Phosphatidylcholine (PtdCho) is the most abundant phospholipid in numerous eukaryotes and is generally thought to be essential for membrane structure and cellular function. We designed a specific test of this idea by using genetic and biochemical manipulation of yeast. Yeast mutants (pem1 pem2Δ) lacking the phosphatidylethanolamine (PtdEtn) methyltransferase enzymes require choline for growth and cannot make N-methylated phospholipids. When these strains are grown on a glucose carbon source supplemented with 20 mM propanolamine (Prn), the PtdCho level declines precipitously to the limits of detection (<0.6%), and the hexagonal phase-forming, primary amine-containing lipids, PtdEtn and PtdPrn, constitute ~60% of the total phospholipid content of the cell. When the lipids were analyzed by mass spectrometry, there was no compensatory shift in unsaturation of the PtdEtn and PtdPrn toward more bilayer-forming species. Thus the majority of the cellular amino phospholipids remained hexagonal phase-forming. The pem1 pem2Δ cells will also grow without choline, in the presence of Prn, on nonfermentable carbon sources (requiring functional mitochondria) and accumulate nearly 70% of their phospholipid as hexagonal phase-forming types. These data provide compelling evidence that the functions of PtdCho and N-methylated lipids in membranes are nonessential in Saccharomyces cerevisiae.

Phosphatidylcholine (PtdCho) is the most abundant phospholipid in many eukaryotic cells and has generally been assumed to be essential for cell viability (1–3). In this study we designed experiments to test whether PtdCho or its N-methylated lipid precursors were truly essential for the yeast Saccharomyces cerevisiae. In S. cerevisiae, PtdCho can be synthesized by the pathway PtdSer → PtdEtn → PtdEtn(Me) → PtdEtn(Me)2 → PtdCho as outlined in Fig. 1. Pem1p and Pem2p are methyltransferases that synthesize PtdCho from PtdEtn (4, 5). When ethanolamine (EtN) and choline (Cho) are present, PtdEtn and PtdCho are synthesized via the Kennedy pathways that use CDP-ethanolamine and CDP-choline intermediates. PtdEtn derived from the Kennedy pathway can also be methylated to form PtdCho (6). Phosphoethanolamine, an intermediate in the Kennedy pathway, can also be synthesized as a consequence of sphingolipid degradation (7, 8). Monoethanolamine (EtN(Me)) and dimethylethanolamine (EtN(Me)2) can also be assimilated into PtdEtn(Me) and PtdEtn(Me)2, respectively, via the Kennedy pathways. Yeast mutants (pem1 pem2Δ) lacking the methyltransferase enzymes cannot make N-methylated phospholipids by the de novo pathway. The growth defect of these mutants can be rescued by choline, EtN(Me)2, or EtN(Me) but not by EtN (4). These findings have led to the conclusions that some form of methylated PtdEtn is essential for yeast growth (1, 4).

To test if PtdCho and N-methylated lipids are critical for cell viability, we examined whether propanolamine (Prn), an ethanamine analogue, could rescue the growth defect of methylation-defective mutant strains. Previous research showed that yeast mutants (psdΔ1 psd2Δ) lacking PtdSer decarboxylases require Cho or EtN for growth but can also grow on Prn (8). Mutant strains supplemented with Prn accumulate a novel lipid, phosphatidylpropanolamine (PtdPrn). PtdPrn can comprise up to 40% of the total phospholipid content in Prn-supplemented cells, at the expense of PtdCho and PtdEtn. In Prn-supplemented cells with psdΔ1 psd2Δ mutations, the PtdCho content was reduced from 38 to 10% total phospholipid, and the PtdEtn content was reduced to 1% of total phospholipid. This result suggests that with appropriate genetic manipulation PtdPrn might be able to replace the entire PtdCho and PtdEtn pools. Under physiological conditions, unsaturated PtdCho, PtdSer, and PtdIns all form bilayers when hydrated, but PtdEtn and PtdPrn form a hexagonal phase in vitro (8–10). These hexagonal phase structures have been proposed to be important for protein translocation, membrane fluidity, and membrane fusion events (11–13).

