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Coordinate Regulation of Phosphatidylserine Decarboxylase in *Saccharomyces cerevisiae*

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Regulation of the activity of the mitochondrial enzyme phosphatidylserine decarboxylase (PSD) was measured in vitro by using membrane preparations from wild-type and mutant strains of *Saccharomyces cerevisiae*. PSD specific activity was not affected by carbon source, and on all carbon sources, the highest specific activity was observed in cells entering the stationary phase of growth. However, PSD activity was found to be regulated in response to soluble precursors of phospholipid biosynthesis. PSD specific activity was reduced to about 63% of the level observed in unsupplemented wild-type cells when the cells were grown in the presence of 75 μM inositol. The presence of 1 mM choline alone had no repressing effect, but the presence of 1 mM choline and 75 μM inositol together led to further repression to a level of about 28% of the derepressed activity. Regulatory mutations known to affect regulation or expression of genes encoding phospholipid-synthesizing enzymes also affected PSD specific activity. *opi1* mutants, which are constitutive for a number of phospholipid-biosynthetic enzymes, were found to have high, constitutive levels of PSD. Likewise, in *ino2* or *ino4* regulatory mutants, PSD activity was found to be at the fully repressed level regardless of growth condition. Regulation of PSD activity was also affected in several structural-gene mutants under conditions of impaired phosphatidylcholine biosynthesis. Together, these data strongly suggest that PSD expression is controlled by the mechanism of general control of phospholipid biosynthesis that regulates many enzymes of phospholipid biosynthesis.

A number of phospholipid-biosynthetic enzymes of *Saccharomyces cerevisiae* are subject to coordinate regulation. These enzymes include cytidine diphosphate (CDP)-diacylglycerol synthase (12), phosphatidylserine (PtdSer) synthase (14, 21), inositol-1-phosphate synthase (11), and the phospholipid *N*-methyltransferases, which convert phosphatidylethanolamine (PtdEtn) into phosphatidylcholine (PtdCho) by three sequential methylations (10, 14, 23, 25, 27). All of these enzymes exhibit a common pattern of regulation (5, 10). They are fully derepressed in the absence of the soluble phospholipid precursors inositol and choline or ethanolamine; they are partially repressed in the presence of inositol and fully repressed in the presence of inositol plus choline.

However, each of these enzymes exhibits a different repression ratio in response to these soluble precursors. For example, CDP-diacylglycerol synthase is repressed only two- to threefold in response to inositol and choline (12), while inositol-1-phosphate synthase is repressed some 30-fold (11). PtdSer synthase, on the other hand, exhibits an intermediate level of repression, some four- to fivefold, in response to inositol and choline (3). Furthermore, the same enzymes are differentially regulated in response to additional soluble phospholipid precursors. For example, PtdSer synthase and CDP-diacylglycerol synthase are repressed not only by inositol or the combination of inositol plus choline, but also by the combination of inositol plus serine or ethanolamine (12, 21).

Several steps of phospholipid biosynthesis in *S. cerevisiae* are also regulated in response to growth stage. In logarithmically growing cells, CDP-diacylglycerol synthase, PtdSer synthase, and the phospholipid *N*-methyltransferases have maximum activities, whereas in stationary growth phase, activities are reduced to 20 to 50% (13).

Each of the enzymes that responds to the presence of soluble phospholipid precursors is also under the control of a common set of regulatory genes. For example, *opi1* mutants, which were originally isolated on the basis of overproduction of inositol (Opi$^-$ phenotype) (9), were later shown to express CDP-diacylglycerol synthase, PtdSer synthase, and the phospholipid *N*-methyltransferases constitutively as well (12, 14). In addition, *ino2* and *ino4* mutants (7), which were originally detected as inositol auxotrophs by their inability to derepress inositol-1-phosphate synthase, were subsequently shown to exhibit low constitutive levels of PtdSer synthase (3) and the phospholipid *N*-methyltransferases (16). It has recently become apparent that the regulatory network within which the *OPI1*, *INO2*, and *INO4* gene products function requires the active synthesis of PtdCho. When the synthesis of PtdCho is blocked by mutations in the structural genes for the phospholipid *N*-methyltransferases, no regulatory signal is generated in response to inositol (19, 23). In such mutants, the regulatory signal can be regenerated if a soluble precursor is present which enters the PtdCho-biosynthetic pathway, bypassing the metabolic lesion in the particular mutant, thus restoring PtdCho biosynthesis.

