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Regulation of Phosphatidylinositol:Ceramide Phosphoinositol Transferase in *Saccharomyces cerevisiae*

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Maximal phosphatidylinositol:ceramide phosphoinositol transferase activity was measured in yeast cells harvested during the exponential phase of growth. The addition of inositol to the growth medium resulted in a twofold increase in IPC synthase activity in cells grown in the presence or absence of exogenous choline. Enzyme activity was not regulated in yeast inositol biosynthesis regulatory mutants by the addition of inositol to the growth medium.

The biosynthesis of inositol-containing sphingolipids in the yeast *Saccharomyces cerevisiae* is essential for the growth and viability of this organism (20). While the reasons for this are not fully understood, sphingolipids are thought to play important roles in various yeast cellular activities, including regulation of phospholipid biosynthesis (22), cell wall biosynthesis (9), anchoring of cell surface glycoproteins (3), membrane signaling pathways (7), and modulation of plasma membrane H^+-ATPase activity (16). The membrane-associated enzyme phosphatidylinositol:ceramide phosphoinositol transferase (IPC synthase) catalyzes an essential step in the biosynthesis of the yeast inositol-containing sphingolipids (1). The regulation of this enzyme should be important in controlling the sphingolipid composition and function of yeast membranes. To date, IPC synthase from a crude membrane preparation of *S. cerevisiae* has been only partially characterized (1), and no detailed information about the regulation of this enzyme is available. In this study, the regulation of IPC synthase activity in response to growth conditions that regulate yeast phospholipid biosynthesis and the ability of various phospholipid metabolism mutants to regulate IPC synthase were investigated.

Growth of strains, preparation of microsomes, and IPC synthase assay conditions. *S. cerevisiae* strains ade5 (*MAT***a** *ade5*), which exhibits normal regulation of phospholipid biosynthesis (2, 4), and ino2 (*MAT*α *ino2-21 lys2*), ino4 (*MAT***a** *ino4-39 lys2*), and opi1 (*MAT***a** *opi1-3 lys1*), inositol biosynthesis mutant strains that are defective in the regulation of phospholipid biosynthesis (2, 4, 8), were used in this study. Cells were grown in complete synthetic medium (12) containing *myo*-inositol (75 μM) and choline (1 mM) where indicated. Yeast cultures were incubated at 28°C on a rotary shaker at 225 rpm. Cells were harvested by centrifugation, and microsomal membranes were prepared as described previously (6).

IPC synthase activity was measured by monitoring the incorporation of phospho-[^{3}H]inositol from phosphatidyl-[^{3}H]inositol ([^{3}H]PI) into alkaline-stable product (IPC). Since the substrates phosphatidylinositol (PI) and ceramide are water insoluble, they were delivered to the assay system as part of a Triton X-100 lipid-mixed micelle. The Triton X-100 micelles effectively diluted the endogenous ceramide and PI present in our membrane preparations, and dependence on the addition of exogenous lipid substrates was observed as long as less than 30 μg of membrane protein was added to the assay system. Reactions were conducted at 30°C in 50 mM Tris-HCl buffer (pH 7.0) containing 0.5 mM [^{3}H]PI (20,000 disintegrations min^{-1} $nmol^{-1}$), 0.2 mM ceramide (*N*-acyl-sphingosine-containing hydroxy fatty acids; Sigma), 5 mM Triton X-100, 1 mM $MnCl_2$, 5 mM $MgCl_2$, and 0.25 mg of yeast microsome protein per ml, all in a final assay volume of 0.1 ml. Assays were terminated by the addition of 0.64 ml of chloroform-methanol (1:1, vol/vol), and the mixtures were then treated with 0.1 ml of methanolic sodium hydroxide (0.6 N sodium hydroxide in methanol) in order to deacylate unreacted [^{3}H]PI. The product of the reaction, [^{3}H]IPC, was identified by chromatography on activated (110°C, 30 min), EDTA-treated silica gel-impregnated paper (SG 81 paper; Whatman) and scintillation counting (1, 18). Since IPC was the only radioactive product detectable after paper thin-layer chromatography analysis, the final organic phase was analyzed routinely by direct scintillation counting. Each sample was assayed in triplicate, along with a blank consisting of all assay reagents inactivated with chloroform-methanol (1:1, vol/vol) before the start of the reaction. One unit of enzymatic activity is defined as the amount of enzyme that catalyzes the formation of 1 nmol of product per min. Specific activity is defined as units per milligram of protein. Purified yeast PI synthase was used to prepare radiolabeled PI from CDP-diacylglycerol (CDP-DAG) and [2-^{3}H]inositol (5). The method of Lowry et al. (13) was used to determine protein concentration, with bovine serum albumin as the standard.