In this study we applied genetic and biochemical approaches to determine whether: 1) PtdCho and its N-methylated phospholipid precursors could be eliminated from cells without loss of viability; 2) PtdCho could also be eliminated for growth on both a fermentable carbon source (glucose) and a nonfermentable carbon source (requiring functional mitochondria); and 3) PtdCho depletion was accompanied by a shift to more saturated species of primary amine-containing phospholipids. The results demonstrate that PtdCho and N-methylated phospholipids are nonessential in yeast and are not required for mitochondrial function.

**EXPERIMENTAL PROCEDURES**

**Construction of Methylation-defective Strains**—Deletion mutants for the PEM2 gene were generated by homologous recombination in the RY52 strain (Mata, trp1, lys2, ura3, leu2, his3, suc2, psdΔ1::TRP1) (14) by transformation with PCR products harboring a KanMX sequence. These PCR products were made by using primers identical to a

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24-nucleotide sequence, 541 bp upstream of PEM2, and a 22-nucleotide sequence, 545 bp downstream of PEM2. Chromosomal DNA templates for the PCR were prepared from a pem2Δ strain (Research Genetics, Inc.) in which the entire PEM2 coding sequence was deleted and replaced by KanMX. The resulting psd1Δ pem2Δ strain was isolated by selection for G418 resistance. Correct replacement of PEM2 by the KanMX sequence was confirmed by PCR analysis using primers outside of the target sequence and a primer specific to the KanMX sequence.

Disruption of the PEM1 gene was accomplished by homologous recombination of the psd1Δ pem2Δ strain using transformation with PCR products containing a 3.2-kb pem1::LEU2 cassette. The pem1::LEU2 module was amplified by PCR from the pem1 disruption strain ESX1-1(4d) obtained from Dr. Susan Henry (Cornell University). The disruption of the PEM1 gene was verified by PCR using one primer within LEU2 and the other outside of the target locus. Strains with a functional Ptd1p were generated from pem1 pem2Δ psd1 mutant strains by transformation with a YCp50-PSD1 plasmid.

Growth Measurement of Lipid Methylation-defective Strains—The methylation-defective strains (pem1::LEU2 pem2::KanMX or pem1::LEU2 pem2Δ::KanMX pem1::TRP1) were pre-grown at 30 °C on synthetic complete medium plates containing Ptn (20 mM) to dilute out cellular PtdCho. The cells were next inoculated into synthetic complete lactate or glucose medium and washed twice with the same medium. Cells were re-inoculated at an A600 of 0.005 in synthetic glucose or 0.02 in synthetic lactate medium plus or minus 20 mM Etn, 20 mM Prn, or 2 mM Cho. Cell growth at 30 °C was monitored by culture absorbance at 600 nm.

Lipid Phosphorus Measurement—Lipid methylation-defective strains were grown to mid-log phase at 30 °C in synthetic glucose or semi-synthetic lactate (2% lactate and 0.05% glucose) media plus Ptn (20 mM) or Cho (2 mM). The cells were harvested by centrifugation and washed twice with water. Mitochondria were prepared as described previously (15). The lipids were extracted from whole cells and mitochondria as described previously (16). The phospholipids were separated by two-dimensional TLC on Silica 60 plates using chloroform/methanol/ammonium hydroxide (65:35:5, v/v/v) followed by chloroform/acetone/methanol/water (75:25:5:2.2, v/v/v). Lipids were visualized with iodine vapor and quantified by measuring phosphorus (17). The results are shown as the percentage of total lipid phosphorus in each phospholipid fraction. Data are means ± S.D. for two or three independent experiments.

