Glycerophosphocholine Catabolism as a New Route for Choline Formation for Phosphatidylcholine Synthesis by the Kennedy Pathway*

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In eukaryotes, neuropathy target esterase (Nte1p in yeast) deacylates phosphatidylcholine derived exclusively from the CDP-choline pathway to produce glycerophosphocholine (GroPCho) and release two fatty acids. The metabolic fate of GroPCho in eukaryotic cells is currently not known. Saccharomyces cerevisiae contains two open reading frames predicted to contain glycerophosphodiester phosphodiesterase domains, YPL110c and YPL206c. Pulse-chase experiments were conducted to monitor GroPCho metabolic fate under conditions known to alter CDP-choline pathway flux and consequently produce different rates of formation of GroPCho. From this analysis, it was revealed that GroPCho was metabolized to choline, with this choline serving as substrate for renewed synthesis of phosphatidylcholine. YPL110c played the major role in this metabolic pathway. To extend and confirm the metabolic studies, the ability of the ypl110cΔ and ypl206cΔ strains to utilize exogenous GroPCho or glycerophosphoinositol as the sole source of phosphate was analyzed. Consistent with our metabolic profiling, the ypl206cΔ strain grew on both substrates with a similar rate to wild type, whereas the ypl110cΔ strain grew very poorly on GroPCho and with moderately reduced growth on glycerophosphoinositol.

Phosphatidylcholine (PtdCho) is the major phospholipid of eukaryotic cells, and regulation of its biosynthesis, degradation, and relative distribution among different membranous structures is critical for cellular function (1). In Saccharomyces cerevisiae, PtdCho is synthesized through two different pathways (2). The CDP-choline (CDP-Chol) or Kennedy pathway involves the activation of choline (Cho) to CDP-Chol via a phosphorylcholine (P-Chol) intermediate for condensation with diacylglycerol to produce PtdCho. Through the methylation pathway, PtdCho is produced through sequential methylation of phosphatidylethanolamine. When Cho is not present in the growth medium, the activity of the CDP-Chol pathway is not reduced; instead, Cho derived from turnover of PtdCho produced by the methylation pathway is used for PtdCho synthesis through the CDP-choline pathway (3). Both the CDP-choline and methylation pathways are subject to regulation by the Lno2p and Lno4p transcription factors (2, 4). When inositol is available as a precursor, the activity of the methylation pathway is reduced by transcriptional repression of the methyltransferase-encoding genes OPI3 and CHO2, as is expression of CKI1 and CPT1 encoding the first and the third enzymes of the CDP-Chol pathway. The addition of Cho to inositol-containing medium further reduces transcription of these genes through an as yet to be determined mechanism.

In contrast to phospholipid biosynthesis, much less is known about their catabolism. In yeast, PtdCho can be degraded by B and D type phospholipases. The major PtdCho phospholipase D is Spol4p, catalyzing the scission of a phosphoester bond to produce phosphatic acid and Cho. Spol4p is essential for sporulation and Sec14p-independent secretion (3, 5, 6). Phospholipase B deacylates PtdCho, producing glycerophosphocholine (GroPCho) and two free fatty acids. Three different genes coding for phospholipase B activities (PLB1 to -3) have been identified in S. cerevisiae whose protein products are located at the plasma membrane and within the periplasmic space. None of the three genes are essential, since the triple mutant strain is viable. Of these phospholipase B activities, Pb1p is the main activity responsible for PtdCho deacylation at the plasma membrane with its production of GroPCho released into the extracellular medium (7–9). Recently, a highly conserved PtdCho deacylating activity responsible for intracellular GroPCho formation was identified in eukaryotes ranging from yeast to humans (10). This phospholipase B, termed neuropathy target esterase (Nte1p in yeast), is a devoted phospholipase B against only CDP-Chol pathway-derived PtdCho. Inhibition of its activity chemically or genetically results in slow neurodegeneration in mice and Drosophila (11, 12). In yeast, Nte1p activity was shown to increase concurrently with high CDP-Chol-derived PtdCho biosynthesis induced by either the addition of exogenous Cho or elevation of temperature (10, 13). In agreement with this observed regulation of Nte1p activity, Dowd et al. (13) reported that GroPCho intracellular content increased 20-fold as exogenous Cho and growth temperature were concomitantly increased.

