32}P -labeled phospholipids were separated by two-dimensional paper chromatography on EDTA-treated SG81 paper (36) with chloroform-methanol–30% ammonium hydroxide–water (66:27:3:0.8) as the solvent system for dimension 1 and chloroform-methanol-glacial acetic acid–water (32:4:5:1) as the solvent system for dimension 2. [2- ^3H]inositol-labeled lipids were separated by one-dimensional paper chromatography on EDTA-treated SG81 paper (36) with chloroform-acetone-methanol-glacial acetic acid–water (40:15:13:12:8) as the solvent system. The positions of labeled lipids on the chromatograms were determined by autoradiography. The chromatograms with [2- ^3H]inositol-labeled lipids were sprayed with En^3Hance prior to autoradiography. The identity of each lipid was determined by comparing its mobility with that of standard lipids. The amount of each labeled lipid was determined by liquid

scintillation counting of the corresponding spots on the chromatogram.

Determination of ATP. Cultures (0.5 ml) were added to 4.5 ml of boiling 50 mM Tris hydrochloride buffer (pH 7.75)–1 mM sodium EDTA in screw-cap test tubes. The cell suspension was boiled for 5 min and then cooled on ice. ATP was measured with luciferin-luciferase reagent, as described in Sigma Technical Bulletin no. BAAB-1, with a Lumac/3M Biocounter.

RESULTS

Effects of growth phase and carbon source on PI kinase activity. Polyphosphoinositide turnover is closely related to ATP levels in *S. cerevisiae* (37). Therefore, it was of interest to examine the effect of cells grown with glucose or glycerol as the carbon source on PI kinase activity. Cells grown with glucose derive their energy from both fermentation and respiration, whereas cells grown with glycerol derive their energy from aerobic respiration. Second, since a number of phospholipid-biosynthetic enzyme activities are reduced in the stationary phase of growth (21), we examined the effect of growth phase on PI kinase activity. For these studies, PI kinase activity was measured by using endogenous PI to assess activity under conditions which would reflect the in vivo concentration of the substrate. When cells were grown in complete synthetic medium with glucose as the carbon source, the specific activity of PI kinase increased twofold in the stationary phase compared with the exponential phase of growth (Fig. 1B). When cells were grown with glycerol as the carbon source, the specific activity of PI kinase increased 2.5-fold in stationary-phase cells compared with exponential-phase cells (Fig. 1D). In the stationary phase of growth, PI kinase activity was 1.5-fold higher in glycerol-grown cells than in glucose-grown cells.

Stationary-phase cells are generally arrested at the G_1 phase of the cell cycle (35). To ensure that cells were arrested in the G_1 phase, exponential-phase glucose-grown cells were washed, suspended in complete synthetic medium containing 0.02% glucose, and incubated for 30 h (22). Approximately 80% of the cells were unbudded. The specific activity of PI kinase was twofold higher in G_1 -phase cells than in exponential-phase cells. The twofold-higher PI kinase activity in arrested and stationary-phase cells compared with exponential-phase cells was also observed when the enzyme was measured with exogenous PI (standard assay conditions).

The water-soluble phospholipid precursor inositol has been shown to repress the levels of cytoplasmic-associated (14) and membrane-associated (20, 23, 34, 41) enzymes of phospholipid metabolism. Therefore, it was of interest to examine the effect of inositol, the water-soluble precursor of the polyphosphoinositides, on PI kinase activity. Unlike the repressive effect of inositol on other phospholipid-biosynthetic enzymes, the addition of inositol to glucose-grown cells resulted in a slight increase in PI kinase activity in the exponential phase (Fig. 1B). The addition of inositol to glycerol-grown cells did not significantly affect PI kinase activity (Fig. 1D).

We examined whether the elevation of PI kinase activity observed in the stationary phase of growth was due to the presence of a soluble activator or the loss of a soluble inhibitor. Cell extracts were prepared for each growth condition, and the extracts were mixed and incubated for 20 min at 30°C. After incubation, PI kinase activity was measured. PI kinase activity in the mixed extracts was the average of

FIG. 1. PI kinase activity during the growth of *S. cerevisiae* (*ade5*). Cells were grown in complete synthetic medium with glucose (A) or glycerol (C) in the absence (■) or presence (□) of inositol. Cells were harvested at the indicated time intervals, and cell extracts were prepared as described in the text. In panels A and C, the cell number was determined by counting under a microscope on a hemacytometer. (B and D) PI kinase activity (in units per milligram) was measured with endogenous PI as substrate.

the specific activity of the enzyme in each cell extract assayed separately (data not shown). These results suggest that the increase in PI kinase activity in the stationary phase of growth was not due to soluble effector molecules. However, these experiments do not rule out the presence of membrane-bound effectors which could have modulated PI kinase activity.

