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# Regulation of Yeast Phospholipid Biosynthetic Genes in Phosphatidylserine Decarboxylase Mutants

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**In the yeast *Saccharomyces cerevisiae*, the products of two genes (*PSD1* and *PSD2*) are able to catalyze the decarboxylation of phosphatidylserine (PS) to produce phosphatidylethanolamine (PE) (C. J. Clancey, S. Chang, and W. Dowhan, J. Biol. Chem. 268:24580–24590, 1993; P. J. Trotter, J. Pedretti, and D. R. Voelker, J. Biol. Chem. 268:21416–21424, 1993; P. J. Trotter, and D. R. Voelker, J. Biol. Chem. 270:6062–6070, 1995). I report that the major mitochondrial PS decarboxylase gene (*PSD1*) is transcriptionaly regulated by inositol in a manner similar to that reported for other coregulated phospholipid biosynthetic genes. The second PS decarboxylase gene (*PSD2*) is not regulated on a transcriptional level by inositol and/or ethanolamine. In yeast, phosphatidylcholine (PC) biosynthesis is required for the repression of the phospholipid biosynthetic genes, including the *INO1* gene, in response to inositol. I show that the presence of a functional major mitochondrial PS decarboxylase encoded by the *PSD1* gene is necessary for proper regulation of *INO1* in response to inositol in the absence of ethanolamine. Disruption of the second PS decarboxylase gene (*PSD2*) does not affect the *INO1* regulation. Analysis of phospholipid content of PS decarboxylase mutants suggests that the proportion of PC on total cellular phospholipids is not correlated to the cell's ability to repress *INO1* in response to inositol. Rather, yeast cells are apparently able to monitor the flux through the phospholipid biosynthetic pathway and modify the transcription of phospholipid biosynthetic genes accordingly.**

Phospholipid biosynthesis is highly regulated in the yeast *Saccharomyces cerevisiae*. Much of this regulation occurs at the transcriptional level (for reviews, see references 5, 8, and 24). The vast majority of genes encoding enzymes involved in phospholipid biosynthesis are transcriptionally regulated by soluble precursors of phospholipid synthesis, inositol and choline. During the active growth of yeast cultures, coordinately regulated phospholipid biosynthetic genes are repressed when inositol is present in the growth medium. If choline is added to medium in which inositol is already present, the coregulated genes are further repressed. However, if choline is present in the growth medium by itself, it has little or no effect on transcription of the phospholipid biosynthetic genes. The derepression-to-repression ratio in structural genes of the CDP-diacylglycerol (CDP-DG) pathway ranges from 2- to 5-fold, whereas the *INO1* gene is subject to 30- to 50-fold repression (4, 10, 13). A consensus element, the inositol-sensitive upstream activation sequence ($UAS_{INO}$), has been found within the 5′ untranslated region of coordinately regulated genes (3, 24). In addition, the response of coordinately regulated genes to inositol and choline is controlled by a single set of regulatory factors: positive regulators, products of the *INO2* and *INO4* genes (1, 19, 22); and a negative regulator, product of the *OPI1* gene (9, 33).

Another aspect of this coordinate regulation is its dependence on ongoing phosphetidylcholine (PC) biosynthesis. Mutants that are defective in the synthesis of PC via the CDP-DG pathway display altered transcriptional regulation of coordinately regulated genes. Thus, yeast strains carrying mutations in the *CHO1*, *CHO2*, and *OPI3* genes show aberrant transcriptional regulation unless a metabolite that enters the PC biosynthetic pathway downstream of the genetic block is supplied exogenously (11, 18, 20, 29) (Fig. 1).

Interestingly, there are two genes (*PSD1* and *PSD2*) (6, 30–32) the products of which are able to catalyze the decarboxylation reaction of phosphatidylserine (PS) to phosphatidylethanolamine (PE). Inactivation of the structural gene for mitochondrially located PS decarboxylase (*PSD1*) has no effect on cell viability even in synthetic media lacking choline and ethanolamine. Despite the fact that disruption of the *PSD1* gene does not lead to the auxotrophic requirement for ethanolamine or choline, mutant cells are not wild type in all of their properties. They display very low decarboxylase activity in an enzymatic analysis, they have altered incorporation of radiolabel into phospholipids during the active phase of growth, and they have a pronounced tendency to form respiratory-deficient mutants (6, 32). Disruption of a second PS decarboxylase gene (*PSD2*) (putatively locatized to Golgi complex and vacuoles) does not result in any detectable phenotype, but simultaneous loss of *PSD1* and *PSD2* results in ethanolamine auxotrophy and severely impaired aminophospholipid metabolism (30).