Mass Spectrometry—Lipids extracted from the cells grown in synthetic complete medium with glucose plus Ptn (20 mM) or Cho (2 mM) were separated by two-dimensional TLC as described above. Lipid spots corresponding to PtdPrn, PtdEtn, or PtdCho were scraped into glass tubes and extracted from the silica gel by the method of Bligh and Dyer (18). Samples dissolved in methanol were analyzed with a PE Sciex API 3000 triple quadrupole mass spectrometer configured to perform multiple experiments during a single flow injection and by using electrospray ionization. Tandem mass spectrometry was performed in the positive ion mode with a first scan event corresponding to the detection of all precursor ions for m/z 184.3, an ion diagnostic for individual molecular species of PtdCho (19). The next scan event involved configuring the tandem mass spectrometer to detect the loss of 141.1 daltons, a neutral loss characteristic of the PtdEtn molecular species (20). The next scan event detected the neutral loss of 155.1 daltons indicative of PtdPrn molecular species, as the homologous ion to the constant neutral loss of 141 for PtdEtn. The instrument next acquired a full mass spectrum of positive ions from m/z 650–850. The final scan event was in the negative ion mode scanning the mass range from m/z 650–850. In separate experiments phospholipid classes were separated by TLC, eluted from the silica gel, and examined by full scan negative ion flow

**Fig. 1. Schematic diagram of pathways leading to phosphatidylcholine synthesis in S. cerevisiae.** PtdCho can be synthesized via multiple pathways in yeast. In the de novo pathway PtdSer is decarboxylated to form PtdEtn by PtdSer decarboxylases (Psd1p and Psd2p), and the resultant PtdEtn is subsequently methylated to form PtdCho by using S-adenosylmethionine as a methyl group donor. The first step of methylation to form PtdEtn(Me) is catalyzed by Pem1p and Pem2p. The second and third steps of methylation for the formation of PtdEtn(Me)2 and PtdCho are catalyzed by Pem2p. When Etn and Cho are present, PtdEtn and PtdCho are synthesized via the Kennedy pathways that use CDP-ethanolamine and CDP-choline intermediates. PtdEtn derived from the Kennedy pathway can also be methylated to form PtdCho. Phosphoethanolamine, an intermediate in the Kennedy pathway, can also be synthesized as a consequence of sphingolipid degradation. Etn(Me) and Etn(Me)2 can also be assimilated into PtdEtn(Me) and PtdEtn(Me)2, respectively, via the Kennedy pathway.

**Fig. 2. Strategy for elimination of PtdCho using Prn supplementation.** In the presence of pem1 and pem2Δ mutations, PtdCho synthesis from Etn and PtdEtn is blocked. Prn enters the Kennedy pathways and is converted to PtdPrn.
injection analysis. Anions present in the negative ion full scan were further analyzed in the subsequent injection of the sample for collision-induced dissociation of \([M + H]^+\) or \([M - 15]^-\) in order to determine the individual fatty acyl components present in each molecular species, using conditions described previously (21). We also conducted experiments to rule out the possibility that the Prn used in these studies was contaminated by Cho. The Prn was analyzed by electrospray mass spectrometry that was sensitive for both the presence of Prn (\([M + H]^+, \text{m/z} 76\)) as well as Cho (\([M + H]^+, \text{m/z} 104\)). There was no evidence for the occurrence of Cho in the commercial Prn at a level less than 1 ppm as evidenced by a lack of any ion at \text{m/z} 104 when the signal for \text{m/z} 76 was 4.4 \times 10^5 intensity units. An equal quantity of choline analyzed after the propanolamine generated 3.2 \times 10^6 intensity units, thus having a higher detectability as expected for this quaternary amine.

RESULTS

Prn Supplementation of Methylation-defective Mutants Supports Growth on Glucose and Completely Depletes PtdCho—Methylation-defective strains that have deletion/disruption in PEM1 and PEM2 genes cannot make PtdCho and N-methylated lipids (4). These mutant cells were grown in the presence of Prn to enable them to make PtdPrn via the Kennedy pathway as shown in Fig. 2. The mutant strains grew on minimal glucose agar media supplemented with Prn. However, when lipids extracted from these cultures were separated by two-dimensional TLC and stained with iodine vapor, there was a minor spot in the PtdCho region. This minor component was identified as PtdCho by mass spectrometry. Quantification of this PtdCho demonstrated that it comprised 2–3% of total phospholipids (data not shown). Consistent with previous findings (8), this result demonstrates marked reduction of PtdCho can occur without affecting cell viability. These data also reveal that trace amounts of choline can be derived from agar plates. Because our goal was to deplete all PtdCho and N-methylated precursors from these yeast strains, all subsequent growth analyses were performed in liquid media.