We present metabolic evidence indicating that intracellular GroPCho is further metabolized and that the Cho moiety is reused for PtdCho biosynthesis. We also present evidence showing that the yeast YPL110c and YPL206c open reading frames (ORFs), the sole two yeast ORFs that are predicted to contain the canonical glycerophosphodiester phosphodiesterase domain, are involved in intracellular GroPCho recycling to PtdCho and as well as in the utilization of exogenous GroPCho and glycerophosphoinositol (GroPIns) as phosphate sources for yeast growth.

**EXPERIMENTAL PROCEDURES**

**Materials**—Radiolabeled [methyl-14C]choline was purchased from American Radiolabeled Chemicals. Silica gel thin layer chromatography plates were purchased from Whatman. Glycerophosphocholine (cadmium salt) was from Sigma. Cadmium was removed by elution through a column (2.7 × 18 mm) of Dowex 50W (Na+ form) at pH 5 and
developed with water. GroPCho eluting in the flow-through was washed from the column, and its concentration was estimated by phosphorus determination (14). Phosphatidylinositol was purchased from Avanti Polar Lipids. Deacylation of phosphatidylinositol was performed with methylamine essentially as described (15). GroPIns was chromatographed as described above for GroPCho, and its concentration was determined by phosphorus assay.

** Yeast Strains and Culture Conditions—** Standard yeast genetic techniques were used (16). The yeast strains used in this study are listed in TABLE ONE. Cells were routinely grown aerobically at 25 °C in synthetic minimal medium containing 2% glucose supplemented as required for cell growth. For yeast growth on alternate phosphate sources, synthetic defined medium was prepared as described (17) with the following modifications. KH₂PO₄ (1 g/liter) was replaced with KCl (1 g/liter), and inositol was added to 75 μM. This medium was then supplemented with KH₂PO₄, GroPIns, or GroPCho. Growth was monitored by turbidity measurement at 600 nm on a Beckman DU 640 spectrophotometer.

**Analysis of GroPCho Metabolism—** Logarithmically growing yeast cells in minimal glucose medium containing the nutritional requirements for cell growth were harvested, washed with fresh medium, and recultured in identical medium containing 1.8 mmol GroPCho (55 μmol/ml) for 30 min at 25 °C. Cells were centrifuged, washed in fresh medium, and recultured for 90 min in identical medium containing 1 mM nonradiolabeled Cho prewarmed to 37 °C. Under these conditions, high CDP-Cho-derived PtdCho synthesis and an accumulation of intracellular GroPCho. Measurements of intracellular GroPCho content in yeast cells range from 0.1 nmol/OD for cells growing at 30 °C without exogenous Cho to 2 nmol/OD for cells growing at 37 °C in the presence of 1 mM Cho in the growth medium (13). Pulse-chase analyses using radiolabeled Cho performed by us (19) and by others (13) showed that there is no significant increase in GroPCho accumulation in the extracellular medium upon temperature elevation, a stimulus that elicits Nte1p-mediated intracellular GroPCho formation. These results suggest that yeast cells control intracellular GroPCho content through a mechanism distinct from excreting GroPCho out of the cell. In order to gain a better understanding of intracellular GroPCho metabolic fate, we performed pulse-chase analysis using [14C]Cho. Wild-type yeast cells were labeled with [14C]Cho for 30 min at 25 °C, and then the labeled cells were incubated for 90 min at 37 °C in the presence of 1 mM unlabeled Cho to chase the radiolabel into GroPCho. Indeed, after this labeling protocol, 70–80% of the label was associated with intracellular GroPCho. A second chase followed in which the cells were cultured at 37 °C in the absence or the presence of 1 mM unlabeled Cho (Fig. 1). The time course analysis of label distribution among Cho-containing metabolites from aqueous and organic intracellular fractions, as well as extracellular medium, during the second chase period revealed two different patterns of radioactivity distribution depending on Cho availability. In the presence of 1 mM Cho, a slight increase in label associated with GroPCho was observed. In stark contrast, when Cho was absent from the medium, a large increase in label associated with PtdCho was observed along with a concomitant diminution of radioactivity associated with GroPCho. Under both conditions, the label recovered in the medium was only 6–7% of the total. The results indicate that intracellular GroPCho is not a refractory end product of PtdCho catabolism but instead can be further metabolized to PtdCho.