In the present study, I show that transcription of the *PSD1* gene is regulated by the availability of soluble precursors inositol and ethanolamine in a fashion similar to that for other structural genes of the CDP-DG pathway (*CHO1*, *CHO2*, and *OPI3*). Transcription of the *PSD2* gene is constitutive under these conditions. The *INO1* gene displays wild-type regulation in a strain with a disrupted *PSD2* gene but is misregulated in a strain with a disrupted *PSD1* gene. This misregulation corresponds to slower than wild-type growth rate of the *PSD1* disruption strain in media without ethanolamine. Steady-state $^{32}P$ labeling of phospholipids reveals that the limiting step in the CDP-DG pathway in the *PSD1* disruption strain is formation of PE from PS.

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## MATERIALS AND METHODS

**Materials.** Materials and sources are as follows: [$^{32}P$]orthophosphate (carrier free) and [$\alpha$-$^{32}P$]CTP (specific activity, 800 Ci/mmol), Du-Pont NEN; nylon membrane, Schleicher & Schüll; SP6/T7 Riboprobe Combination System, Pro-

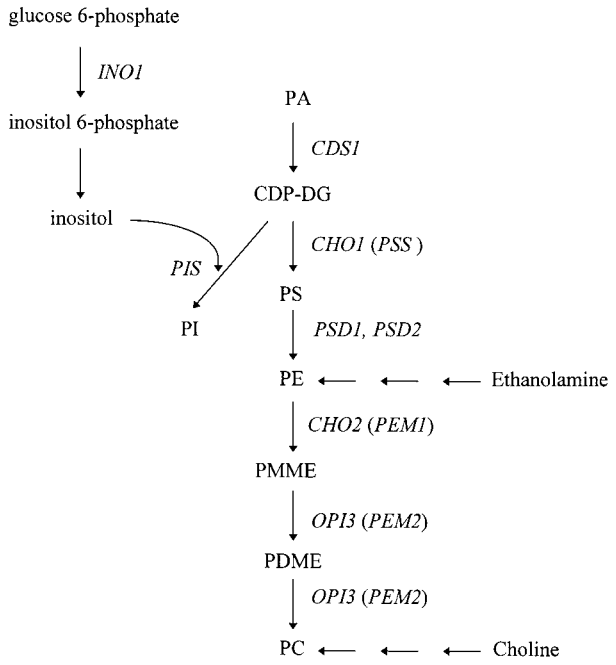


FIG. 1. Phospholipid biosynthetic pathways in yeast. For a more complex description of glycerophospholipid biosynthesis in yeast, see recent reviews by Paltauf et al. (24) and Carman and Zeimetz (5). Abbreviations not given in the text: PA, phosphatidic acid; PI, phosphatidylinositol; PMME, phosphatidylmonomethylethanolamine; PDME, phosphatidyldimethylethanolamine. The structural genes are *INO1* (encoding inositol-1-phosphate synthase), *CDS1* (encoding CDP-DG synthase), *PIS* (encoding phosphatidylinositol synthase), *CHO1* (*PSS*) (encoding PS synthase), *PSD1* and *PSD2* (encoding PS decarboxylases), *CHO2* (*PEM1*) (encoding PE *N*-methyltransferase), and *OPI3* (*PEM2*) (encoding phospholipid *N*-methyltransferase).

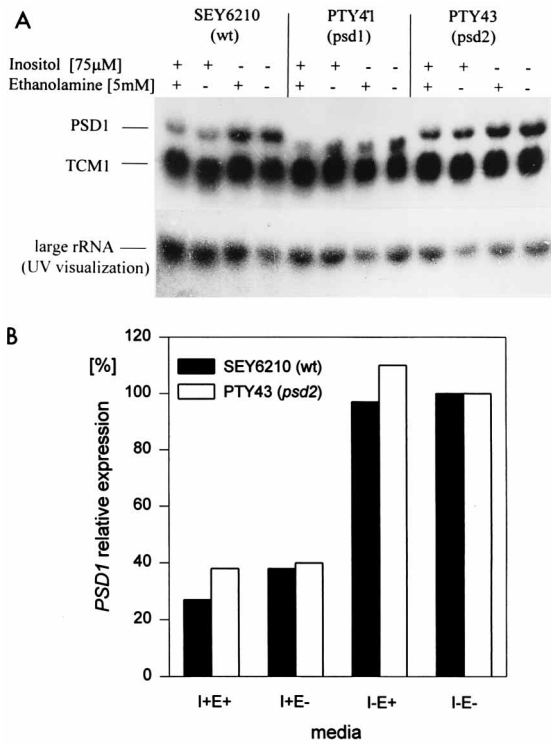


FIG. 2. Northern blot analysis of *PSD1* expression. (A) Autoradiogram of a Northern blot of RNA extracted from wild-type (wt; SEY6210), *psd1* (PTY41), and *psd2* (PTY43) strains. Yeast strains were grown in vitamin-defined media with or without inositol and ethanolamine as indicated. Samples for RNA analysis were taken from the exponential phase of growth, and RNA was isolated as described in Materials and Methods. UV visualization of large rRNA serves as a control of the quality and integrity of RNA. (B) quantification of the Northern blot depicted in panel A. One hundred percent represents the amount of *PSD1* mRNA in the wild-type strain (SEY6210) under derepressing growth conditions (absence of inositol and ethanolamine). Amounts of *PSD1* mRNA were normalized to *TCM1* as a loading control.

mega; restriction and modification enzymes, Promega and Boehringer Mannheim. All other materials were reagent grade or better.