In liquid medium, yeast mutants (pem1 pem2) lacking the
Mass spectrometry analysis reveals that there is no marked shift in the degree of saturation in PtdEtn and PtdPrn when the PtdCho pool is eliminated. Lipid classes of pem1 pem2Δ mutant strains were purified by TLC, and the areas corresponding to PtdPrn, PtdEtn, or PtdCho were recovered, extracted from the silica gel, and dissolved in methanol prior to mass spectrometric analysis. 

A, negative ion electrospray mass spectrometry of PtdEtn of mutant cells supplemented with choline. [M – H]⁻ ions indicate the relative abundance of each molecular ion species. The identity of the molecular species was determined by collisional activation of the indicated ion in the inset that reveals the fatty acyl group as a carboxylate anion. 

B, molecular species of PtdCho in choline-supplemented cells with the inset showing the collision-induced decomposition of the [M – H]⁻ ion. 

C, molecular species of PtdEtn from propanolamine-supplemented cells with the inset showing the collision-induced decomposition of the [M – H]⁻ ion. 

D, molecular species of PtdPrn from propanolamine-supplemented cells with the inset showing the collision-induced decomposition of the [M – H]⁻ ion.
methyltransferase enzymes cannot make N-methylated phospholipids. The growth defect of the mutants can be rescued by choline, Etn(Me)$_2$, or Etn(Me) but not by Etn. To examine further whether PtdCho and N-methylated lipids are critical for cell viability, we investigated the growth of the lipid methylation-defective mutant strain on minimal glucose media sup-
were analyzed by negative ion mass spectrometry. A relatively individual molecular species of PtdEtn, PtdPrn, and PtdCho toward more bilayer-forming species. To test this idea the might result in fatty acid compositional changes to make a —

The dramatic increase of total hexagonal phase lipids Marked Shift in the Degree of Unsaturation in PtdEtn and

extracted from the TLC plates also confirmed the above observation (Fig. 4B). PtdCho was reduced to the limits of detection. The PtdIns pool more than doubled, and PtdPrn accounted for 30% of the total phospholipid. Furthermore, with Prn supplementation, the hexagonal phase lipids PtdEtn and PtdPrn comprised 58% of the total phospholipid.

Mass Spectrometry Analysis Reveals That There Is No Marked Shift in the Degree of Unsaturation in PtdEtn and PtdPrn—The dramatic increase of total hexagonal phase lipids (PtdEtn and PtdPrn) in the mutants supplemented with Prn might result in fatty acid compositional changes to make a compensatory shift in the degree of saturation of these lipids toward more bilayer-forming species. To test this idea the individual molecular species of PtdEtn, PtdPrn, and PtdCho were analyzed by negative ion mass spectrometry. A relatively simple mixture of molecular species was found in yeast grown in the presence of choline, because only four major molecular species of PtdEtn were observed at m/z 686, 688, 714, and 716 (Fig. 5A). The identity of these molecular species was determined after collisional activation of each of these ions, which yielded the carboxylic anion corresponding to each fatty acyl substituent (Fig. 5A, inset). Collisional activation of m/z 686 generated only one carboxylic anion at m/z 253, indicative of the fatty acyl group palmitoleoyl at both sn-1 and sn-2 of this PtdEtn molecular species (16:1/16:1-PtdEtn). However, collision-induced decomposition of m/z 688 revealed a mixture of two isobaric components, the major species corresponding to 16:0/16:1-PtdEtn, but a somewhat less abundant species at the same mass corresponding to 14:0/18:1-PtdEtn was also present in the precursor [M − H]− at m/z 688.4. Similarly, analysis of m/z 714 and 716 revealed the molecular species 16:1/18:1-PtdEtn and 16:0/18:1 with somewhat smaller amounts of 18:0/ 16:1-PtdEtn. Abundance of the fatty acyl substituents with the corresponding phosphatidylethanolamine molecular species isolated from the yeast cultures was indicated by the [M − 15]− ion, revealing a similar small number of molecular species with glycerophosphocholine as the polar moiety (Fig. 5B). A slightly more abundant species was observed, corresponding to 16:0/16: 1-PtdCho relative to the corresponding molecular species in the PtdEtn class. When cells were incubated with propanolamine, there was no change in the relative molecular species distribution within the phosphatidylethanolamine species either at the level of [M − H]− ion abundances or their collision-induced decomposition products (Fig. 5C). New PtdPrn molecular species were now observed at m/z 700, 702, 728, and 730 which corresponded to 16:1/16:1-PtdPrn, 16:0/16:1-PtdPrn, 16:1/18:1- PtdPrn, and 16:1/18:0-PtdPrn plus 16:0/18:1-PtdPrn, respectively (Fig. 5D). The identities of these were confirmed again by tandem mass spectrometry (inset, Fig. 5D). This direct determination of the individual molecular species and fatty acyl substituents (Fig. 5) demonstrated that the saturated fatty acyl species were not increased in the PtdPrn and PtdEtn classes of the mutant supplemented with Prn.

