Inositol Regulates Phosphatidylglycerolphosphate Synthase Expression in *Saccharomyces cerevisiae*

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The enzyme phosphatidylglycerolphosphate synthase (PGPS; CDPdiacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase; EC 2.7.8.5) catalyzes the committed step in the synthesis of cardiolipin, a phospholipid found predominantly in the mitochondrial inner membrane. To determine whether PGPS is regulated by cross-pathway control, we analyzed PGPS expression under conditions in which the regulation of general phospholipid synthesis could be examined. The addition of inositol resulted in a three- to fivefold reduction in PGPS expression in wild-type cells in the presence or absence of exogenous choline. The reduction in enzyme activity in response to inositol was seen in minutes, suggesting that inactivation or degradation of the enzyme plays an important role in inositol-mediated repression of PGPS. In *cho2* and *opi3* mutants, which are blocked in phosphatidylcholine synthesis, inositol-mediated repression of PGPS did not occur unless choline was added to the media. Three previously identified genes that regulate general phospholipid synthesis, *INO2*, *INO4*, and *OPI1*, did not affect PGPS expression. Thus, *ino2* and *ino4* mutants, which are unable to derepress biosynthetic enzymes involved in general phospholipid synthesis, expressed wild-type levels of PGPS activity under derepressing conditions. PGPS expression in the *opi1* mutant, which exhibits constitutive synthesis of general phospholipid biosynthetic enzymes, was fully repressed in the presence of inositol and partially repressed even in the absence of inositol. These results demonstrate for the first time that an enzymatic step in cardiolipin synthesis is coordinately controlled with general phospholipid synthesis but that this control is not mediated by the same genetic regulatory circuit.

Mitochondria are functionally complex, and their biogenesis is subject to multiple regulatory constraints. As mitochondrial membranes are crucial for mitochondrial function and biogenesis, study of these membranes is clearly essential to understanding the organelle as a whole. A potential problem for such studies might lie in the identification of mitochondrion-specific membrane regulation. However, the phospholipid cardiolipin (CL) is found only in the mitochondrial membrane (5, 8, 17) (predominantly in the inner membrane), so that its synthesis can serve as a marker for the study of mitochondrion-specific membrane biogenesis. CL itself plays a unique role in mitochondrial function. Trivedi et al. have shown that cytochrome oxidase activity is dependent upon CL availability: reducing the available CL in mitochondrial membranes either genetically or by the addition of adriamycin (which forms undissociable complexes with CL) results in a concomitant reduction in cytochrome oxidase activity (22, 23).

It seems likely that mitochondrial membrane biogenesis is subject to regulation not only by factors that affect general mitochondrial development but also by factors that influence the synthesis of the major membrane components, the phospholipids. In this study we have focused on the interplay between the phospholipid synthesis required for all cell membranes and the phospholipid synthesis specific for the mitochondrion, i.e., CL synthesis.

Eucaryotic phospholipid synthesis is known to proceed via a three-branch pathway from the central intermediate CDP-diglyceride (CDP-DG) (reviewed in reference 13) (see Fig. 1). The phospholipid products of the phosphatidylinositol (PI) and phosphatidylcholine (PC) branches are common to all cell membranes, and the biosynthesis of many of

the enzymes in these two branches is subject to coordinate control by the phospholipid precursors inositol and choline (4, 12-14). A variety of regulatory effectors, including at least two positive effectors encoded by the *INO2* and *INO4* genes and the negative effector encoded by the gene *OPI1*, mediate this control (9, 19, 21). Regulation of the third branch, the CL branch, has not been extensively studied. Early studies indicated that total membrane CL content is dependent upon mitochondrial development: factors such as carbon source, oxygen concentration, and genetic lesions affecting mitochondrial function all affect the observed levels of CL (17). How these factors affect the CL biosynthetic enzymes is not understood.

In this paper we present evidence that expression of the enzyme phosphatidylglycerolphosphate synthase (PGPS; CDPdiacylglycerol:sn-glycerol-3-phosphate 3-phosphatidyltransferase; CDPdiacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase; EC 2.7.8.5), which catalyzes the committed step in the CL pathway, is regulated by the precursors inositol and choline in the same manner as are enzymes of the PI and PC branches. However, this process is not mediated by the same genetic regulatory circuit as that which controls the PI and PC branches. These results represent the first demonstration of coordinate control of a CL branch enzyme with the PI and PC branches of phospholipid synthesis.