**Strains and culture conditions.** The following laboratory strains of *S. cerevisiae* were generously provided by D. Voelker (National Jewish Center for Immunology and Respiratory Medicine, Denver, Colo.): SEY6210 (*Matα ura3 trp1 his3 lys2 leu2 suc2*), PTY41 (*Matα ura3 trp1 his3 lys2 leu2 psd1-Δ1::TRP1*), and PTY43 (*Mat***a** *ura3 trp1 his3 leu2 ade2 suc2 psd2-Δ1::HIS3*). All *psd* mutant strains were maintained on YEPD medium (1% yeast extract, 2% Bacto Peptone, 3% glucose) supplemented with 5 mM ethanolamine. Vitamin-defined synthetic media were prepared as described previously (25). Where indicated, media were supplemented with 75 μM inositol (I+) and/or 5 mM ethanolamine (E+). All yeast cultures were grown aerobically at 30°C with shaking. Bacterial media were made by standard methods (26).

**RNA analyses.** PS decarboxylases RNA probes for Northern blot hybridization were synthesized according to the manufacturer's recommendations for the SP6/T7 Riboprobe Combination System (Promega) from plasmids pPG108 (*PSD1*) and pPG109 (*PSD2*). To prepare plasmid pPG108, a *Eco*RI-*Hin*dIII fragment (nucleotides 1097 to 2109) from plasmid YEp352-PSD1 (generously provided by D. Voelker) was inserted into *Eco*RI-*Hin*dIII polylinker sites of vector pGEM4z (Promega). Similarly, plasmid pPG109 was prepared by inserting a *Bam*HI-*Bam*HI fragment (nucleotides 1874 to 3363) from plasmid YEp352-PSD2-6 (generously provided by D. Voelker) into the *Bam*HI polylinker site of vector pGEM3zf(−) (Promega). The orientation of the fragment was determined by *Pst*I and *Sph*I restriction digestion. To synthesize the *PSD1* probe, plasmid pPG108 was linearized with *Eco*RI, and RNA was transcribed by using T7 RNA polymerase. To synthesize the *PSD2* probe, plasmid pPG109 was linearized with *Hin*dIII, and RNA was transcribed by using T7 RNA polymerase. The *TCM1* and *INO1* probes were prepared from plasmids described by Hudak et al. (13), linearized with a restriction enzyme, and transcribed with an RNA polymerase as follows (plasmid/restriction enzyme/RNA polymerase): pAB309Δ/*Eco*RI/SP6 (*TCM1*) and pJH310/*Hin*dIII/T7 (*INO1*). The *TCM1* ribosomal protein gene, expression of which is unaffected by the availability of inositol and choline, was used as a standard for RNA loading as described previously (11). The integrity and quality of RNA after fractionation in a formaldehyde agarose gel were monitored by using short-wave UV epi-illumination against a fluorochrome-coated thin-layer chromatography plate (21). Northern hybridization was performed essentially as described by Hirsch and Henry (11), and the results were visualized by autoradiography and quantified with an AMBIS 4000 phosphorimager (AMBIS, Inc.) or by cutting the radioactive spots and liquid scintillation counting.

**Phospholipid composition.** Steady-state labeling with [$^{32}$P]orthophosphate was performed by the method of Atkinson et al. (2). Cells were labeled overnight for at least six generations with 5 μCi of [$^{32}$P]orthophosphate/ml in vitamin-defined synthetic media, as described above, and harvested in the late logarithmic phase of growth. Labeled lipids were extracted as described by Atkinson et al. (2). Following chloroform-methanol extraction, 10 μl from each sample was removed and counted in a scintillation counter to assess total radioactivity incorporation into the lipid fraction. Two-dimensional paper chromatography on silica-impregnated paper was carried out by the method of Steiner and Lester (28). Labeled spots corresponding to specific lipids were quantified with an AMBIS 4000 phosphorimager.