Glycerophosphodiester phosphodiesterases have been characterized from several sources (20–23). They exhibit hydrolytic activity against different glycerophosphoalcohols producing sn-glycerol 3-phosphate and the corresponding alcohol (EC 3.1.4.46). S. cerevisiae possesses two ORFs, *YPL110c* and *YPL206c*, that probably code for glycerophosphodiester phosphodiesterase activities, since their ORFs are predicted to contain a glycerophosphodiester phosphodiesterase motif. An intracellular GroPIns glycerophosphodiester phosphodiesterase activity in

**TABLE ONE**

| Yeast strains used in this study | Genotype | Source |
|----------------------------------|----------|--------|
| BY4741                           | MATα *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 | Euroscaf |
| *ypl110cΔ*                       | BY4741 *ypl110cΔ*::kanMX | Euroscaf |
| *ypl206cΔ*                       | BY4741 *ypl206cΔ*::kanMX | Euroscaf |
| *git1Δ*                          | BY4741 *git1Δ*::kanMX | Euroscaf |
| *ypl110cΔ ypl206cΔ*             | BY4741 *ypl110cΔ*::kanMX *ypl206cΔ*::kanMX | This study |

**RESULTS**

Deacylation of PtdCho mediated by Nte1p leads to intracellular GroPCho formation, and the extent of this reaction correlates well with the activity of the CDP-Cho pathway. The addition of Cho into the growth medium and temperature elevation promote both a high rate of CDP-Cho-derived PtdCho synthesis and an accumulation of intracellular GroPCho. Measurements of intracellular GroPCho content in yeast cells range from 0.1 nmol/OD for cells growing at 30 °C without exogenous Cho to 2 nmol/OD for cells growing at 37 °C in the presence of 1 mM Cho in the growth medium (13). Pulse-chase analyses using radiolabeled Cho performed by us (19) and by others (13) showed that there is no significant increase in GroPCho accumulation in the extracellular medium upon temperature elevation, a stimulus that elicits Nte1p-mediated intracellular GroPCho formation. These results suggest that yeast cells control intracellular GroPCho content through a mechanism distinct from excreting GroPCho out of the cell. In order to gain a better understanding of intracellular GroPCho metabolic fate, we performed pulse-chase analysis using [14C]Cho. Wild-type yeast cells were labeled with [14C]Cho for 30 min at 25 °C, and then the labeled cells were incubated for 30 min at 37 °C in the presence of 1 mM unlabeled Cho to chase the radiolabel into GroPCho. Indeed, after this labeling protocol, 70–80% of the label was associated with intracellular GroPCho. A second chase followed in which the cells were cultured at 37 °C in the absence or the presence of 1 mM unlabeled Cho (Fig. 1). The time course analysis of label distribution among Cho-containing metabolites from aqueous and organic intracellular fractions, as well as extracellular medium, during the second chase period revealed two different patterns of radioactivity distribution depending on Cho availability. In the presence of 1 mM Cho, a slight increase in label associated with GroPCho was observed. In stark contrast, when Cho was absent from the medium, a large increase in label associated with PtdCho was observed along with a concomitant diminution of radioactivity associated with GroPCho. Under both conditions, the label recovered in the medium was only 6–7% of the total. The results indicate that intracellular GroPCho is not a refractory end product of PtdCho catabolism but instead can be further metabolized to PtdCho.

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**Glycerophosphocholine Metabolism**
yeast has been proposed, since it was clearly demonstrated that S. cerevisiae can use GroPIns both as a phosphate and inositol source with only intact GroPIns imported into the cell (17, 24, 25). A second type of enzymatic activity toward the ester bond of glycerophosphoalcohols was described in crude cellular extracts that resulted in the production of glycerol and P-Cho (26). Cleavage of GroPCho by these types of glycerophosphodiester phosphodiesterase activities could release Cho (EC 3.1.4.46) or P-Cho (EC 3.1.4.38) for potential use by the CDP-Cho pathway for PtdCho synthesis. From our results, the observed change of radioactivity distribution during the second chase period in the absence of Cho is consistent with the presence of an intracellular glycerophosphodiester phosphodiesterase. The results are consistent with a pathway of circular flux whereby Cho released from GroPCho is reused for PtdCho biosynthesis.