Phosphatidylserine decarboxylase (PSD), which catalyzes the conversion of PtdSer to PtdEtn, while not as extensively studied as the phospholipid-biosynthetic enzymes discussed above, has been shown to be repressed in response to choline (6). PSD is unique among the enzymes involved in PtdCho biosynthesis in that it cofractionates with the inner mitochondrial membrane (15, 29), whereas the phospholipid *N*-methyltransferases, for example, which catalyze the sequential methylation of PtdEtn that may be produced by the PSD reaction, are localized in the microsomal membrane.

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TABLE 1. Strains used in this study

Strain	Relevant genotype	Remarks	Source or reference
ade5	*MATα ade5*	Wild type	7
D273-10B		Wild type	ATCC 25657
cho1	*MATa cho1::TRP1 ura3 leu2*	PtdSer synthase deficient	3
cho2	*MATa cho2::LEU2 leu2-3,112 ura3-1 lys2 ade6*	PtdEtn methyltransferase I deficient	23
opi1	*MATa opi1-1 ura3-1 his3 leu2-3,112*	Regulatory mutant	9
ino2	*MATa ino2-21 ade2-1 leu2-3,112 trp1-1 ura3-1*	Regulatory mutant	7
ino4	*MATa ino4-39 leu2-3,112 trp1-1 ura3-1*	Regulatory mutant	7

Localization of these enzymes in different membrane compartments may well play an important role in the control of substrate flow through the biosynthetic pathways. In the present study, we have further analyzed the regulation of PSD in order to determine whether it is regulated in coordination with other enzymes involved in PtdCho biosynthesis despite different subcellular distributions. To address this question, we have explored four aspects of the regulation of PSD: response to carbon sources affecting mitochondrial proliferation; response to growth in the presence or absence of soluble phospholipid precursors; effect of the regulatory mutations *opi1*, *ino2*, and *ino4*; and requirement for ongoing PtdCho biosynthesis.

MATERIALS AND METHODS

Yeast strains and culture conditions. The *S. cerevisiae* strains used in this study and their sources are summarized in Table 1. Cells were grown in liquid culture medium (11), with or without 75 μM inositol, 1 mM choline, or 1 mM ethanolamine or with no supplementation, as indicated. Galactose (2%), 3% lactate, or 5% glucose was used as the carbon source, as indicated. Cell density was monitored by cell counting and nephelometry at 548 nm.

Preparation of membranes and enzyme assays. Cells were harvested by centrifugation at the times indicated in the figure legends. Membrane fractions were obtained by disintegration of cells with glass beads as described before (8) and centrifugation at 100,000 × *g* for 60 min. The enzymatic activity of PSD was determined in vitro by measuring the conversion of phosphatidyl-L-[3-^{3}H]serine into radioactively labeled PtdEtn. The assay was based on the system described by Carson et al. (6) with several modifications as indicated below. Labeled PtdSer was synthesized in vitro by using total membranes from a transformed yeast strain overexpressing the *CHO1* structural gene encoding PtdSer synthase (22) and L-[3-^{3}H]serine in the presence of CDP-(*sn*-1 palmitoyl, *sn*-2 oleoyl)-glycerol, Mn^{2+} ions, and hydroxylamine (2). Labeled PtdSer was purified by preparative thin-layer chromatography on silica gel H60 plates (Merck). The specific activity of radioactively labeled PtdSer was in the range of 0.50 to 2.0 mCi/mmol.

PSD assays were routinely performed at 30°C. The basic incubation assay mix contained, in a total volume of 300 μl, 15 μmol of Tris-Cl (pH 7.2), 0.12% Triton X-100, 150 nmol of radioactively labeled PtdSer (1.9 μCi/μmol), 37.5 μmol of sucrose, 1.5 μmol of EDTA, and 3 μmol of dithiothreitol (6). In order to determine the dependence of the enzyme activity on the detergent, the concentration and the ratio of Triton X-100 to substrate were varied as indicated (see also Fig. 1). Prior to incubation with membrane protein, this incubation mixture was subjected to extensive vortexing and ultrasonication for 5 min at 100 W in a water bath. The reaction was initiated by addition of 60 to 150 μg of membrane protein and vigorous vortexing. The reaction was terminated by removing 100-μl aliquots from the incubation mixture and extracting lipids with 0.5 ml of chloroform-methanol (2:1, vol/vol) containing 1 mg of unlabeled yeast total lipids per ml as a carrier. Extracts were dried under a stream of nitrogen, and lipids were dissolved in 50 μl of chloroform-methanol (2:1, vol/vol) and subjected to thin-layer chromatography on silica gel H60 plates in a solvent system containing chloroform–methanol–28% ammonia (50:25:6, by vol). Lipid spots were visualized by exposure to iodine vapor and scraped off with a scalpel, and radioactivity in the PtdSer and PtdEtn fractions was determined by liquid scintillation counting. The specific activity of succinate dehydrogenase (1) and cytochrome oxidase (18), which were used as mitochondrial markers, was assayed as described before. The protein concentration was determined by the assay of Lowry et al. (17) with bovine serum albumin as the standard. The glucose concentration in the medium was measured with a glucose oxidase test kit from Boehringer, Mannheim, Germany.