## RESULTS

**Transcriptional regulation of PS decarboxylases.** Expression of the yeast PS decarboxylase structural genes, *PSD1* and *PSD2*, was assessed by Northern blot analysis of RNA extracted from exponentially growing cell cultures. Parental strain SEY6210 together with strains disrupted in the major, mitochondrial PS decarboxylase gene *PSD1* (PTY41) and in the *PSD2* gene encoding the second, nonmitochondrial PS decarboxylase (PTY43) were grown to the mid-exponential phase of growth in media with or without the precursors of phospholipid biosynthesis, inositol and ethanolamine. Figure 2 shows a Northern blot illustrating the expression of *PSD1* together with quantification of this expression. *PSD1* expres-

sion shows an expression pattern typical of other structural genes of phospholipid biosynthesis. In the absence of inositol, the *PSD1* gene is fully derepressed; in the presence of inositol, the *PSD1* gene is repressed. Ethanolamine plays no significant role in the regulation of *PSD1*. The absence of a functional second PS decarboxylase encoded by the *PSD2* gene does not influence the expression of *PSD1*. The pattern of *PSD1* regulation is the same in the PTY43 (*psd2*) strain as in the parental wild-type strain (SEY6210). Also, the derepressed levels of *PSD1* in the absence of inositol are the same in a *psd2* disruption mutant (PTY43) as in the wild-type strain (SEY6210). A faster-moving band that hybridizes with the *PSD1* probe can be seen in the PTY41 (*psd1*) strain. For disruption of the *PSD1* gene, a 0.27-kb fragment close to the end of the gene containing the putative catalytic subunit was removed and replaced with the *TRP1* gene in the direction opposite that of the *PSD1* gene (for details, see reference 32). Therefore, transcription of the *PSD1* gene terminated about 0.3 kb from the end of the gene, and the *PSD1* probe recognized this mRNA as a slightly faster moving band on a Northern blot. It was shown previously that this disruption of the *PSD1* gene abolished all PS decarboxylase activity derived from the *PSD1* gene (30).

In contrast to *PSD1* regulation, levels of *PSD2* expression show no response to inositol and/or ethanolamine in the wild-type strain (SEY6210) (Fig. 3). Absence of the functional mitochondrial PS decarboxylase encoded by the *PSD1* gene has no significant effect on *PSD2* transcription except for some increase of *PSD2* expression in the absence of ethanolamine. Expression of many phospholipid biosynthetic genes has been shown to be highly sensitive to the growth phase of the yeast culture (14, 17). Therefore, this increase in *PSD2* transcription may be due to the slower than wild-type growth rate of the PTY41 strain in the absence of ethanolamine and the partially decreased viability under these conditions (data not shown).

**Effect of mutations in genes encoding PS decarboxylases on *INO1* expression.** *INO1* expression was assessed in wild-type strain SEY6210 and in mutant strains disrupted in *PSD1* (PTY41) and *PSD2* (PTY43) (Fig. 4). All strains were grown to the mid-logarithmic phase of growth in vitamin-defined synthetic yeast media with or without inositol and ethanolamine. Northern blot analysis of *INO1* expression revealed normal, wild-type regulation of *INO1* expression in the parental strain SEY6210. The *INO1* gene is fully derepressed in the absence of inositol and ethanolamine; addition of ethanolamine causes only a slight decrease in *INO1* expression. Supplementation of the growth medium with inositol (with or without ethanolamine) causes the repression of *INO1*. In the strain with the disrupted *PSD2* gene (PTY43), *INO1* expression follows a pattern similar to that for the wild-type strain, with slightly higher derepressed levels. In the strain carrying the disrupted *PSD1* gene (PTY41), transcription of the *INO1* gene is misregulated. The derepressed levels of *INO1* expression are higher than corresponding levels in the wild-type strain, and inositol by itself is not able to repress the *INO1* gene to the wild-type repressed levels unless ethanolamine is also present in the growth medium.

**Phospholipid analysis.** The steady-state phospholipid composition of the PS decarboxylase mutants was determined by using [$^{32}$P]orthophosphoric acid labeling. Cells were incubated overnight with the label to the mid- to late logarithmic phase of growth to achieve uniform labeling. Lipids were extracted and separated as described in Materials and Methods. The parental strain SEY6210 displays (at least in I+ media) a lower than usual PC percentage of total phospholipids (phospholipid content reviewed in reference 24) and a corresponding increase in the percentages of PE, phosphatidylmonomethylethanolamine, and phosphatidyldimethylethanolamine (Table 1). Disruption of the mitochondrial PS decarboxylase gene *PSD1* causes a shift of the label from PE to PC (strain PTY41 in the I+E− medium), suggesting that the limiting step in the de novo synthesis of PC in this strain is located upstream of PE. Disruption of the second PS decarboxylase gene *PSD2* does not result in any change of phospholipid composition compared to the parental strain, SEY6210.