MATERIALS AND METHODS

***Saccharomyces cerevisiae* strains.** The strains used in this study are listed in Table 1. All mutant strains have been characterized previously and were generously provided by Susan Henry.

Growth media. Strains were maintained in 15% glycerol at -80°C for long-term storage and on 1% yeast extract-2%

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

Strain	Genotype	Reference(s)
Wild type	<i>ade5 MATα</i>	12–15
$\Delta 4d$	<i>cho2::LEU2 ura3 his3 ade6 MATα</i>	12
A38	<i>opi3-3 ade5 MATα</i>	9, 10
NO128	<i>opi3-4 ade5 MATα</i>	9, 10
ino1	<i>ino1 adel MATα</i>	7
it17	<i>ino2-21 thr1 MATα</i>	21
it8	<i>ino2-21 ura3 lys2 MATα</i>	21
it3	<i>ino4-40 thr1 MATα</i>	21
it20	<i>ino4-40 leu2 ura3 lys2 MATα</i>	21
opi1	<i>opi1-1 ade5 MATα</i>	9, 11

peptone–2% glucose slants at 4°C for short-term storage. Media for experiments consisted of vitamin-free yeast base (0.6%; Difco Laboratories), vitamins (described in reference 7), glycerol (3%), ethanol (0.95%), adenine (0.15 mM), and other components as required to supplement additional auxotrophies. When needed, inositol was added to a concentration of 10 or 75 μ M, and choline was added to 1 mM.

Materials. Components of growth media were obtained from Difco, buffer and enzyme assay components were purchased from Sigma Chemical Co., CDP-DG was obtained from Life Science Resources, and L-[2-³H(N)]glycerol 3-phosphate (5.8 Ci/mmol) was purchased from Dupont, NEN Research Products.

Growth conditions. Liquid cultures were inoculated from YEPD slants or plates and grown overnight at 30°C. Experimental cultures were inoculated from these overnight cultures and grown to the mid-log stage to the A_{550} indicated for each experiment. Overnight cultures were always grown in the same medium as the experimental cultures. Cells were harvested by centrifugation at 4°C and washed once with a buffer containing 50 mM Tris hydrochloride (pH 7.5), 1 mM EDTA, 300 mM sucrose, and 10 mM β -mercaptoethanol (buffer 1), and pellets were immediately frozen at –80°C.

Preparation of mitochondrial extracts. Mitochondrial extracts were prepared as described by Carman and Belunis (3) with the following modifications. Pellets were suspended in buffer 1 to a concentration of 1 g/ml (wet weight), and cells were broken open by being vortexed with glass beads for five 1-min intervals, with cooling of the cells on ice between intervals. Extracts were centrifuged in a Sorvall SS34 rotor at 5,000 rpm for 5 min, and supernatants were transferred to 15-ml Corex tubes. We repeated the 5,000-rpm spin twice more, transferring the supernatants each time and discarding the pellets. The supernatants of the third spin were transferred to small Oak Ridge tubes, and mitochondria were pelleted by centrifugation for 10 min at 15,000 rpm in a Sorvall SS34 rotor. The mitochondrial pellets were washed twice in buffer 1, suspended in 50 mM Tris hydrochloride (pH 7.5)–20% glucose–10 mM β -mercaptoethanol (buffer 2) to a concentration of 2.5 mg/ μ l (wet weight), and stored at –80°C.

Great care was taken to treat all samples within an experiment identically during every stage of preparation of mitochondrial extracts. This was particularly important during the 5,000-rpm-centrifugation steps, since contamination of mitochondrial extracts with even small amounts of material sedimenting at this rate invalidates the comparison of protein determinations in different samples. Assays of PGPS activity in mitochondrial extracts prepared as described were consistent and reproducible. The figures shown in Results are representative of three or more independent experiments.