Ptn Supplementation Supports Growth of Methylation-defective Mutants and Eliminates the PtdCho Pool Under Respiratory Conditions—Functional mitochondria are required when yeast cells grow on nonglycolytic carbon sources and must use respiration for ATP generation. We analyzed cell growth under respiratory conditions to determine whether PtdPrn could support mitochondrial function. For this analysis, the methylation-defective mutants were grown on minimal lactate media either without supplementation or with Etn, Prn, or Cho (Fig. 6). Both unsupplemented cultures and those provided with Etn failed to support the growth of methylation-defective strains. In contrast, the mutants grew normally when supplied with Cho. In addition, after a significant lag, the mutants also grew when supplemented with Prn.

Lipids extracted from the cells grown on minimal lactate medium supplemented either with Cho or Prn were separated by two-dimensional thin layer chromatography. As with glucose grown cells, the mutants grown in minimal lactate medium supplemented with Prn showed dramatic alterations in lipid composition. Quantification of phospholipids resolved on TLC plates, by phosphorus assay (Fig. 7), revealed that PtdCho levels declined precipitously, to the limits of detection. The loss of PtdCho was accompanied by a modest increase in PtdIns and the appearance of PtdPrn as greater than 20% of the total phospholipid. Under these conditions the hexagonal phase lipids PtdEtn and PtdPrn accounted for more than 60% of the phospholipid pool.

We also examined the consequences of the methyltransferase mutations and Prn supplementation upon the lipid composition of isolated mitochondria. The results of this analysis are shown in Fig. 8 and demonstrate that the mitochondrial phospholipid composition closely parallels that found for the whole cell. The findings clearly demonstrate that PtdCho is not essential for mitochondrial function and that the organelle can tolerate a hexagonal phase lipid content of greater than 60% of its total phospholipid.

![Fig. 6. Ptn supplementation supports growth of methylation-defective mutants under respiratory conditions.](http://www.jbc.org/Downloaded from)
Cells Lacking PtdCho and the Ability to Synthesize Mitochondrial PtdEtn Remain Viable with Prn Supplementation on Glucose-containing Medium—Previous research (8, 22) has demonstrated that mitochondrial PtdEtn generated by Psd1p is critical for the respiratory growth of yeast strains. We performed experiments to test if mitochondrial PtdEtn as well as PtdCho can be replaced by PtdPrn (Fig. 9). We used triple mutant strains defective in PEM1 and PEM2 for phospholipid methylation and defective in PSD1 for PtdEtn synthesis in mitochondria. The mutation in the PSD1 gene results in a decrease in the PtdEtn pool in mitochondria. The triple mutants were grown on minimal glucose media either lacking supplements or with Etn, Prn, or Cho (2 mM). Cells were harvested by centrifugation, washed twice with distilled H2O, and mitochondria isolated. Lipids were extracted from mitochondria and separated by two-dimensional TLC on Silica 60 plates. Lipid classes were visualized with iodine vapor and quantified by measuring phosphorus. Results are the percentage of total lipid phosphorus in each phospholipid fraction. Values shown are means ± S.D. for three experiments. The total amount of phospholipids per mg of protein was 301.47 ± 22.79 nmol/mg for Prn-supplemented cells and 376.22 ± 44.45 nmol/mg for Cho-supplemented cells, respectively.