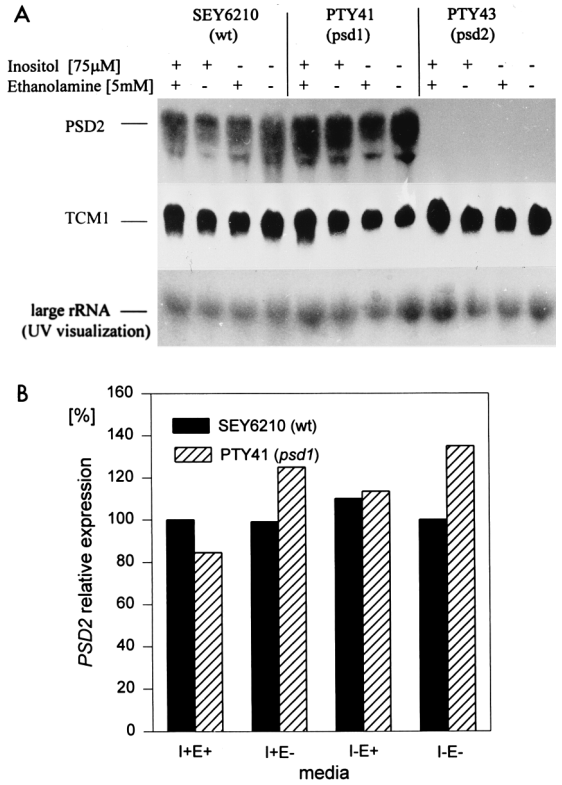


FIG. 3. Northern blot analysis of *PSD2* expression. (A) Autoradiogram of a Northern blot of RNA extracted from wild-type (wt; SEY6210), *psd1* (PTY41), and *psd2* (PTY43) strains. Yeast strains were grown in vitamin-defined media with or without inositol and ethanolamine as indicated. Samples for RNA analysis were taken from the exponential phase of growth, and RNA was isolated as described in Materials and Methods. UV visualization of large rRNA serves as a control of quality and integrity of RNA. (B) Quantification of *PSD2* expression. One hundred percent represents the amount of *PSD2* mRNA in the wild-type strain (SEY6210) under derepressing growth conditions (absence of inositol and ethanolamine). Amounts of *PSD2* mRNA were normalized to *TCM1* as a loading control. Quantification represents an average from two independent experiments, one of which is depicted in panel A.

## DISCUSSION

Computer-assisted analysis of the promoter region of the mitochondrial PS decarboxylase gene (*PSD1*) revealed three sequences homologous to the 10-bp $UAS_{INO}$ consensus sequence, 5′-CATGTGAAAT-3′ (3, 6, 32). Therefore, it is not surprising that expression of the *PSD1* gene is regulated in the same fashion as expression of other coregulated genes of the CDP-DG pathway leading to the formation of PC, namely, *CHO1* (PS synthase) (4), *CHO2*/*PEM1* (PE methyltransferase), and *OPI3*/*PEM2* (phospholipid methyltransferase) (7, 15). CDP-DG synthase activity is also repressed by the availability of inositol in the growth medium (12), and the promoter of the corresponding gene, *CDS1*, contains the $UAS_{INO}$ consensus sequence (27). Therefore it is likely that the *CDS1* gene

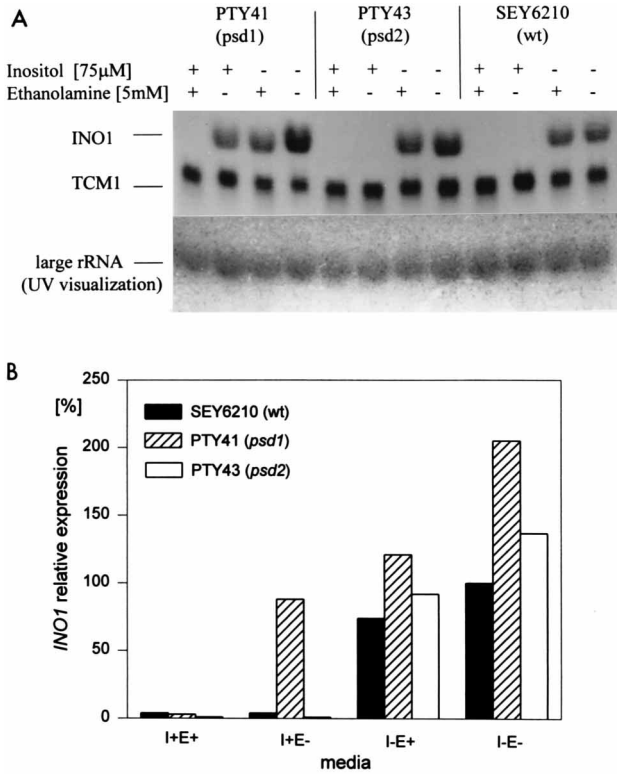


FIG. 4. Northern blot analysis of *INO1* expression in PS decarboxylase mutants. Wild-type (wt; SEY6210), *psd1* (PTY41), and *psd2* (PTY43) strains were grown in vitamin-defined media with or without 75 μM inositol and 5 mM ethanolamine as indicated. RNA samples from exponentially growing cultures were subjected to Northern blot analysis (A) and subsequent quantification (B); 100% represents the amount of *INO1* mRNA in the wild-type strain (SEY6210) under derepressing growth conditions (absence of inositol and ethanolamine). Amounts of *INO1* mRNA were normalized to *TCM1* as a loading control. Quantification represents an average from two independent experiments, one of which is depicted in panel A.