Assays for protein and enzyme activity. Mitochondrial extracts were assayed for protein concentration by the method of Bradford (2) with a protein assay kit (BioRad Laboratories) with bovine serum albumin as a standard. PGPS activity was assayed at 30°C by the method of Carman and Belunis (3). Incorporation of 0.5 mM [2-³H]glycerol 3-phosphate (4,000 cpm/nmol) into chloroform-soluble material was measured in the presence of 50 mM morpholineethanesulfonic acid HCl (pH 7.0)–0.1 mM $MnCl_2$ –0.2 mM CDP-DG–1 mM Triton X-100 and mitochondrial extract corresponding to 50 μ g of protein in a total volume of 0.1 ml. The reaction was stopped at 20 min with 0.5 ml of 0.1 N HCl in methanol. Chloroform (1 ml) and 1 M $MgCl_2$ (1 ml) were added. The tubes were vortexed, and the phases were separated by centrifugation for 3 min at low speed; 0.5 ml was transferred from the chloroform layer into a scintillation vial. The chloroform was evaporated, organic counting scintillant (3 ml; Amersham Corp.) was added, and the radioactivity in each sample was determined with an LS-3801 liquid scintillation counter (Beckman Instruments, Inc.).

Triplicate tubes were used for each sample, and a blank tube containing crude extract which was inactivated before the reaction was included for each sample. Specific activities were defined as units per milligram of protein, where 1 U is the amount of enzyme that catalyzes the formation of 1 nmol of product per min at 30°C. Phosphatidylglycerol was identified as the main product of the reaction by thin-layer chromatography with the solvents described by Carman and Belunis (3), indicating that the phosphatidylglycerolphosphate initially synthesized is rapidly dephosphorylated.

RESULTS

Repression of PGPS expression by inositol. Inositol is a key regulatory substance that represses the enzymes of PI and PC synthesis, including inositol-1-phosphate (I-1-P) synthase, CDP-DG synthase, phosphatidylserine synthase, phosphatidylserine decarboxylase, and the methyltransferases (12, 15, 16, 24, 25) (Fig. 1). If PGPS expression were coordinately controlled with PI and PC synthesis, we would expect PGPS activity to be repressed in cells grown in the presence of inositol. Wild-type cells grown in the presence of ≥ 10 μ M inositol contained decreased amounts of PGPS activity (Fig. 2). Derepressed PGPS levels were fourfold greater than repressed levels (Fig. 2).

Dependence of inositol-mediated regulation of PGPS upon the synthesis of PC. Henry and co-workers have shown that inositol repression of PI and PC branch enzymes is dependent upon PC synthesis. Thus, in mutants defective in methyltransferase activity and therefore unable to convert phosphatidylethanolamine (PE) to PC, PI and PC branch enzymes are not fully repressed in the presence of inositol unless choline is also present (15). One such mutant is *cho2*, which is blocked in the conversion of PE to phosphatidylmonomethylethanolamine (13). We measured PGPS activity in mitochondrial extracts of the *cho2* mutant to determine whether inositol repression of PGPS is also dependent upon PC synthesis. The kinetics of inositol repression in the wild type and the *cho2* mutant are shown in Fig. 3. Inositol repression of PGPS expression in the wild type was apparent within five min following the addition of inositol in the presence or absence of choline. In the *cho2* mutant, inositol repressed PGPS within 5 min only if choline was present in the medium.

To determine whether the choline requirement for inositol-mediated repression is unique to the *cho2* mutant or is a

FIG. 1. Pathways for de novo synthesis of phospholipids in *S. cerevisiae*. Inositol (I) is synthesized from glucose 6-phosphate (Glc-6-P) and utilized with CDP-DG in the synthesis of PI. The committed step in PC synthesis is the conversion of CDP-DG and serine (S) to phosphatidylserine (PS), which is decarboxylated to PE. PE is thrice methylated to form PC. The committed step in CL synthesis is the conversion of CDP-DG and glycerol 3-phosphate (G-3-P) to phosphatidylglycerolphosphate (PGP), which is dephosphorylated to phosphatidylglycerol (PG). CDP-DG is a substrate in the conversion of PG to CL. *ino1*, *cho1*, *cho2*, and *opi3* mutants have been characterized previously (1, 6, 10, 12, 15). PSD, Phosphatidylserine decarboxylase; PMME, phosphatidylmonomethylethanolamine; PDME, phosphatidylmethylethanolamine.

property of cells blocked in the PC pathway, we measured PGPS levels in the *opi3* mutant, which is blocked in the second and third methylation steps of the PC pathway (10). Figure 4 shows PGPS levels in *opi3-4* cells grown in the presence or absence of inositol and choline. We have observed that *opi3* cultures grown in the absence of choline segregate increasing numbers of petite colonies as the cultures approach the stationary phase. Therefore, in the experiment shown in Fig. 4, great care was taken to ensure that experimental cultures were inoculated from very-low-density overnight cultures and harvested at the early to mid-log phase. The results of this experiment indicate that PGPS levels in the *opi3-4* mutant are not fully repressed by 75 μ M inositol unless choline is added to the medium. A strain bearing the *opi3-3* allele exhibited the same pattern (data not shown).