The results clearly show that Prn-supplemented mutants still can grow despite genetic elimination of PtdCho synthesis and mitochondrial PtdEtn synthesis.

Lipids extracted from the cells grown on either Cho- or Prn-containing media were separated by two-dimensional TLC. When the lipids were visualized by iodine staining the triple mutant strain grown on Prn lacked detectable PtdCho and accumulated PtdPrn (Fig. 11A). In addition, the PtdIns spot appeared much more prominent in the triple mutant supplemented with Prn when compared with this strain supplemented with choline. Quantification of the phospholipids (Fig. 11B) demonstrated the elimination of PtdCho and a 4-fold increase in PtdIns, and an accumulation of PtdPrn to 33% of the total phospholipid pool. Unlike Cho-supplemented cells, the Prn-supplemented cells did not accumulate relatively high levels of PtdSer.

We also isolated mitochondria from glucose-grown cells harboring pem1 pem2Δ psd1Δ mutations and quantified the phospholipids of these organelles. The results shown in Fig. 11C demonstrate that choline supplementation of the triple mutant produces mitochondria in which PtdCho is the most abundant phospholipid (44.3%), and PtdIns, PtdEtn, and PtdSer are nearly equivalent, ranging from 12 to 17% of the total phospholipids. As expected for a strain lacking Psl1p, the PtdEtn content of the organelle is reduced by 49% when compared with the level of this phospholipid in total cell membranes (see Fig. 11B). Analysis of the mitochondria derived from the triple mutant supplemented with 2 mM Prn demonstrates the or-

**Fig. 7.** Propanolamine supplementation eliminates PtdCho from methylation-defective mutants under respiratory growth conditions. The pem1 pem2Δ mutant strains were grown to mid-log phase at 30 °C in semi-synthetic lactate (2% lactate and 0.05% glucose) media plus Prn (20 mM) or Cho (2 mM). Cells were harvested by centrifugation and washed twice with distilled H2O. Lipids were extracted from whole cells. Lipids were separated by two-dimensional TLC on Silica 60 plates, visualized with iodine vapor, and quantified by measuring phosphorus. Results are the percentage of total lipid phosphorus in each phospholipid fraction. Values shown are means ± S.D. for three experiments. The total amount of phospholipids per mg of cell protein was 147.90 ± 21.82 nmol/mg for Prn-supplemented cells and 114.44 ± 7.26 nmol/mg for Cho-supplemented cells, respectively.

**Fig. 8.** PtdPrn is incorporated into mitochondrial membranes and eliminates the requirement for PtdCho. Lipid methylation-defective strains were grown to mid-log phase at 30 °C in semi-synthetic lactate (2% lactate and 0.05% glucose) media plus Prn (20 mM) or Cho (2 mM). Cells were harvested by centrifugation, washed twice with distilled H2O, and mitochondria isolated. Lipids were extracted from mitochondria and separated by two-dimensional TLC on Silica 60 plates. Lipid classes were visualized with iodine vapor and quantified by measuring phosphorus. Results are the percentage of total lipid phosphorus in each phospholipid fraction. Values shown are means ± S.D. for three experiments. The total amount of phospholipids per mg of protein was 301.47 ± 22.79 nmol/mg for Prn-supplemented cells and 376.22 ± 44.45 nmol/mg for Cho-supplemented cells, respectively.
Ganelles are devoid of PtdCho and contain PtdPrn as the most abundant phospholipid (35.2%). These mitochondria also contain relatively high levels of PtdIns (30.1%) and lower levels of PtdEtn (18.7%) and PtdSer (8%).

We next sought to determine whether PtdPrn could support the growth of the triple mutant under respiratory conditions. The results of these experiments are shown in Fig. 12. Cells lacking both mitochondrial PtdCho and the pool of PtdEtn generated by Psd1p fail to grow with Prn supplementation under conditions requiring functional mitochondria. These findings demonstrate that PtdPrn is unable to fulfill both Ptd-Cho and PtdEtn requirements for mitochondrial function during respiration.