In the experiments performed in the presence of 1 mM exogenous Cho, the specific radioactivity of [14C]Cho-containing metabolites would continuously decrease during the chase. This fact, together with the slight increase in labeling of GroPCho probably reflects an increase in GroPCho levels. This interpretation would be in agreement with previous data (13). On the other hand, the specific activity of [14C]Cho-containing metabolites would remain almost unchanged when the cells were incubated in the absence of external Cho. Our observed net transfer of label from GroPCho to PtdCho under conditions of no external Cho and growth at 37 °C reflects a reduction in intracellular GroPCho and indicates that under these conditions the rate of GroPCho production mediated by Nte1p is slower than the rate of GroPCho consumption mediated by a presumptive glycerophosphodiester phosphodiesterase activity.

Similar pulse-chase analyses of Cho-containing metabolites were performed for ypl110cΔ and ypl206cΔ strains (Fig. 1). The radioactivity distribution profiles of these strains were similar to that observed for wild type strain in the absence or presence of 1 mM Cho. This was surprising, since these are the only two yeast ORFs with a predicted glycerophosphodiester phosphodiesterase motif. We reasoned that the elevated temperature of 37 °C used throughout the chase could mask any potential biochemical effect derived from the absence of these genes, since under this condition there is high CDP-Cho-derived PtdCho biosynthesis and consequent Nte1p-mediated PtdCho deacylation, and thus a further increase in label associated with GroPCho due to the absence of a GroPCho consuming pathway would be barely detectable. Indeed, in the absence of Cho, resulting in a diminution of CDP-choline pathway flux, a small decrease in the rate of transfer of label from GroPCho to PtdCho was consistently observed for ypl110cΔ and ypl206cΔ strains in comparison with wild type cells.

To circumvent this problem, we performed another set of pulse-chase analyses, schematically outlined in Fig. 2A, but monitored labeled

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**FIGURE 1.** GroPCho accumulation upon temperature elevation depends on Cho availability. Wild type, ypl110cΔ::kanMX, and ypl206cΔ::kanMX strains were grown to midlog phase in minimal glucose medium. Cells were centrifuged, washed, and resuspended in fresh medium containing 0.1 μCi/ml [14C]Cho (55 mCi/mmole) for 30 min at 25 °C. Cells were harvested, washed, and recultured for 90 min in fresh medium containing 1 mM nonradioactive Cho prewarmed to 25 °C. At the indicated times, aliquots of the culture were analyzed for radioactivity distribution among the indicated Cho-containing metabolites. Total radioactivity incorporated by the three strains was essentially identical. Data are expressed as a percentage of total label recovered in each fraction at each time point. This experiment was repeated three times with qualitatively similar results. The radiolabel associated with Cho and P-Cho is not shown, since it was less than 1% of the total. Ext, radioactivity associated with extracellular medium.