RESULTS

Enzymatic properties of PSD. The properties of PSD were analyzed in membrane preparations of wild-type cells grown under different nutritional conditions. The protein was stable at 0°C for several hours; at the standard assay temperature (30°C) in the presence of Triton X-100, the activity dropped to about 35% within 1 h. The reaction was linear with time between 0 and 10 min and with protein concentrations between 0.2 and 3.0 mg/ml. The pH dependence and inhibition by divalent cations and by reagents affecting sulfhydryl and carbonyl groups were the same as described by Carson et al. (6). When the detergent-to-substrate ratio was kept constant (Triton X-100 to PtdSer ratio of 3.7:1), a linear dependence of S/v versus S was observed in a Hanes-Langmuir plot (Fig. 1). The apparent K_m was 0.16 ± 0.05 mM and the V_{max} was 9.8 ± 0.8 nmol min^{-1} mg^{-1} in membrane preparations of wild-type cells grown without lipid precursors, inositol, and choline.

When the concentration of Triton X-100 was held constant at 0.12%, thus varying the molar ratio of detergent to substrate between 0.9:1 and 18.4:1, a significant alteration in the kinetics of the reaction was observed. At a detergent-to-substrate ratio above 5:1, PSD activity was significantly reduced and a Michaelis-Menten-type of reaction kinetics could no longer be observed (Fig. 1). These rather complex kinetics of the PSD reaction are obviously due to the facts that both the substrate and product of the reaction are unsoluble in water and that the enzyme is a membrane protein. Therefore, in order to obtain reliable results for the PSD activity in different membrane preparations, much attention was paid to using identical amounts of substrate, detergent, and protein in each experiment. For the experiments reported here, the substrate concentration was 0.5

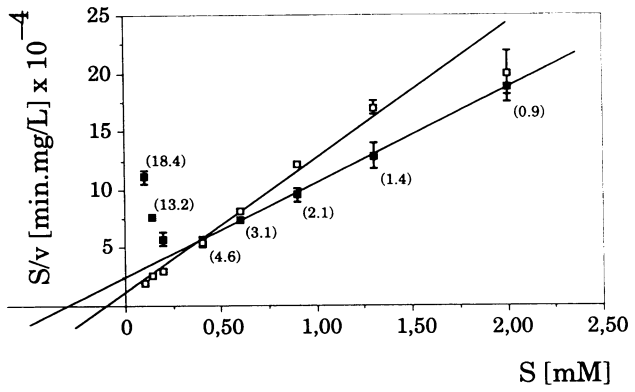

FIG. 1. Kinetics of the PSD reaction in membrane preparations from wild-type cells (strain ade5) grown in the absence of inositol and choline. Media contained 2% galactose as the carbon source. The assay conditions were as described in the Materials and Methods section at a constant Triton X-100-to-PtdSer molar ratio of 3.7:1 (□) or a constant concentration of Triton X-100 of 0.12% (■). Numbers in parenthesis indicate the molar ratio of Triton X-100 to PtdSer.

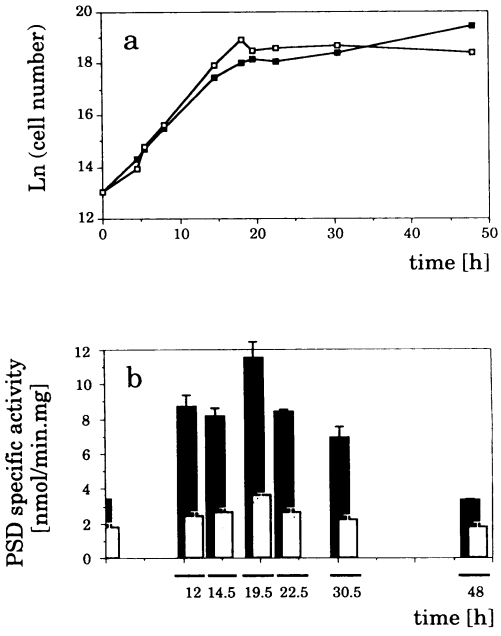

FIG. 2. (a) Growth dependence of wild-type cells (strain ade5) in media containing 2% galactose as the carbon source without supplementation (■) or supplemented with 75 μM inositol and 1 mM choline (□). (b) Specific activity of PSD during growth in cells grown without supplementation (black boxes) or with inositol and choline (white boxes). Growth was monitored by cell counting.

mM (about five times the apparent K_m) and the Triton X-100 concentration was 0.12% (molar ratio of Triton X-100 to PtdSer of 3.7:1). Under these conditions, the assay results were highly reproducible, and triplicate determinations were routinely performed with a standard error below 10%.