DISCUSSION

In experiments described in this report we tested the essentiality of PtdCho and other methylated forms of PtdEtn for the growth and mitochondrial function of *S. cerevisiae*. In many well described eukaryotes typically used for biochemical experiments, PtdCho is the most abundant phospholipid and is often assumed to be essential for membrane structure and function and cell growth (1, 3, 11). The results of our studies provide clear evidence that neither PtdCho, PtdEtn(Me), nor PtdEtn(Me)_2 is required for either cell viability or mitochondrial function in yeast.

Our general approach to examining the role of methylated forms of PtdEtn, including PtdCho, in supporting cell growth was to use the Etn analog Prn. Prn, like Etn, contains a primary amine. Previous studies have shown that Prn is readily incorporated into phospholipid in both *Saccharomyces* and *Tetrahymena* species (8, 23). The physical properties of PtdPrn are similar to those of PtdEtn. Most notably, PtdPrn forms hexagonal HII phase structures. NMR studies reveal that PtdPrn has a higher propensity to form hexagonal phase structures than PtdEtn (8). Although PtdPrn forms hexagonal phase structures, it cannot fully substitute for the cellular requirements for PtdEtn in yeast. In addition, in earlier studies examining PtdPrn metabolism, we demonstrated that this novel lipid replaced much of the PtdCho present in cells and simultaneously enabled cells to survive with drastically reduced levels of PtdEtn, created by mutations in PtdSer decarboxylases (8). These observations led to the initial experiments described in this study designed to test whether PtdPrn formation would be permissive for cell growth in the absence of PtdCho synthesis.

The experiments described in Figs. 3 and 6 demonstrate that Prn supplementation enables strains harboring deletions in *PEM1* and *PEM2* genes to grow, albeit at a slower initial rate than the same cells supplemented with Cho. Analysis of the lipid composition of these cells reveals several interesting findings. Unexpectedly, cells grown on minimal agar plates lacking choline, but supplemented with Prn, are able to synthesize low levels of PtdCho. This result indicates that agar contains trace
levels of Cho that the cells are able to successfully scavenge. With growth in liquid medium, no PtdCho was formed above the background levels of our assay system. These data clearly demonstrate that yeast does not require PtdCho or the mono- or di-methylated forms of PtdEtn for growth. Previous work by Summers et al. (4) demonstrated that yeast cells could grow moderately well with PtdEtn(Me)$_2$ replacing PtdCho and poorly with PtdEtn(Me) replacing PtdCho but implicated a specific need for at least some methylation of PtdEtn as essential for growth. Additional work in mammalian cells also provided evidence that PtdEtn(Me)$_2$ could support growth of mammalian cells containing greatly diminished levels of PtdCho.
synthetic lactate plates containing Prn (20 mM) to dilute out cellular ptdEtn and PtdCho under respiratory conditions. A by supplemented with Prn. As expected, PtdPrn replaced PtdCho, pronounced lag but reach normal levels of saturation when supplemented with Prn. This PtdPrn can replace mitochondrial PtdCho only when there is adequate PtdEtN present in the organelle. The PtdPrn appears unable to replace both PtdCho and PtdEtN pools within the mitochondria. These findings are consistent with other observations indicating that PtdEtN plays an essential role in mitochondrial membrane structure and function during respiration (8, 22).

In all conditions described in this report, the supplementation of cells with Prn resulted in elevated levels of PtdIns when compared with cells supplemented with Cho. This consistent finding was most pronounced when analyzing the phospholipids of the pem1 pem2Δ psd1Δ triple mutant. The elevation of membrane PtdIns content may reflect the biophysical need to replace some of the eliminated PtdCho with the bilayer forming PtdIns to counteract the propensity of PtdPrn and PtdEtN to form nonbilayer structures (8).

In summary, this work demonstrates that it is possible to alter dramatically the phospholipid composition of yeast and its mitochondria with a combination of genetic and biochemical manipulations. PtdCho, the major lipid of many eukaryotes, can be eliminated along with other N-methylated forms of PtdEtN, indicating that these phospholipids are nonessential. The dispensability of PtdCho is true under both respiratory and nonrespiratory growth conditions.

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