**FIGURE 2.** YPL110c and YPL206c gene products differentially affect GroPCho turnover. A, diagram of the experimental design. Wild type, ypl110cΔ::kanMX, and ypl206cΔ::kanMX strains were grown to midlog phase in minimal supplemented glucose medium. Cells were centrifuged, washed, and resuspended in fresh medium containing 0.1 μCi/ml [14C]Cho (55 mCi/mmole) for 30 min at 25 °C. Cells were harvested, washed, and recultured for 90 min in fresh medium containing 1 mM nonradioactive Cho prewarmed to 25 °C. Cells were harvested and resuspended in minimal glucose medium containing 1 mM nonradioactive Cho prewarmed to 25 °C and incubated for 60 min. This second chase period at 25 °C was intercalated to allow for a reduction of CDP-Cho-derived PtdCho biosynthesis rate and Nte1p-mediated GroPCho formation. At time 0, cells were harvested and exhaustively washed with and recultured in medium with or without 1 mM nonradioactive Cho prewarmed to 25 °C. At the indicated times, aliquots of the culture were analyzed for radioactivity distribution among the indicated Cho-containing metabolites. B, radioactivity distribution among Cho-containing metabolites. C, radioactivity distribution profiles of intracellular Cho and P-Cho. Total radioactivity incorporated by the three strains was essentially identical. Data are expressed as a percentage of total label recovered in each fraction at each time point. This experiment was repeated three times with qualitatively similar results. Ext, radioactivity associated with extracellular medium. The data indicated with the asterisks represent the radioactivity distribution profile corresponding to samples taken at the end of the 37 °C chase period.
GroPCho metabolism at 25 °C. Yeast cells were labeled with [14C]Cho at 25 °C for 30 min and then cultured at 37 °C for 90 min in the presence of 1 mM Cho to accumulate 70–80% of the radiolabel into GroPCho. In order to decrease the sustained PtdCho synthesis and degradation that takes place at 37 °C, the cells were transferred into identical medium containing 1 mM Cho prewarmed to 25 °C and cultured for a further 60 min. This was followed by continued growth at 25 °C but in the absence or the presence of 1 mM unlabeled Cho (Fig. 2, B and C). When the cells were cultured in the absence of Cho, a transfer of radioactivity from GroPCho to PtdCho was observed for wild type, ypl110cΔ, and ypl206cΔ strains (Fig. 2B). As was observed when the chase was performed at 37 °C (Fig. 1), the rate of radioactivity transfer from GroPCho to PtdCho was slightly reduced for ypl110cΔ and ypl206cΔ strains in comparison with wild type cells.

Under Cho-replete conditions, differing metabolic profiles for each strain were very evident. These results illustrate that YPL110c is a major contributor of GroPCho consumption, whereas YPL206c plays a far less prominent role (Fig. 2B). Also, since Cho uptake and flux through the CDP-Cho pathway measured in ypl110cΔ and ypl206cΔ strains were essentially similar to those parameters for wild type cells (data not shown), we can conclude first that the rate of diminution of the specific activity of Cho-containing metabolites is the same for all three strains, and second, the rate of PtdCho deacylation mediated by Nte1p is also the same for the three strains. The transfer of label from GroPCho to PtdCho observed for each strain suggests that the GroPCho pool is not at steady state upon temperature reduction and would progress to reach the same for the three strains.

Remarkably, in the double mutant ypl110cΔ ypl206cΔ cells the Cho pool could be regarded as an index that the other ester bond is cleaved. We do not favor such an interpretation. First, further auxiliary hypotheses should explain the minor but consistent expansion of the Cho pool observed in the presence of the YPL110c gene product. Second, all of the biochemically characterized enzymes that possess canonical glycerophosphodiester phosphodiesterase domains and exhibit such an activity in vitro cleave glycerophosphoalcohols, producing the corresponding alcohol and glycerol 3-phosphate (20–23). Our attempts to directly measure glycerophosphodiester phosphodiesterase activity against GroPCho or GroPIns in whole cell extracts, soluble or particulate fractions prepared from wild type, ypl110cΔ, ypl206cΔ, and ypl110cΔ ypl206cΔ strains have yet to meet with success. Furthermore, our use of purified recombinant Ypl110c and Ypl206c as sources of enzyme did not render any positive results. Several parameters were manipulated in order to measure enzyme activity including buffer composition and pH, the presence of various divalent cations, the presence of detergents, substrate concentration, incubation time, and amount of protein. Astonishingly, under any combination analyzed, we could not detect in vitro glycerophosphodiester phosphodiesterase activity, although our results clearly indicate that this activity exists in vivo. An elusive required cofactor is obviously required for enzymatic activity that we have yet to identify and will be the subject of future studies.

To analyze whether the products of YPL110c and YPL206c genes constitute the whole complement responsible for GroPCho consumption, a double mutant strain was obtained by crossing the corresponding single mutants of both genes. Pulse-chase analyses similar to those depicted in Fig. 2A were performed for two independent ypl110cΔ ypl206cΔ double mutants. The radioactivity distribution profiles obtained in the absence or presence of 1 mM Cho were essentially similar to those obtained for the ypl110cΔ single mutant (data not shown).