Effect of growth phase and inositol and choline on IPC synthase activity. Yeast cells (*ade5*) grown on complete synthetic medium were harvested in the mid- and late exponential phase, early and mid-retardation phase, and stationary phase, and IPC synthase activity was determined in microsomal membrane preparations. Like other enzymes in the primary pathway of phosphatidylcholine biosynthesis (2, 12, 17, 21), IPC synthase activity was affected by the growth phase of the yeast cells. The results, summarized in Table 1, showed that the highest IPC synthase activity occurred in the mid- to late exponential phase and that a sevenfold decrease in enzyme activity occurred in cells harvested in the stationary phase of growth. These results are similar to those reported previously for the activities of the yeast phospholipid biosynthetic enzymes CDP-DAG synthase, phosphatidylserine synthase, and the phospholipid *N*-methyltransferases, which also decrease, 2.5- to 5-fold, during the stationary phase of growth (10).

Since a number of the yeast phospholipid biosynthetic

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TABLE 1. Effect of growth phase on IPC synthase activity[a]

Growth phase	Mean IPC synthase activity (U/mg) ± SD	Relative activity (%)
Mid-exponential	0.21 ± 0.01	100
Late exponential	0.19 ± 0.01	90
Early retardation	0.14 ± 0.02	67
Mid-retardation	0.08 ± 0.01	38
Stationary	0.03 ± 0.01	14

[a] Cells were grown in complete synthetic medium and harvested at the indicated phases of growth. Microsomal membranes were prepared from cell extracts, and IPC synthase activity was determined in triplicate as described in the text. Values are the means ± standard deviations for at least three independent growth studies.

enzymes are regulated by water-soluble phospholipid precursors (2), we investigated the effect of inositol and choline on IPC synthase activity. The addition of inositol to the growth medium of yeast cells resulted in a twofold increase in IPC synthase specific activity (Table 2). The addition of choline to the growth medium, in the absence or presence of inositol, did not significantly affect IPC synthase activity (Table 2). These findings are of significance because inositol is a key regulator of yeast phospholipid biosynthesis (2, 15, 21). The addition of inositol to the growth medium of yeast cells is known to result in the repression, to various degrees, of the entire set of enzymes leading from the synthesis of CDP-DAG to phosphatidylcholine (CDP-DAG → phosphatidylserine → phosphatidylethanolamine → phosphatidylmonomethylethanolamine → phosphatidyldimethylethanolamine → phosphatidylcholine) (2, 15, 21). Choline has little or no repressive effect alone; however, in combination with inositol, the level of repression is greater than when inositol is present alone (2, 15, 21). Our results show that, unlike the enzymes leading to phosphatidylcholine biosynthesis, IPC synthase activity was not repressed in cells grown in the presence of water-soluble phospholipid precursors. The only other phospholipid biosynthetic enzymes that are not repressed by inositol in *S. cerevisiae* are phosphatidic acid phosphatase (14), cardiolipin synthase (19), and PI synthase (6, 12). Both cardiolipin synthase (19) and PI synthase (6, 12) are unaffected by inositol, while the expression of phosphatidic acid phosphatase, activity and protein level, has been shown to be increased twofold when yeast cells are grown in medium supplemented with inositol (14). To determine whether inositol was directly affecting IPC synthase activity, we measured IPC synthase activity in the presence of inositol (0.05 to 2 mM) and found no effect on enzyme activity.