PI synthase catalyzes the formation of PI (33), the phospholipid substrate for the PI kinase reaction. We examined whether the activity of this enzyme was influenced by the growth phase of cells and the carbon source of the medium. The specific activity of PI synthase was not significantly affected in cells grown in glucose or glycerol with or without inositol in the growth medium (data not shown).

Effects of growth phase and carbon source on phospholipid composition. The composition of the major phospholipids of cells grown with glucose or glycerol is presented in Table 1. As previously reported (21), when glucose-grown cells entered the stationary phase of growth, the major change in the composition of the major phospholipids was an increase in PI. However, in glycerol-grown cells the PI content did not change significantly in the stationary phase. The major changes in the phospholipid composition of exponential-phase cells grown in glycerol compared with exponential-phase cells grown in glucose were a 1.5-fold increase in the PI content and a 1.2-fold decrease in the phosphatidylcholine content. The PI and phosphatidylcholine contents of stationary-phase cells were similar in glucose-grown and glycerol-grown cells. Addition of inositol to the glucose-containing growth medium resulted in elevated levels of PI in the exponential phase as previously reported (23). On the other hand, addition of inositol to glycerol-grown cells had little effect on the PI content. The PI content of stationary-phase cells grown in glucose or glycerol in the presence of inositol

decreased compared with that of exponential-phase cells. This is most probably due to a depletion of inositol in the growth medium (21).

The steady-state composition of the inositol-containing phospholipids of cells grown with glucose and glycerol is

TABLE 1. Phospholipid composition of cells grown with glucose or glycerol in the absence or presence of inositol^a

Growth conditions	% of total phospholipid ^b for:							
	PC	PE	PI	PS	PMME	PDME	PA	Others
Glucose								
Exponential	41.8	17.5	17.3	8.1	0.7	3.2	1.1	10.6
Stationary	36.5	17.8	26.0	6.6	1.8	2.4	0.9	8.0
Glycerol								
Exponential	34.6	13.5	26.4	8.6	2.2	3.4	0.9	10.4
Stationary	30.7	16.0	29.5	6.2	3.2	1.5	0.2	12.6
Glucose + inositol								
Exponential	29.0	11.8	35.9	6.0	2.0	1.4	0.7	13.0
Stationary	29.3	20.0	26.7	8.2	1.5	2.6	0.9	10.7
Glycerol + inositol								
Exponential	24.0	16.1	31.3	8.1	2.3	1.5	1.1	15.6
Stationary	35.0	15.2	25.9	3.5	2.5	1.9	0.4	15.6

^a Cells were grown in complete synthetic medium with glucose (2%) or glycerol (2%) as the carbon source in the absence or presence of inositol (50 μ M). Cells were harvested in the exponential (2×10^7 to 4×10^7 CFU/ml) or stationary (7×10^7 to 9×10^7 CFU/ml) phase of growth. The phospholipid composition was determined by ³²P labeling as described in the text.

^b The data presented are the averages of three determinations. Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PMME, phosphatidylmonomethylethanolamine; PDME, phosphatidylmethylethanolamine; PA, phosphatidate. Others, Pooled percentages of minor phospholipid species.

TABLE 2. Inositol-containing lipid composition and ATP content of cells grown with glucose or glycerol in the absence or presence of inositol^a

Growth conditions	% of total inositol-containing lipid ^b for:				ATP (fM)/CFU ^c
	PI	PIP	PIP ₂	Others	
Glucose					
Exponential	68	15	13	5	0.17
Stationary	72	17	8	3	0.17
Glycerol					
Exponential	78	12	9	1	0.42
Stationary	61	27	10	1	0.24
Glucose + inositol					
Exponential	92	5	1	2	0.24
Stationary	84	14	2		0.17
Glycerol + inositol					
Exponential	96	3	1	ND ^d	0.31
Stationary	89	10	1	ND	0.17

^a Cells were grown and harvested as described in Table 1, footnote *a*. The inositol-containing lipid composition was determined by [2-³H]inositol labeling as described in the text.