S. cerevisiae can use exogenous GroPIns as the sole source of phosphate and inositol. It has been demonstrated that the Git1p transporter specifically mediates GroPIns uptake, and it was anticipated that the utilization of GroPIns as a source of phosphate or inositol would require an intracellular glycerophosphodiester phosphodiesterase activity (25). To this end, we analyzed the role of YPL110c and YPL206c gene products in the utilization of GroPIns as a source of phosphate. Wild type, ypl110cΔ, ypl206cΔ, and ypl110cΔ ypl206cΔ cells were tested for growth in minimal synthetic liquid medium containing 75 μM PO4 or 75 μM GroPIns (Fig. 3). As expected, all of the strains grew at similar rates in 75 μM PO4. Mutant ypl110cΔ cells exhibited a reduced growth rate using GroPIns as a source of phosphate in comparison with wild type cells, whereas the ypl206cΔ strain grew as fast as the wild type. However, the double mutant ypl110cΔ ypl206cΔ strain grew slightly slower than ypl110cΔ cells. It should be noted that for the ypl110cΔ strain, growth of these cells on GroPIns as the sole source of phosphate would reach similar cell densities as that observed for growth on PO4 but required at least 80 h to do so (data not shown).

We extended this study analyzing the participation of YPL110c and
YPL206c gene products in the utilization of external GroPCho as a source of phosphate. Kinetic characterization of the Git1p transporter showed that GroPCho was a poor competitor of GroPIns, since when Git1p-mediated uptake was assayed at 10 μM labeled GroPIns, a 40-fold excess of GroPCho reduced transport activity by only 25% (24). We observed that wild type cells barely grew using 75 μM GroPCho as phosphate source (Fig. 3), but faster growth rates were reached on higher GroPCho concentrations (Fig. 4). The fact that the git1Δ strain grew poorly on GroPCho at any concentration (Fig. 4) suggests that Git1p is the major transporter for GroPCho into the cell. In an overall comparison, the patterns of GroPCho utilization displayed by each strain analyzed closely resembled those observed when GroPIns was used as a source of phosphate.

**DISCUSSION**

We present evidence that intracellular GroPCho is not a refractory end product of PtdCho catabolism but is metabolized intracellularly. Pulse-chase experiments using radiolabeled Cho were conducted to promote the accumulation of intracellular GroPCho, and then its metabolic fate was monitored under different culture conditions. Under conditions that promoted a high rate of Nte1p-mediated PtdCho deacylation, most of the label remained associated with intracellular GroPCho, with the percentage of label associated with extracellular GroPCho never exceeding 10% even after extended incubations. Reduction of CDP-Cho-mediated PtdCho biosynthesis by deprivation of external Cho and/or temperature diminution resulted in lower GroPCho production rates. Under these circumstances, a transfer of label from GroPCho to PtdCho was clearly observed, indicating that the labeled Cho moiety of GroPCho was reused for PtdCho biosynthesis. It was known...
that Cho molecules produced via Spo14p activity are reused for PtdCho biosynthesis (3), and our data support a role for a second pathway whereby accumulated intracellular GroPCho is not excreted from the cells but instead is recycled back into PtdCho by YPL110c and YPL206c. Our new observations extend our knowledge of how intracellular Cho molecules are rerouted from intact PtdCho into the CDP-Chol pathway.

We observed that a small amount of [14C]Cho label associated with GroPCho was transferred to PtdCho in ypl110cΔ ypl206cΔ yeast cells. This indicates that these two ORFs do not constitute the entire complement that facilitates GroPCho reutilization for PtdCho synthesis. Another hypothetical and unpredicted protein could fulfill a role of intracellular glycerophosphodiester phosphodiesterase, producing free Cho and glycerol-3 phosphate from GroPCho, and indeed examples of completely unrelated polypeptides that catalyze the same molecular transformation are not scarce. Alternatively, a glycerophosphodiester phosphodiesterase activity toward the other ester bond of GroPCho producing glycerol and P-Chol might exist. Recently, a mammalian member of the nucleotide pyrophosphatase/phosphodiesterase family was shown to possess such hydrolytic activity against GroPCho (26).