Lack of dependence of PGPS expression upon the synthesis of inositol. The experiments shown in Fig. 3 and 4 indicated that a functional PC pathway is required for inositol repression of PGPS. We sought to determine whether a defect in

the inositol biosynthetic pathway might also affect PGPS expression. The first step in inositol biosynthesis is the conversion of glucose 6-phosphate to I-1-P (Fig. 1). This reaction is catalyzed by I-1-P synthase, the product of the gene *INO1* (13). Transcription of the *INO1* gene is derepressed in medium lacking inositol and repressed in the presence of 50 μ M inositol (15). Inositol at 10 μ M is sufficient to allow the growth of auxotrophs and elicits only partial derepression of the *INO1* gene (15). We measured PGPS expression in *ino1* mutant cells grown in medium containing 10 or 75 μ M inositol. PGPS expression in the *ino1* mutant was similar to that seen in the wild type and is therefore unaffected by a block in the inositol pathway (Fig. 5).

Derepression of PGPS expression in the positive regulatory mutants *ino2* and *ino4*. The *INO2* and *INO4* genes encode positive regulatory effectors of the PI and PC branches of phospholipid synthesis (21). These genes are required for the derepression of a variety of phospholipid biosynthetic genes, including *INO1*, *CHO1*, *CHO2*, and *OPI3*, in the absence of inositol (1, 15, 21). Since PGPS expression is controlled by inositol in a manner similar to that of the other enzymes of phospholipid synthesis, we sought to determine whether PGPS is also subject to *INO2* and *INO4* control. PGPS expression was measured in the *ino2* mutant strain it17 grown in repressing or derepressing concentrations of inositol. (Since the *ino2* mutant cannot grow in inositol-free medium, 10 μ M inositol was used for derepressing conditions.) This experiment (Fig. 6) (and similar experiments not shown here with strain it8) had two very striking results. First, the *INO2* gene did not affect the capacity to make PGPS, as wild-type levels of derepressed PGPS expression were observed in medium lacking choline in the *ino2* mutant. Second, although 10 μ M inositol completely repressed wild-type PGPS expression, concentrations as high as 75 μ M inositol did not completely repress PGPS expression in the *ino2* mutant unless choline was present.

Similar results were observed with the *ino4* mutant (Fig. 6). However, we noted differences between the *ino4* strains it3 and it20 with respect to PGPS expression in medium containing 10 μ M inositol and 1 mM choline. In it3, PGPS

FIG. 2. Inositol-mediated repression of PGPS (PGP Synthase) in wild-type cells. Cells were grown for six to eight generations in synthetic medium lacking inositol, inoculated into synthetic medium containing the indicated concentrations of inositol (0 to 75 μ M), and grown for six to eight generations to an A_{550} of 0.3. PGPS activity was determined in mitochondrial extracts. This figure is representative of seven experiments.

FIG. 3. Kinetics of inositol repression of PGPS (PGP Synthase) in the wild type and the *cho2* mutant. Wild type (A and B) and *cho2* mutant (C and D) cells were grown for 10 to 14 generations in synthetic medium lacking inositol in the presence (B and D) or absence (A and C) of 1 mM choline. When cultures reached an A_{550} of 0.3 to 0.5, inositol (75 μ M) was added to half the flasks at time zero (arrows), and cultures were harvested at the indicated times. PGPS activity in mitochondrial extracts was determined as described in the text. Symbols: ○, no inositol; ●, inositol.

expression was repressed in this medium (Fig. 6), while in strain *it20*, PGPS expression was derepressed (data not shown). This result can most likely be explained by slight differences between the two *ino4* strains in the concentration of inositol which is required for PGPS repression.

Nonconstitutive PGPS expression in the *opil* mutant. The *OP11* gene is a negative regulatory gene which mediates the repression of PI and PC branch enzymes (including I-1-P synthase, phosphatidylserine synthase, and the methyltrans-

ferases) in the presence of inositol (9, 19). In the *opil* mutant, these enzymes are constitutively expressed, although the levels of expression of the different enzymes vary (19). To determine whether PGPS is subject to *OP11* control, we grew the *opil* mutant in the presence and absence of inositol and measured PGPS activity in *opil* and wild-type cells. The results of these experiments (Fig. 7) indicate that

FIG. 4. PGPS (PGP Synthase) activity in the *opi3-4* mutant. Cells were grown for 10 to 14 generations in the presence or absence of inositol and choline to an A_{550} of 0.3. PGPS activity was determined in mitochondrial extracts. This figure is representative of four experiments. Symbols: ■, wild type; ▨, *opi3-4* mutant.