is subject to the same type of transcriptional regulation. The *PSD1* gene is derepressed in the absence of inositol and repressed in the presence of inositol. Ethanolamine plays no significant role in regulation of the *PSD1* gene. The level of repression of the *PSD1* gene is in a good agreement with previous observations of Lamping et al. (16) and Overmayer and Waechter (23), who studied the regulation of PS decarboxylase activity in yeast by using in vitro enzymatic analysis. They found that the repressed levels of PS decarboxylase enzymatic activity in the presence of inositol represent about 50 to 60% of the derepressed activity in the absence of inositol. The addition of choline, in the presence of inositol, further repressed the PS decarboxylase activity. Ethanolamine had no effect on the PS decarboxylase activity. This finding suggests that the PS decarboxylase activity in yeast derived from the *PSD1* gene is subject to the same type of regulation as activities of other enzymes of the CDP-DG pathway leading to the formation of PC.

In contrast to the inositol-responsive expression of *PSD1*, the expression of the second PS decarboxylase gene (*PSD2*) is not regulated by the availability of the precursors of phospholipid biosynthesis, inositol and ethanolamine. There are apparently no sequences homologous to the $UAS_{INO}$ consensus sequence in the region upstream from the *PSD2* coding region (31). More than 95% of in vitro PS decarboxylase activity has been attributed to the mitochondrial PS decarboxylase (prod-

TABLE 1. Phospholipid composition of wild-type and PS decarboxylase mutant strains[a]

| Strain | Genotype | Medium | Doubling time (h) | $^{32}P$ incorporation into lipid ($10^3$ cpm/OD/ml) | Proportion of phospholipids (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | PA | PI | PS | PE | PMME | PDME | PC | Other[b] |
| SEY6210 | Wild type | I+E− | 2.0 | 43.6 | 1.2 ± 0.1 | 26.5 ± 0.7 | 7.7 ± 0.3 | 28.2 ± 0.3 | 3.0 ± 0.3 | 3.1 ± 0.2 | 24.4 ± 0.7 | 5.9 ± 0.4 |
| | | I+E+ | 1.9 | 43.8 | 0.9 ± 0.1 | 24.8 ± 0.8 | 7.1 ± 0.3 | 35.9 ± 0.9 | 3.8 ± 1.1 | 4.8 ± 0.3 | 17.8 ± 0.8 | 5.1 ± 0.9 |
| PTY41 | *psd1* | I+E− | 3.0 | 38.6 | 1.0 ± 0.1 | 33.9 ± 0.4 | 8.1 ± 0.2 | 7.4 ± 0.2 | 1.2 ± 0.6 | 1.1 ± 0.7 | 44.2 ± 0.6 | 3.1 ± 0.3 |
| | | I+E+ | 2.1 | 40.4 | 0.9 ± 0.1 | 26.6 ± 1.0 | 8.7 ± 0.4 | 24.8 ± 0.8 | 4.4 ± 0.9 | 8.9 ± 1.5 | 19.4 ± 0.6 | 6.4 ± 1.1 |
| PTY43 | *psd2* | I+E− | 1.9 | 34.6 | 1.1 ± 0.2 | 27.6 ± 0.7 | 9.6 ± 0.6 | 22.7 ± 0.6 | 1.9 ± 0.7 | 3.1 ± 1.1 | 27.8 ± 0.5 | 6.0 ± 0.8 |
| | | I+E+ | 1.8 | 41.6 | 1.0 ± 0.1 | 25.6 ± 1.2 | 9.0 ± 0.5 | 31.6 ± 1.4 | 3.0 ± 0.6 | 4.0 ± 0.4 | 18.9 ± 0.4 | 6.8 ± 1.9 |

[a] Yeast strains were grown in vitamin-defined media with 75 μM inositol (I+) and with (E+) or without (E−) 5 mM ethanolamine. Cells were harvested in late exponential phase of growth; glycerophospholipids were extracted, resolved, and quantitated as described in Materials and Methods. OD, optical density. For other abbreviations, see the footnote to Fig. 1.
[b] Includes mainly polar lipids migrating with the origin and other minor lipids, like phosphatidylglycerol, cardiolipin, and lysophospholipids.

uct of the *PSD1* gene) (6, 32). Therefore, it was of interest to check *PSD2* expression in a strain with a disrupted *PSD1* gene (PTY41). The levels of *PSD2* expression are roughly the same in the *PSD1* disruption strain (PTY41) as in the wild-type strain (SEY6210), except for a slight increase in the level of transcript in cells grown in medium without ethanolamine. This result shows not only that inositol does not affect *PSD2* transcription but also that transcription of the *PSD2* gene is not significantly influenced by the functionality of the major mitochondrial PS decarboxylase (*PSD1*). Hybridization conditions necessary to detect the *PSD2* transcript suggest that during exponential growth, *PSD2* mRNA is much less abundant than *PSD1* mRNA.