Relationship of PSD activity to growth phase in the wild-type strain. Wild-type cells (strain ade5) grown on 2% galactose medium with or without 75 μM inositol and 1 mM choline were harvested at several points of growth, in log phase, late log phase, and early and late stationary phase, and PSD activity was determined in membrane preparations as described in the Materials and Methods section. The results, summarized in Fig. 2, showed a significant increase of PSD activity in cells entering the stationary phase. This modulation of PSD activity was identical for cells grown without and with supplementation with inositol and choline; however, the level of activity was about two- to threefold higher in cells grown in the absence of inositol and choline, regardless of growth phase.

Regulation of PSD activity in response to carbon source. The activity of PSD was determined in strain D273-10B during growth in medium without inositol and choline and containing 5% glucose as the carbon source. In order to correlate PSD activity to the state of respiratory activity, the mitochondrial enzymes succinate dehydrogenase and cytochrome *c* oxidase were analyzed as markers. Figure 3 summarizes the specific activities of PSD and the marker enzymes during cellular growth in the presence of initially high glucose concentrations. Succinate dehydrogenase and cytochrome *c* oxidase activities were low as long as the glucose concentration in the growth medium was high. Maximum activities of succinate dehydrogenase and cytochrome *c* oxidase were observed in stationary-phase cells. PSD specific activity was very similar between the wild-type strains D273-10B grown on 5% glucose and ade5 grown on 2% galactose and did not correlate with the activities of the marker enzymes, indicating that PSD does not respond to glucose repression.

Regulation of PSD activity in response to inositol, ethanolamine, and choline. The data summarized in Table 2 show that PSD specific activity in wild-type cells grown with inositol in the growth medium was reduced to about 63% of the level observed in cells grown without supplementation. The absence or presence of 1 mM ethanolamine or choline did not significantly affect PSD specific activity. The presence of a combination of either ethanolamine or choline with inositol, however, reduced the specific activity to about 4.4 or 2.6 nmol min^{-1} mg^{-1}, respectively (48 and 28% of the derepressed level, respectively). Apparent K_m and V_{max} values for the conversion of PtdSer to PtdEtn were determined for preparations from cells grown in the presence or absence of inositol and choline. The apparent K_m did not vary significantly between the different preparations and was 0.11 ± 0.05 mM for membranes from cells grown in the presence of inositol and choline versus 0.16 ± 0.05 mM for those from unsupplemented cells. However, the V_{max} was 9.8 ± 0.8 nmol min^{-1} mg^{-1} in cells grown without supplementation, compared with 2.9 ± 0.3 nmol min^{-1} mg^{-1} in cells grown in the presence of inositol and choline. The maximal rate for the PSD reaction was identical when Triton X-100 was added in a constant molar ratio to PtdSer of 3.7:1 or 1:1 (data not shown). Addition of inositol and/or choline or ethanolamine to the assay mixture did not affect the enzymatic properties of PSD. Mixing of membrane preparations from wild-type cells grown under different supplementation conditions resulted in the expected average specific activity (data not shown).

Regulation of PSD specific activity in structural and regulatory mutants defective in phospholipid biosynthesis. In order to analyze further whether regulation of PSD activity occurs via the mechanisms of general control of phospholipid synthesis, enzyme activity was analyzed in different structural and regulatory yeast mutants defective in phospholipid biosynthesis. *chol* mutants (3) are defective in the synthesis of PtdSer, the endogenous substrate for the PSD reaction. In