^b The data presented are the averages of three determinations. Others, Pooled percentages of inositol-containing sphingolipids. The minus-inositol medium contained 0.1 μ M inositol from the label. This concentration of inositol does not affect the regulation of phospholipid biosynthesis (12).

^c The ATP concentration in cells was determined as described in the text.

^d ND, Not detected.

presented in Table 2. As glucose-grown cells entered the stationary phase, the relative amounts of PI and PIP did not change significantly; however, the relative amount of PIP₂ decreased about 1.6-fold. On the other hand, as glycerol-grown cells entered the stationary phase, the amount of PI decreased about 1.3-fold, the amount of PIP increased about 2.2-fold, and the amount of PIP₂ remained the same. In the exponential phase of glucose-grown and glycerol-grown cells supplemented with inositol, the amount of PI increased 1.2- to 1.3-fold and that of PIP decreased 3- to 4-fold compared with amounts in cells grown in the absence of inositol. In the stationary phase of inositol-supplemented cells, the amount of PI decreased about 1.1-fold and the amount of PIP increased 2.8- to 3.3-fold. The amount of PIP₂ in exponential- and stationary-phase glucose- and glycerol-grown cells supplemented with inositol decreased to 1 to 2% of the amount of total inositol-containing lipids, compared with the amounts in cells grown in the absence of inositol.

Effects of growth phase and carbon source on ATP concentration. The ATP content of cells grown with glucose or glycerol is presented in Table 2. As expected, the ATP content per cell in glycerol-grown cells was about 2.5-fold higher than that in glucose-grown cells. In glucose- and glycerol-grown cells, the ATP content per cell generally decreased in the stationary phase of growth. The addition of inositol to glucose-grown cells resulted in a 1.4-fold increase in the ATP content, whereas the ATP content decreased 1.3-fold when inositol was added to glycerol-grown cells.

Effect of phosphorylation-dephosphorylation conditions on PI kinase activity. Reversible covalent modification of enzymes by phosphorylation-dephosphorylation is a major mechanism of cellular regulation (10). Since a number of yeast enzymes are regulated by phosphorylation via cAMP-dependent protein kinase (4, 17, 42), we examined the effect on PI kinase activity of preincubation conditions favoring protein phosphorylation. PI kinase activity decreased 1.75-

TABLE 3. Effect of phosphorylation-dephosphorylation conditions on PI kinase activity^a

Preincubation conditions	Relative activity (%) ^b			
	With glucose		With glycerol	
	Exponential	Stationary	Exponential	Stationary
No addition	100	100	100	100
+ cAMP, ATP, MgCl ₂	86	97	57	107
+ cAMP, ATP, MgCl ₂ , protein kinase inhibitor	100	100	100	100
+ NaF	89	100	65	95
+ Alkaline phosphatase	ND ^c	ND	125	100

^a Cells were grown and harvested as described in Table 1, footnote *a*. Cell extracts were preincubated for 10 min under the indicated conditions, and PI kinase activity was measured as described in the text.

^b The data presented are the averages of three determinations.

^c ND, Not determined.

fold when the cell extract of exponential-phase glycerol-grown cells was preincubated under the assay conditions for *S. cerevisiae* cAMP-dependent protein kinase activity (Table 3). This effect could be abolished by the addition of protein kinase inhibitor to the preincubation mixture (Table 3). The decrease in PI kinase activity under the preincubation phosphorylation conditions was time dependent (Fig. 2). When the cell extract of exponential-phase glycerol-grown cells was preincubated with NaF, PI kinase activity decreased 1.5-fold (Table 3). Similar results were found with the cell extract of exponential-phase glucose-grown cells; however, the reduction in PI kinase activity was not as great (Table 3). Preincubation of cell extracts from stationary-phase cells grown with glucose or glycerol under phosphorylation conditions or with NaF did not affect PI kinase activity (Table 3). When the cell extract of exponential-phase glycerol-grown cells was preincubated with alkaline phosphatase, PI kinase activity increased 1.25-fold (Table 3).

DISCUSSION

Glucose starvation of wild-type and respiratory-deficient cells results in a rapid decline in the levels of ATP, PIP, and

FIG. 2. Time-dependent inactivation of PI kinase activity after preincubation under phosphorylation conditions. The cell extract of exponential-phase glycerol-grown cells was preincubated with no additions (□) and under conditions which favor cAMP-dependent protein kinase activity (■) for the indicated time intervals. Following incubation, PI kinase activity was measured as described in the text.