FIG. 5. PGPS (PGP Synthase) activity in the *ino1* mutant. Cells were grown for 12 to 16 generations in synthetic medium containing inositol (10 or 75 μ M) in the presence or absence of 1 mM choline to an A_{550} of 0.3. PGPS activity was determined in mitochondrial extracts. This figure is representative of three experiments. Symbols: ■, wild type; ▨, *ino1* mutant.

FIG. 6. PGPS (PGP Synthase) activity in *ino2* and *ino4* mutants. Cells were grown for 10 to 14 generations in synthetic medium containing inositol (10 or 75 μM) in the presence or absence of 1 mM choline to an A_{550} of 0.3 to 0.5. PGPS activity was determined in mitochondrial extracts. This figure is representative of three experiments. Symbols: ■, wild type; □, *ino2* mutant; ▨, *ino4* mutant.

PGPS is fully repressed in *opil* cells grown in the presence of inositol and almost fully repressed in *opil* cells grown in the absence of inositol.

DISCUSSION

Yeast cells contain an elaborate genetic regulatory circuit to control the relative composition of membrane phospholipids. The products of at least three regulatory genes, *INO2*, *INO4*, and *OPH1*, ensure that synthesis of the enzymes of the PI and PC branches is repressed by inositol. This repression is observed only if the PC branch of phospholipid synthesis is functional. Because we expected that the CL branch of phospholipid synthesis might be influenced by cross-pathway control, we measured the effects of inositol, perturbation of the PC pathway, and mutation of phospholipid regulatory genes on PGPS expression. From the experiments reported in this paper, we conclude the following. (i) Inositol represses PGPS expression; (ii) inositol repression of PGPS requires the presence of a functional PC pathway; and (iii) inositol repression of PGPS is not mediated by the regulatory genes *OPH1*, *INO2*, and *INO4*.

Inositol repression of PGPS activity was observed in all strains tested. Inositol at 10 μM is sufficient to fully repress

PGPS activity in the wild-type strain (Fig. 2). This concentration is consistent with inositol concentrations which repress the PI and PC branch enzymes. Similar differences between repressed and derepressed levels are commonly observed for yeast biosynthetic enzymes. In the wild type, the difference between repressed and derepressed levels is greater in media containing choline.

That inositol repression requires PC synthesis is demonstrated by the fact that PGPS activity in mutants blocked in the ability to methylate PE is not repressed by inositol unless choline is also present (Fig. 3 and 4), similar to the nature of inositol repression reported for PI and PC branch enzymes (15).

Particularly intriguing is the speed with which inositol acts as a repressor of PGPS (Fig. 3). A drop in specific activity is observed within minutes of inositol addition and is therefore too rapid an effect to be explained by repression of enzyme synthesis alone. This rapid effect is more likely the result of inactivation and/or degradation of the enzyme and can thus be compared to glucose-triggered catabolite inactivation, which affects enzymes of gluconeogenesis and the glyoxylate cycle, among others (reviewed in reference 18). The kinetics of PGPS repression by inositol differ significantly from those observed by Culbertson et al. (7) for repression of I-1-P synthase, the *INO1* gene product (Fig. 1). No decrease in I-1-P synthase activity was observed until 1 h after the addition of inositol, and the much more dramatic 50-fold repression of I-1-P synthase activity was not fully apparent until 10 h after inositol addition. Thus, although both enzymes are repressed by inositol, it appears that the mechanisms of repression are markedly different. Further analysis of the roles of transcription and protein modification in the regulation of PGPS expression will follow from a molecular analysis of the cloned gene encoding PGPS, and this avenue of investigation is currently in progress.