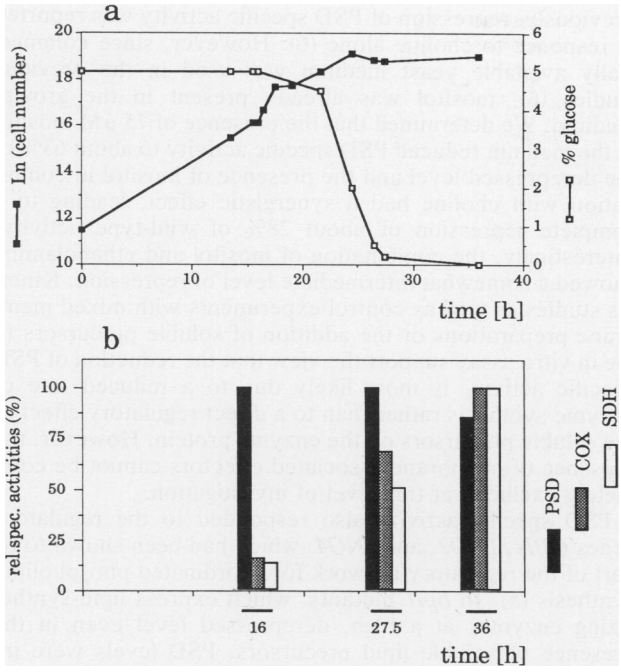

FIG. 3. (a) Growth of wild-type D273-10B cells in semisynthetic medium without inositol and choline and containing 5% glucose as the carbon source. The number of cells and the concentration of glucose present in the medium are shown. (b) Relative specific activities of PSD (black boxes) and the mitochondrial marker enzymes cytochrome *c* oxidase (COX, hatched boxes) and succinate dehydrogenase (SDH, white boxes) in wild-type D273-10B. Cells were grown in semisynthetic medium containing glucose as the carbon source and without inositol and choline. The cells were harvested at the times indicated, and enzymes were analyzed by the procedures described in the Materials and Methods section.

cho1 mutants grown on ethanolamine or choline, the specific activity of PSD was significantly lower (about 50%) than in the wild type. This reduction could be due to the significantly reduced growth rate of the mutant even under supplementation conditions. Addition of inositol to the growth medium of *cho1* mutants supplemented with ethanolamine or choline resulted in a reduction of PSD specific activity to about 28% of the derepressed level, which parallels the reduction in wild-type cells under the same nutritional conditions.

In *cho2* mutants, defective in the monomethylation of PtdEtn to phosphatidylmonomethylethanolamine, PSD activity levels did not respond significantly to the addition of inositol. However, restoration of PtdCho synthesis by the addition of choline to the inositol-containing growth medium again resulted in reduced PSD activity, to a level almost equivalent to the fully repressed level observed in the wild-type strain (Table 2). Despite an accumulation of PtdEtn in unsupplemented *cho2* mutants of up to 50% of total phospholipids, no effect on PSD specific activity was observed. In vitro, PSD specific activity also did not respond to the addition of PtdEtn to the assay mix. Therefore, feedback control of the enzyme by its immediate product, PtdEtn, can be excluded as a regulatory mechanism.

opi1 regulatory mutants are known to express phospholipid-synthesizing enzymes at a high, derepressed level in the presence of lipid precursors (9). PSD activity in *opi1* mutants was about 1.5-fold higher than in wild-type cells when both inositol and choline were absent from the growth medium. In contrast to the wild type, PSD activity in the *opi1* mutant remained at a high, unregulated level in the presence of inositol and/or choline (Table 2).

In the *ino2* and *ino4* regulatory mutants, defective in the derepression of phospholipid-synthesizing enzymes, PSD activity remained at the fully repressed level (Table 2) whether or not choline was added to the inositol-supplemented medium.

This pattern of regulation of PSD specific activity in wild-type cells and regulatory mutants in response to soluble phospholipid precursors is equivalent to the pattern observed for other phospholipid-biosynthetic enzymes that have been analyzed. This result strongly suggests that PSD is controlled by the same regulatory mechanism that controls other enzymes of phospholipid biosynthesis.

DISCUSSION

PSD catalyzes a central step in phospholipid biosynthesis in *S. cerevisiae*. This reaction provides cells with PtdEtn, which in itself is a major membrane component and, in addition, serves as the substrate for the de novo synthesis of PtdCho via the methylation pathway. From studies with rat brain (4) and perfused hamster heart (28), it was estimated that in mammalian cells, the contribution of the PSD reaction accounted for less than 10% of the overall production of PtdEtn. However, these assumptions most likely underestimate the significance of PSD for phospholipid synthesis in higher organisms (20, 24).