TABLE 2. IPC synthase activity in microsomal membrane extracts from *S. cerevisiae* grown in the presence and absence of phospholipid precursors[a]

Growth medium	Mean IPC synthase activity (U/mg) ± SD	Relative activity (%)
Complete synthetic	0.20 ± 0.01	100
Complete synthetic plus:		
Inositol	0.41 ± 0.02	205
Choline	0.21 ± 0.01	105
Inositol and choline	0.40 ± 0.03	200

[a] Cells were grown in complete synthetic medium with and without inositol (75 μM) and choline (1 mM), as indicated. Cells were harvested in the mid-exponential phase of growth, and microsomal membranes were prepared from cell extracts as described in the text. Values are the means ± standard deviations for at least three independent growth studies.

TABLE 3. IPC synthase activity in microsomal membrane extracts from inositol biosynthesis regulatory mutants grown in the presence and absence of inositol[a]

Mutation	Medium	Mean IPC synthase activity (U/mg) ± SD	Relative activity (%)
ade5	CS	0.20 ± 0.01	100
	CS + inositol	0.40 ± 0.01	200
ino2	CS + inositol	0.32 ± 0.02	160
ino4	CS + inositol	0.20 ± 0.01	100
opi1	CS	0.39 ± 0.01	190
	CS + inositol	0.42 ± 0.01	210

[a] Cells were grown in complete synthetic (CS) medium with and without 75 μM inositol, as indicated. Cells were harvested in the mid-exponential phase of growth, and microsomal membranes were prepared from cell extracts as described in the text. Values are the means ± standard deviations for at least three independent growth studies.

IPC synthase activity in inositol biosynthesis regulatory mutants. In order to determine whether inositol was affecting the expression of IPC synthase activity, we measured IPC synthase activity in yeast inositol biosynthesis regulatory mutants (*ino2*, *ino4*, and *opi1* mutants) that were grown in the presence or absence of inositol. The *opi1* mutant yeast cells express the coordinately regulated yeast phospholipid biosynthetic enzymes at high levels regardless of the growth conditions of the cells (21). IPC synthase activity in the *opi1* mutant was elevated twofold in the absence or presence of inositol (Table 3). Therefore, like other enzymes in the primary pathway of phosphatidylcholine biosynthesis (2, 12, 17, 21), IPC synthase activity was not regulated by inositol in the *opi1* mutant. The *ino2* and *ino4* mutant cells are inositol auxotrophs (4) and express repressed levels of the coordinately regulated phospholipid biosynthetic enzymes (15, 21). IPC synthase activity in the *ino4* mutant did not respond to the presence of inositol in the growth medium and was expressed at the same level as in *ade5* cells grown in the absence of inositol (Table 3). In the *ino2* mutant grown in the presence of inositol, IPC synthase activity was 1.6-fold higher than in *ade5* cells grown in the absence of inositol (Table 3). These results suggest that IPC synthase is differentially regulated by inositol in the *ino2* and *ino4* regulatory mutants.

In summary, IPC synthase activity was found to be affected by the growth phase of the cells, by the addition of inositol to the growth medium, and by mutations that affect the regulation of yeast phospholipid biosynthesis. These results suggest that IPC synthase activity may be regulated by the same mechanism that controls yeast phospholipid biosynthesis and regulates many of the yeast phospholipid biosynthetic enzymes. The inability of inositol to affect IPC synthase activity in inositol biosynthesis regulatory mutants suggests that regulation of IPC synthase occurs at the genetic level. Further evidence for the genetic regulation of IPC synthase by inositol awaits in vivo analysis of the IPC synthase mRNA and protein levels. The regulation of IPC synthase by inositol also suggests that synthesis of the yeast inositol-containing sphingolipids may be coordinately regulated with the synthesis of PI through the control of inositol synthesis. The modulation of IPC synthase activity by inositol may have further physiological significance, since the amount of PI in yeast membranes is elevated in cells grown in the presence of exogenous inositol (11). Therefore, increased levels of PI and IPC synthase may be one way in which yeast cells regulate the synthesis of their inositol-

containing sphingolipids. Future studies are planned to gain a better understanding of the mechanism that regulates IPC synthase expression and activity in yeast cells. Towards these goals, we are currently developing a purification procedure for the membrane-associated IPC synthase of *S. cerevisiae.*

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