An interesting feature of the transcriptional regulation of phospholipid biosynthetic genes is its dependence on ongoing PC biosynthesis. When PC synthesis is disrupted, inositol itself cannot repress the coordinately regulated genes. Only when inositol is present in the medium together with a precursor of PC biosynthesis that enters the biosynthetic pathway downstream of the genetic block is it able to repress those genes (reviewed by Greenberg and Lopes [8] and Paltauf et al. [24]). There are two PS decarboxylases in yeast, either of which by itself can support the growth of yeast cells even in the absence of ethanolamine or choline (6, 30, 32). This situation permitted an investigation of which of the PS decarboxylases (if either) is required for proper regulation of coordinately regulated phospholipid biosynthetic genes. To answer this question, I studied the regulation of *INO1* in the wild-type (SEY6210), *psd1* (PTY41), and *psd2* (PTY43) strains. The results clearly show proper *INO1* regulation in the wild-type and *psd2* disrupt strains. However, the *INO1* gene is misregulated in a strain with a disruption of mitochondrial PS decarboxylase (*psd1* strain). In this strain, inositol is not able to repress the *INO1* gene unless ethanolamine is present in the growth medium. Analysis of the phospholipid composition of strains used in this study revealed that the parental wild-type strain SEY6210 had a lower than usual PC content as well as corresponding increase in PE and the methylated PC precursors. However, this lower PC content in the wild-type strain does not lead to any apparent phenotype (e.g., change in growth rate, cell morphology, or regulation of phospholipid biosynthetic genes). Under conditions where the *INO1* gene is misregulated (*psd1* strain PTY41 in I+E− medium [Fig. 4]), the phospholipid composition is changed markedly compared to the parental strain SEY6210 grown under the same conditions. There is a dramatic decrease in PE content and an increase in PC content to 45% of total phospholipid. Previous studies (18, 20, 29) suggested that PC biosynthesis is required for proper regulation of phospholipid biosynthesis. However, the PC content of the *psd1* disrupt strain (PTY41) under conditions where the phospholipid biosynthetic genes are misregulated (I+E− medium [Table 1]) is higher than the PC content of the parental strain. Thus, the proportion of PC in cellular phospholipid composition is not correlated to the cell's ability to repress *INO1* in response to inositol. In a previous study (10), we found that none of the precursors of PC biosynthesis, either in the CDP-DG pathway or in the CDP-choline pathway, are required for proper regulation of the coordinately regulated genes. It seems more likely that yeast cells are able to monitor the flux through the phospholipid biosynthetic pathway. When the flux through the CDP-DG pathway leading to formation of PC is inadequate (e.g., when the *CDS1, CHO1, PSD1, CHO2,* or *OPI3* gene is disrupted or modified) and the CDP-choline or CDP-ethanolamine pathway does not contribute significantly to the formation of phospholipids due to the lack of free precursors choline or ethanolamine, the regulation of coordinate phospholipid biosynthesis genes is disturbed. The mechanism by which the rate of lipid synthesis is monitored and transmitted to the transcriptional apparatus remains to be determined.