The aim of this study was to determine the regulatory effects of growth phase, carbon source, lipid precursors, and mutations in the phospholipid biosynthetic pathway on the

TABLE 2. Specific activity of PSD in wild-type and mutant strains of *S. cerevisiae* grown with 2% galactose as the carbon source and in the presence or absence of inositol, ethanolamine, and choline[a]

Strain	PSD sp act (nmol of PtdEtn generated/min/mg of membrane protein)					
	No supplementation	Ethanolamine (1 mM)	Choline (1 mM)	Inositol (75 μM)	Inositol (75 μM) + ethanolamine (1 mM)	Inositol (75 μM) + choline (1 mM)
ade5	9.3 ± 1.1	10.1 ± 0.2	9.4 ± 0.1	5.8 ± 0.1	4.4 ± 0.3	2.6 ± 0.3
cho1	ND	5.2 ± 0.1	4.7 ± 0.1	ND	2.3 ± 0.1	1.26 ± 0.1
cho2	10.5 ± 0.7	ND	9.6 ± 0.4	11.3 ± 0.3	ND	3.6 ± 0.3
opi1	14.2 ± 0.4	ND	13.7 ± 0.7	16.4 ± 0.3	ND	18.2 ± 1.1
ino2	ND	ND	ND	2.7 ± 0.2	ND	2.6 ± 0.1
ino4	ND	ND	ND	2.5 ± 0.1	ND	2.9 ± 0.3

[a] Membranes were prepared from early-stationary-phase cells as described in the Materials and Methods section. Values are the mean ± standard deviation of triplicate determinations.
[b] ND, not determined.

activity of PSD in *S. cerevisiae*. For obvious reasons, Michaelis-Menten kinetics cannot be applied to a system in which the enzyme is membrane bound and the substrate forms micellar aggregates. Thus, the K_m values obtained from the kinetic studies can only be interpreted as apparent K_m. At a constant Triton X-100-to-PtdSer ratio of 3.7 and with a relatively small amount of membrane protein, substrate concentrations could be varied over a wide range without deviation from linearity in a Hanes plot (Fig. 1). When PSD activity was measured at a constant Triton X-100 concentration of 0.12%, the reaction rates became very low at low substrate concentrations, which could be explained by surface dilution of PtdSer in the presence of excess detergent (26).

The assay conditions used in this study are somewhat different from those employed in a previous study on the effects of choline on PSD activity (6). The main differences are the amount of enzyme preparation, which was 10- to 100-fold higher in the previous study, and the type of substrate and detergent used. Small amounts of membrane preparation appeared to be advantageous because membrane lipids contain PtdSer, which dilutes the labeled PtdSer added to the assay mixture. In our study, the amount of endogenous PtdSer in the membrane preparation was determined and accounted for less than 4% of the total substrate in the assay. The semisynthetic PtdSer used for the in vitro assay reflects the fatty acyl composition of the endogenous yeast PtdSer. We have used Triton X-100 as the detergent, whereas Cutscum was used in the previous study (6). These differences, taken together, probably account for the significantly lower apparent K_m (0.11 ± 0.05 versus 3.1 mM [6]) and the approximately twofold-higher V_{max} observed in the present study.

PSD cofractionates with the inner mitochondrial membrane in *S. cerevisiae* (15, 29). We have shown, however, that the specific activity of PSD does not respond to glucose repression. This result was not unexpected because a high glucose concentration promotes growth, leading to an increased phospholipid requirement for membrane synthesis. A reduction of PSD activity to the extent observed for repressible mitochondrial enzymes would presumably limit the overall rate of aminophospholipid synthesis and, as a consequence, reduce the rate of cellular growth.

PSD activity is regulated in response to the growth phase of cells. Maximum activities were found when cells entered the stationary growth phase regardless of supplementation with the soluble lipid precursors inositol and choline. In the late stationary phase, PSD specific activity was reduced to about 35 to 50% of the maximum level under both growth conditions. Somewhat different results had been reported previously for CDP-diacylglycerol synthase, PtdSer synthase, and the phospholipid *N*-methyltransferases (13). In cells grown in the absence of inositol and choline, maximum activities of these enzymes were observed in the logarithmic growth phase, and a two- to fivefold reduction occurred in the stationary phase. In wild-type cells supplemented with inositol and choline, these enzyme activities were repressed maximally and did not differ significantly between growing and nongrowing cells (13).

The presence in the growth medium of the soluble lipid precursors inositol and inositol plus ethanolamine or choline affected PSD specific activity in a fashion similar to that reported for other enzymes involved in phospholipid biosynthesis in *S. cerevisiae* (5). For wild-type cells, addition of ethanolamine or choline to the growth medium in the absence of inositol had no effect on PSD specific activity. Previously, repression of PSD specific activity was reported in response to choline alone (6). However, since commercially available yeast medium was used in the previous studies (6), inositol was already present in the growth medium. We determined that the presence of 75 μM inositol in the medium reduced PSD specific activity to about 63% of the derepressed level and the presence of inositol in combination with choline had a synergistic effect, leading to a complete repression of about 28% of wild-type activity. Interestingly, the combination of inositol and ethanolamine showed a somewhat intermediate level of repression. Kinetics studies as well as control experiments with mixed membrane preparations or the addition of soluble precursors to the in vitro assay support the view that the reduction of PSD specific activity is most likely due to a reduced rate of enzyme synthesis rather than to a direct regulatory effect of the soluble precursors on the enzyme protein. However, the presence of membrane-associated effectors cannot be completely excluded at this level of investigation.