PIP₂. However, within a short period the levels of ATP, PIP, and PIP₂ increase, approaching the prestarvation levels (37). The increase in these compounds with time in glucose-starved cells is presumably due to the mobilization of endogenous energy reserves (37). The addition of glucose to glucose-starved cells arrested at the G₁ phase of the cell cycle results in cell proliferation and rapid synthesis and turnover of inositol phospholipids (22). The elevation of the steady-state levels of PIP in stationary-phase cells observed in this study is not inconsistent with previous findings (22, 37). The accumulation of the relative amount of PIP in stationary-phase cells might result from the lack of inositol phospholipid turnover during cell proliferation (22). On the other hand, the increase in PIP levels in the stationary phase correlated with an increase in PI kinase activity. If the synthesis of the polyphosphoinositides is stimulated during cell proliferation, why is PI kinase activity greatest when cells enter the G₁ phase of the cell cycle? Dahl et al. (13) have shown that PI kinase activity increases 1.3-fold as glucose-grown cells enter the G₁ phase of the cell cycle as a result of ergosterol starvation in an *S. cerevisiae* sterol auxotroph. PI kinase activity is also influenced by the growth phase in higher eucaryotic organisms. For example, PI kinase activity is elevated 1.6- to 2-fold in Swiss 3T3 cells during the G₁ phase of growth (24) and 2-fold in *Dictyostelium discoideum* 4 h after removal of the food source (40).

PI, the phospholipid substrate for PI kinase, increased in the stationary phase of glucose-grown cells. The increase in the PI content of stationary-phase glucose-grown cells correlated with an increase in PI kinase activity. However, this increase in activity could not be attributed to the availability of the substrate PI. Activity increased in the stationary phase of glucose-grown cells when PI kinase activity was measured with either endogenous or exogenous PI. The increase in PI kinase activity in the stationary phase of glycerol-grown cells could not be attributed to the availability of PI, since the PI content was essentially the same in exponential- and stationary-phase cells. Furthermore, the increase in PI kinase activity did not correlate with the ATP levels in the cell. The results of the mixing experiments indicated that the increase in PI kinase activity in the stationary phase was not due to the presence of soluble effector molecules in cell extracts.

Cell extracts of exponential-phase cells preincubated under conditions which favor cAMP-dependent protein kinase activity had reduced PI kinase activity. The reduction in PI kinase activity was most dramatic with glycerol-grown cells. These results suggest that PI kinase activity in exponential-phase cells may be regulated by phosphorylation. The reduction in PI kinase activity in cell extracts preincubated with NaF, a phosphoprotein phosphatase inhibitor (3), may suggest that a phosphorylated form of PI kinase is less active than a dephosphorylated form of the enzyme. Furthermore, preincubation of the cell extract from exponential-phase glycerol-grown cells with alkaline phosphatase resulted in a slight increase in PI kinase activity. It is also possible that PI kinase activity is regulated by a modulator protein that is subject to control by phosphorylation. Conclusive evidence for covalent modification of PI kinase awaits the purification of PI kinase, the preparation of antibodies, and the demonstration of ³²P incorporation into this enzyme in vitro and in vivo.

It is known that cAMP-dependent protein phosphorylation plays a major regulatory role in the control of cell proliferation and differentiation in *S. cerevisiae*. Tripp et al. (38) have recently identified a number of phosphoproteins in *S.*

cerevisiae that are catalyzed by cAMP-dependent protein kinase and are correlated with proliferation and cell cycle arrest. Results of genetic studies have shown that cAMP-dependent protein phosphorylation is required for cell cycle initiation and vegetative growth, whereas cAMP-dependent protein kinase must be inactivated before cell cycle arrest or sporulation can take place (26–28). The fact that PI kinase activity was not affected in cell extracts of stationary-phase cells preincubated under phosphorylation conditions was not surprising. This may provide an explanation of why PI kinase activity was elevated in the stationary phase. It has been suggested that enhanced synthesis of the polyphosphoinositides via PI kinase at the G₁ phase of the cell cycle may be important for increased formation of the plasma membrane (25). It is also possible that increased PI kinase activity is required for cell differentiation. It is clear that purification of PI kinase to homogeneity is required for a study of the regulation of this important enzyme under well-defined conditions. Work is currently in progress toward this end.

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