Another striking difference between PGPS regulation and the regulation of PI and PC branch enzymes is that the regulatory effectors of the latter branches do not affect PGPS control. The *ino2* and *ino4* mutants are fully capable of PGPS derepression in 10 μM inositol (Fig. 6). (This concentration of inositol is sufficient to allow the growth of inositol auxotrophs such as *ino2* and *ino4* but insufficient to fully repress steady-state levels of inositol-sensitive enzymes in

FIG. 7. PGPS (PGP Synthase) activity in the *opil* mutant. Cells were grown for 12 to 16 generations in synthetic medium in the presence or absence of inositol and choline to an A_{550} of 0.3. PGPS activity was determined in mitochondrial extracts. This figure is representative of three experiments. Symbols: ■, wild type; ▨, *opil* mutant.

inositol auxotrophs). In addition, the *opil* negative regulatory mutant, which is constitutive for PI and PC branch enzymes, exhibits fully repressed PGPS levels in medium containing inositol (Fig. 7). Interestingly, PGPS levels are repressed in the *opil* mutant even in cells grown in the absence of inositol. This could be explained by the hypothesis that an increased endogenous level of inositol in the *opil* mutant is sensed by a negative regulatory effector that represses PGPS expression. Previous experiments indicated that *opil* mutant cells do have endogenous inositol levels higher than those seen in wild-type cells. (i) The *opil* mutant excretes inositol into the medium in amounts sufficient to allow the growth of inositol auxotrophs present in the medium. (ii) The rates of PI synthesis (relative to total phospholipid synthesis) in wild-type cells are 11% in medium lacking inositol and 38% in medium containing 75 μ M inositol. In the *opil* mutant, however, the rate of PI synthesis is 31% in medium lacking inositol, consistent with the presence of a high level of endogenous inositol (9). The ability of *opil* cells to repress PGPS expression in the presence of both exogenous inositol and high levels of endogenous inositol argues for the presence of a functional negative regulatory molecule in these cells.

It is noteworthy that the *ino2* and *ino4* mutants exhibit fully derepressed levels of PGPS activity in 75 μ M inositol in the absence of choline. The most likely explanation for this finding is that since these mutants are defective in the derepression of PC branch enzymes (21), inositol repression of PGPS does not occur. An alternative hypothesis, that PGPS levels are sensitive to progress through the inositol biosynthetic pathway, is unlikely, since PGPS levels in the *ino1* mutant are fully repressed in 75 μ M inositol in the presence or absence of choline (Fig. 5). Thus, the effects of *ino2* and *ino4* mutations on PGPS expression are very likely indirect: progress through the PC pathway, which is under the direct control of *ino2* and *ino4*, is deficient, and this deficiency is sensed by some as-yet-unidentified mediator(s) of PGPS regulation.

These observations suggest that while PI and PC branch enzyme activity must be balanced with PGPS activity for mitochondrial membrane synthesis, a different genetic regulatory circuit is needed to mediate this balance. If this hypothesis is correct, we would expect to identify genes other than *OPII*, *INO2*, and *INO4* which affect PGPS regulation. Experiments are currently under way to isolate regulatory mutants which may help to identify these genes. That inositol repression of PGPS activity is not mediated by the *INO2-INO4-OPII* regulatory circuit is surprising. One possible explanation is that phospholipid biosynthetic enzymes localized in the mitochondrion evolved by a different route than did those localized in the cytoplasm and microsomes and are consequently regulated by different regulatory genes. If this is the case, we would expect that PGPS and phosphatidylserine decarboxylase, which is also found only in the mitochondrial inner membrane (20), are regulated in a similar manner. This is not the case, however, since phosphatidylserine decarboxylase expression, unlike PGPS expression, is constitutive in the *opil* mutant (S. Henry, personal communication). An alternative hypothesis is that enzymes that synthesize phospholipid products which are found only in the mitochondrion are subject to a common genetic control system. While there is no evidence for the subcellular localization of phosphatidylglycerolphosphate and phosphatidylglycerol, it has been demonstrated that CL occurs predominantly in the mitochondrial inner membrane (8, 17). If this hypothesis is correct, then we might expect

that the enzymes catalyzing the second and third steps of the CL pathway (phosphatidylglycerolphosphate phosphatase and CL synthase) are coordinately controlled with PGPS. Analysis of the regulation of these enzymes is currently in progress.

ACKNOWLEDGMENTS

We are grateful to Susan Henry, George Carman, and John Pringle for many useful discussions and for critical review of the manuscript; we acknowledge members of the Henry lab (especially John Hill and Pat McGraw) and members of the Greenberg lab for useful discussions; and we thank Brian Bonish for preparation of the figures.

This work was supported by Public Health Service grant GM 37723-02 from the National Institutes of Health.