PSD specific activity also responded to the regulatory genes *OPI1*, *INO2*, and *INO4*, which had been shown to be part of the regulatory network for coordinated phospholipid synthesis (5). In *opi1* mutants, which express lipid-synthesizing enzymes at a high, derepressed level even in the presence of soluble lipid precursors, PSD levels were increased rather than reduced when inositol and/or choline was present in the growth medium. In *ino2* and *ino4* mutants, which are defective in positive regulatory factors required for expression of lipid-synthesizing enzymes, the PSD specific activity remained at a low, repressed level regardless of supplementation. This pattern of regulation of PSD is similar to the pattern of regulation of other enzymes of phospholipid biosynthesis, including PtdSer synthase and inositol-1-phosphate synthase (5), and strongly suggests that PSD is under the same regulatory control as the other coordinately regulated enzymes. At least two of the structural genes encoding coregulated enzymes (*INO1*, encoding inositol-1-phosphate synthase [11], and *CHO1*, encoding PtdSer synthase [3]) have been shown to be regulated at the transcriptional level. Thus, we conclude that PSD activity is also, at least in part, regulated at the level of gene expression.

Two of the strains analyzed in this study are structural mutants defective in phospholipid-synthesizing enzymes. *cho1* mutants lack PtdSer synthase and are therefore devoid of PtdSer, the substrate for the PSD reaction. In these mutants, which are ethanolamine or choline auxotrophs, the PSD activity levels responded to the presence of inositol in the growth medium, comparable to the wild type. The lack of repression of PSD specific activity in *cho2* mutants in response to inositol and the restoration of regulation upon further addition of choline to the growth medium show that in order for repression to occur, ongoing synthesis of PtdCho is required. A similar requirement has been observed previously for the expression of inositol-1-phosphate synthase, the product of the yeast *INO1* gene (11, 19). This most interesting phenomenon, that ongoing synthesis of PtdCho is required for the cell to respond to the presence of inositol, is as yet unexplained. Further progress in the understanding of the control of expression of PSD in *S. cerevisiae* awaits the isolation of the protein and the structural analysis of the *PSD* gene, including the regulatory sequences.