## ACKNOWLEDGMENTS

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## REFERENCES

1. **Ambroziak, J., and S. A. Henry.** 1994. *INO2* and *INO4* gene products, positive regulators of phospholipid biosynthesis in *Saccharomyces cerevisiae*, form a complex that binds to the *INO1* promoter. J. Biol. Chem. **269:**15344–15349.
2. **Atkinson, K. D., B. Jensen, A. I. Kolat, E. M. Storm, S. A. Henry, and S. Fogel.** 1980. Yeast mutants auxotrophic for choline or ethanolamine. J. Bacteriol. **141:**558–564.
3. **Bachhawat, N., Q. Ouyang, and S. A. Henry.** 1995. Functional characterization of an inositol-sensitive upstream activation sequence in yeast: a *cis*-regulatory element responsible for inositol-choline mediated regulation of phospholipid biosynthesis. J. Biol. Chem. **270:**25087–25095.
4. **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.
5. **Carman, G. M., and G. M. Zeimetz.** 1996. Regulation of phospholipid biosynthesis in the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. **71:**13293–13296.
6. **Clancey, C. J., S. Chang, and W. Dowhan.** 1993. Cloning of a gene (*PSD1*) encoding phosphatidylserine decarboxylase from *Saccharomyces cerevisiae* by complementation of an *Escherichia coli* mutant. J. Biol. Chem. **268:**24580–24590.
7. **Gaynor, P. M., T. Gill, S. Toutenhoofd, E. F. Summers, P. McGraw, M. J. Homann, S. A. Henry, and G. M. Carman.** 1991. Regulation of phosphatidylethanolamine methyltransferase and phospholipid methyltransferase by phospholipid precursors in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **1090:**326–332.
8. **Greenberg, M. L., and J. M. Lopes.** 1996. Genetic regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae*. Microbiol. Rev. **60:**1–20.
9. **Greenberg, M. L., P. Goldwasser, and S. A. Henry.** 1982. Characterization of a yeast regulatory mutant constitutive for inositol-1-phosphate synthase. Mol. Gen. Genet. **186:**157–163.
10. **Griac, P., M. J. Swede, and S. A. Henry.** 1996. The role of phosphatidylcholine biosynthesis in the regulation of the *INO1* gene of yeast. J. Biol. Chem. **271:**25692–25698.
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. **Hudak, K. A., J. M. Lopes, and S. A. Henry.** 1994. A pleiotropic phospholipid biosynthetic regulatory mutation in *Saccharomyces cerevisiae* is allelic to *sin3* (*sdi1*, *ume4*, *rpd1*). Genetics **136:**475–483.
14. **Jiranek, V., J. A. Graves, and S. A. Henry.** 1997. Unpublished data.
15. **Kodaki, T., K. Hosaka, J. I. Nikawa, and S. Yamashita.** 1991. Identification of the upstream activation sequences responsible for the expression and regulation of the *PEM1* and *PEM2* genes encoding the enzymes of the phosphatidylethanolamine methylation pathway in *Saccharomyces cerevisiae*. J. Biochem. **109:**276–287.
16. **Lamping, E., S. D. Kohlwein, S. A. Henry, and F. Paltauf.** 1991. Coordinate regulation of phosphatidylserine decarboxylase in *Saccharomyces cerevisiae*. J. Bacteriol. **173:**6432–6437.
17. **Lamping, E., F. Paltauf, S. A. Henry, and S. D. Kohlwein.** 1995. Isolation and characterization of a mutant of *Saccharomyces cerevisiae* with pleiotrophic deficiencies in transcriptional activation and repression. Genetics **137:**56–65.
18. **Letts, V. A., and S. A. Henry.** 1985. Regulation of phospholipid synthesis in phosphatidylserine synthase-deficient (*cho1*) mutants of *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **163:**560–567.
19. **Lopes, J. M., and S. A. Henry.** 1991. Interaction of *trans* and *cis* regulatory elements in the *INO1* promoter of *Saccharomyces cerevisiae*. Nucleic Acids Res. **19:**3987–3994.

20. **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.
21. **Mukhopadhyay, T., and J. A. Roth.** 1994. Visualization of RNA in gel by shortwave UV epi-illumination without staining. BioTechniques **17:**619–620.
22. **Nikoloff, D. M., and S. A. Henry.** 1994. Functional characterization of the *INO2* gene of *Saccharomyces cerevisiae*. J. Biol. Chem. **269:**7402–7411.
23. **Overmayer, J. H., and C. J. Waechter.** 1991. Regulation of phosphatidylserine decarboxylase in *Saccharomyces cerevisiae* by inositol and choline: kinetics of repression and derepression. Arch. Biochem. Biophys. **290:**511–516.
24. **Paltauf, F., S. D. Kohlwein, and S. A. Henry.** 1992. Regulation and compartmentalization of lipid synthesis in yeast, p. 415–500. *In* J. Broach, E. Jones, and J. Pringle (ed.), Molecular biology of the yeast *Saccharomyces cerevisiae*, vol. II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Patton, J. L., L. Pessoa-Brandao, and S. A. Henry.** 1995. Production and reutilization of an extracellular phosphatidylinositol catabolite, glycerophosphoinositol, by *Saccharomyces cerevisiae*. J. Bacteriol. **177:**3379–3385.
26. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Shen, H., P. N. Heacock, C. J. Clancey, and W. Dowhan.** 1996. The *CDS1* gene encoding CDP-diacylglycerol synthase in *Saccharomyces cerevisiae* is essential for cell growth. J. Biol. Chem. **271:**789–795.
28. **Steiner, M. R., and R. L. Lester.** 1972. *In vitro* studies of phospholipid biosynthesis in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **260:**222–243.
29. **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.
30. **Trotter, P. J., and D. R. Voelker.** 1995. Identification of a non-mitochondrial phosphatidylserine decarboxylase activity (*PSD2*) in the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. **270:**6062–6070.
31. **Trotter, P. J., J. Pedretti, R. Yates, and D. R. Voelker.** 1995. Phosphatidylserine decarboxylase 2 of *Saccharomyces cerevisiae*. J. Biol. Chem. **270:**6071–6080.
32. **Trotter, P. J., J. Pedretti, and D. R. Voelker.** 1993. Phosphatidylserine decarboxylase from *Saccharomyces cerevisiae*. J. Biol. Chem. **268:**21416–21424.
33. **White, M. J., J. P. Hirsch, and S. A. Henry.** 1991. The *OPI1* gene of *Saccharomyces cerevisiae*, a negative regulator of phospholipid biosynthesis, encodes a protein containing polyglutamine tracts and a leucine zipper. J. Biol. Chem. **266:**863–872.