LITERATURE CITED

- 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.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248-254.
- Carman, G. M., and C. J. Belunis. 1983. Phosphatidylglycerophosphate synthase activity in *Saccharomyces cerevisiae*. *Can. J. Microbiol.* 29:1452-1457.
- 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.* 259:6297-6273.
- Cobon, G. S., P. D. Crowfoot, and A. W. Linnane. 1974. Biogenesis of mitochondria: phospholipid synthesis *in vitro* by yeast mitochondrial and microsomal fractions. *Biochem. J.* 144:265-275.
- Culbertson, M. R., T. F. Donahue, and S. A. Henry. 1976. Control of inositol biosynthesis in *Saccharomyces cerevisiae*: properties of a repressible enzyme system in extracts of wild-type (*Ino*⁺) cells. *J. Bacteriol.* 126:232-242.
- Culbertson, M. R., and S. A. Henry. 1975. Inositol requiring mutants of *Saccharomyces cerevisiae*. *Genetics* 80:23-40.
- Daum, G. 1985. Lipids of mitochondria. *Biochim. Biophys. Acta* 822:1-42.
- 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.
- Greenberg, M. L., L. S. Klig, V. A. Letts, B. S. Loewy, and S. A. Henry. 1983. Yeast mutant defective in phosphatidylcholine synthesis. *J. Bacteriol.* 153:791-799.
- Greenberg, M. L., B. Reiner, and S. A. Henry. 1982. Regulatory mutations of inositol biosynthesis in yeast: isolation of inositol excreting mutants. *Genetics* 100:19-33.
- Henry, S., D. Hoshizaki, A. Bailis, M. Homann, and G. Carman. 1986. Genetic regulation of phospholipid synthesis in yeast, p. 623-632. In L. Freysz, H. Dreyfus, R. Massarelli, and S. Gatt (ed.), *Enzymes of lipid metabolism II*. Plenum Publishing Corp., New York.
- Henry, S. A. 1982. Membrane lipids of yeast: biochemical and genetic studies, p. 101-158. In J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), *Molecular biology of the yeast *Saccharomyces cerevisiae*: metabolism and gene expression*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- 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.
- 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.
- 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.

17. Jakovcic, S. G., S. Getz, M. Rabinowitz, H. Jakob, and H. Swift. 1971. Cardiolipin content of wild type and mutant yeasts in relation to mitochondrial function and development. *J. Cell Biol.* **40**:490-502.
18. Jones, E. W. 1984. The synthesis and function of proteases in *Saccharomyces*: genetic approaches. *Annu. Rev. Genet.* **18**: 233-270.
19. Klig, L. S., M. J. Homann, G. M. Carman, and S. A. Henry. 1985. Coordinate regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae*: pleiotropically constitutive *opil* mutant. *J. Bacteriol.* **162**:1135-1141.
20. Kuchler, K., G. Daum, and F. Paltauf. 1986. Subcellular and submitochondrial localization of phospholipid-synthesizing enzymes in *Saccharomyces cerevisiae*. *J. Bacteriol.* **165**:901-910.
21. 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.
22. Trivedi, A., M. Schwab, D. Fantin, and E. R. Tustanoff. 1987. Relationship of cardiolipin to cytochrome *c* oxidase kinetics as probed by adriamycin, p. 319-328. In K. W. A. Wirtz (ed.), *Membrane Receptors, Dynamics and Energetics*. Plenum Publishing Corp., New York.
23. Trivedi, A., A. V. Wearring, S. D. Kohlwein, F. Paltauf, and E. R. Tustanoff. 1988. Functional importance of mitochondrial cardiolipin in yeast cytochrome *c* oxidase activity, p. 87-94. In J. J. Lemasters, C. R. Hackenbrock, R. G. Thurman, and H. V. Weterhoff (ed.), *Integration of mitochondrial function*. Plenum Publishing Corp., New York.
24. Waechter, C. J., and R. L. Lester. 1973. Differential regulation of the N-methyl transferases responsible for phosphatidylcholine synthesis in *Saccharomyces cerevisiae*. *Arch. Biochem. Biophys.* **158**:401-410.
25. Yamashita, S., A. Oshima, J. Kawa, and K. Hosaka. 1982. Regulation of the phosphatidylethanolamine methylation pathway in *Saccharomyces cerevisiae*. *Eur. J. Biochem.* **128**:589-595.