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REFERENCES

1. **Ackrell, B. A. C., E. B. Kearney, and T. P. Singer.** 1978. Mammalian succinate dehydrogenase. Methods Enzymol. **53:** 466–483.
2. **Bae-Lee, M. S., and G. M. Carman.** 1984. Phosphatidylserine synthesis in *Saccharomyces cerevisiae*. Purification and characterization of membrane-associated phosphatidylserine synthase. J. Biol. Chem. **259:**10857–10862.
3. **Bailis, A. M., M. A. Poole, G. M. Carman, and S. A. Henry.** 1987. The membrane-associated enzyme phosphatidylserine synthase is regulated at the level of mRNA abundance. Mol. Cell. Biol. **7:**167–176.
4. **Butler, M., and P. Morell.** 1983. The role of phosphatidylserine decarboxylase in brain phospholipid metabolism. J. Neurochem. **41:**1445–1454.
5. **Carman, G. M., and S. A. Henry.** 1989. Phospholipid biosynthesis in yeast. Annu. Rev. Biochem. **58:**635–669.
6. **Carson, M. A., M. Emala, P. Hogsten, and C. J. Waechter.** 1984. Coordinate regulation of phosphatidylserine decarboxylase activity and phospholipid *N*-methylation in yeast. J. Biol. Chem. **259:**6267–6273.
7. **Culbertson, M. R., and S. A. Henry.** 1975. Inositol requiring mutants of *Saccharomyces cerevisiae*. Genetics **80:**23–40.
8. **Daum, G., E. Heidorn, and F. Paltauf.** 1986. Intracellular transfer of phospholipids in the yeast, *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **878:**93–101.
9. **Greenberg, M., P. Goldwasser, and S. A. Henry.** 1982. Regulatory mutations of inositol biosynthesis in yeast: isolation of inositol excreting mutants. Genetics **100:**19–33.
10. **Henry, S. A., L. S. Klig, and B. S. Loewy.** 1984. The genetic regulation of biosynthetic pathways in yeast: amino acid and phospholipid synthesis. Annu. Rev. Genet. **18:**207–231.
11. **Hirsch, J. P., and S. A. Henry.** 1986. Expression of the *Saccharomyces cerevisiae* inositol-1-phosphate synthase (*INO1*) gene is regulated by factors that affect phospholipid synthesis. Mol. Cell. Biol. **6:**3320–3328.
12. **Homann, M. J., S. A. Henry, and G. M. Carman.** 1985. Regulation of CDP-diacylglycerol synthase activity in *Saccharomyces cerevisiae*. J. Bacteriol. **163:**1265–1266.
13. **Homann, M. J., M. A. Poole, P. M. Gaynor, C.-T. Ho, and G. M. Carman.** 1987. Effect of growth phase on phospholipid biosynthesis in *Saccharomyces cerevisiae*. J. Bacteriol. **169:** 533–539.
14. **Klig, L. S., M. J. Homann, G. M. Carman, and S. A. Henry.** 1985. Coordinate regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae*: pleiotropically constitutive *opi1* mutant. J. Bacteriol. **162:**1135–1141.
15. **Kuchler, K., G. Daum, and F. Paltauf.** 1986. Subcellular and submitochondrial localization of phospholipid-synthesizing enzymes in *Saccharomyces cerevisiae*. J. Bacteriol. **165:**901–910.
16. **Loewy, B. S., and S. A. Henry.** 1984. The *INO2* and *INO4* loci of *Saccharomyces cerevisiae* are pleiotropic regulatory genes. Mol. Cell. Biol. **4:**2479–2485.
17. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
18. **Mason, T. L., R. O. Poyton, D. C. Wharton, and G. Schatz.** 1973. Cytochrome c oxidase from baker's yeast. J. Biol. Chem. **248:**1346–1354.
19. **McGraw, P., and S. A. Henry.** 1989. Mutations in the *Saccharomyces cerevisiae opi3* gene: effects on phospholipid methylation, growth and cross-pathway regulation of inositol synthesis. Genetics **122:**317–330.
20. **Miller, A. A., and C. Kent.** 1986. Characterization of the pathways for phosphatidylethanolamine biosynthesis in Chinese hamster ovary mutant and parental cell lines. J. Biol. Chem. **261:**9753–9761.
21. **Poole, M. A., M. J. Homann, M. S. Bae-Lee, and G. M. Carman.** 1986. Regulation of phosphatidylserine synthase from *Saccharomyces cerevisiae* by phospholipid precursors. J. Bacteriol. **168:**668–672.
22. **Sperka-Gottlieb, C., E.-V. Fasch, K. Kuchler, A. M. Bailis, S. A. Henry, F. Paltauf, and S. D. Kohlwein.** 1990. The hydrophilic and acidic N-terminus of the integral membrane enzyme phosphatidylserine synthase is required for efficient membrane insertion. Yeast **6:**331–343.
23. **Summers, E. F., V. A. Letts, P. McGraw, and S. A. Henry.** 1988. *Saccharomyces cerevisiae cho2* mutants are deficient in phospholipid methylation and cross-pathway regulation of inositol synthesis. Genetics **120:**909–922.
24. **Voelker, D. R.** 1984. Phosphatidylserine functions as the major precursor of phosphatidylethanolamine in cultured BHK-21 cells. Proc. Natl. Acad. Sci. USA **81:**2669–2673.
25. **Waechter, C. J., and R. J. Lester.** 1973. Differential regulation of the *N*-methyltransferases responsible for phosphatidylcholine synthesis in *Saccharomyces cerevisiae*. Arch. Biochem. Biophys. **158:**401–410.
26. **Warner, T. G., and E. A. Dennis.** 1975. Action of the highly purified, membrane-bound enzyme phosphatidylserine decarboxylase of *Escherichia coli* toward phosphatidylserine in mixed micelles and erythrocyte ghosts in the presence of surfactant. J. Biol. Chem. **250:**8004–8009.
27. **Yamashita, S., A. Oshima, J. Nikawa, and K. Hosaka.** 1982. Regulation of the phosphatidylethanolamine methylation pathway in *Saccharomyces cerevisiae*. Eur. J. Biochem. **104:**611–616.
28. **Zelinski, T. A., and P. C. Choy.** 1982. Phosphatidylethanolamine biosynthesis in isolated hamster heart. Can. J. Biochem. **60:**817–823.
29. **Zinser, E., C. D. M. Sperka-Gottlieb, E.-V. Fasch, S. D. Kohlwein, F. Paltauf, and G. Daum.** 1991. Phospholipid synthesis and lipid composition of subcellular membranes in the unicellular eukaryote *Saccharomyces cerevisiae*. J. Bacteriol. **173:** 2026–2034.