However, we have yet to find buffer conditions whereby the hydrolysis of GroPCho can be observed in vitro in yeast extracts or for purified YPL110c- or YPL206c-encoded proteins. The enzymology of GroPCho metabolism will require further investigation.

Ntl1p is an integral membrane phospholipase localized at the endoplasmic reticulum and exhibits remarkable substrate specificity: it deacylates PtdCho derived from the CDP-Chol pathway but not from the methylthion pathway. Furthermore, its activity is stimulated under conditions of increased CDP-Chol-derived PtdCho biosynthesis via the provision of exogenous Cho and/or elevated temperature. Our data revealed that intracellular GroPCho pool size decreased when yeast cells were cultured at 37 °C in the absence of exogenous Cho due to uneven rates of GroPCho production and consumption. We cannot rule out the possibility that Cho or some Cho-containing metabolite could exert a negative effect over the rate of GroPCho consumption, but we favor the notion that a high rate of GroPCho formation is reached upon temperature elevation, provided that sustained CDP-Chol derived PtdCho biosynthesis is occurring.

Glycerophosphodiester phosphodiesterase activities have been characterized from several sources (20–23), and genomic analyses reveal a wide distribution of this protein family from bacteria and Archaea to metazoans, plants, and fungi. S. cerevisiae YPL110c and YPL206c ORFs code for glycerophosphodiester phosphodiesterase domain-containing polypeptides. YPL110c ORF codes for a 138-kDa soluble product that, in addition to the glycerophosphodiester phosphodiesterase domain localized at the C terminus, also contains an SPX N-terminal domain along with several ankyrin repeats. The SPX domain is named after the yeast proteins Sgg1p and Pho81p and the mammalian XPR1 protein (xenotropic and polytropic retrovirus receptor). This domain is characteristically localized at the N terminus of proteins, but its function is not known. Pho81p is a cyclin-dependent kinase inhibitor that modulates the activity of the cyclin-kinase complex Pho80p–Pho85p in response to phosphate availability (27, 28). Interestingly, Pho81p and the YPL110c product share domain architecture beyond the N-terminal SPX domain, since both proteins possess ankyrin repeats following the SPX domain. This implies that YPL110c may play a role in maintaining cellular phosphate levels. Indeed, the evidence linking YPL110c to phosphate metabolism can be extended, since Pho91p, Pho90p, Pho87p, Vtc2p, Vtc3p, and Vtc4p also contain an SPX domain, and their functions are related with phosphate metabolism (29, 30). It was reported that VTC2, VTC3, and VTC4 as well as YPL110c expression is regulated by phosphate (29). Accordingly, using a tagged allele of YPL110c, we detected by Western blotting a significant increase in YPL110c expression when yeast cells were grown under low phosphate conditions (data not shown).

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Glycerophosphodiester phosphodiesterase activities have been characterized from several sources (20–23), and genomic analyses reveal a wide distribution of this protein family from bacteria and Archaea to metazoans, plants, and fungi. S. cerevisiae YPL110c and YPL206c ORFs code for glycerophosphodiester phosphodiesterase domain-containing polypeptides. YPL110c ORF codes for a 138-kDa soluble product that, in addition to the glycerophosphodiester phosphodiesterase domain localized at the C terminus, also contains an SPX N-terminal domain along with several ankyrin repeats. The SPX domain is named after the yeast proteins Sgg1p and Pho81p and the mammalian XPR1 protein (xenotropic and polytropic retrovirus receptor). This domain is characteristically localized at the N terminus of proteins, but its function is not known. Pho81p is a cyclin-dependent kinase inhibitor that modulates the activity of the cyclin-kinase complex Pho80p–Pho85p in response to phosphate availability (27, 28). Interestingly, Pho81p and the YPL110c product share domain architecture beyond the N-terminal SPX domain, since both proteins possess ankyrin repeats following the SPX domain. This implies that YPL110c may play a role in maintaining cellular phosphate levels. Indeed, the evidence linking YPL110c to phosphate metabolism can be extended, since Pho91p, Pho90p, Pho87p, Vtc2p, Vtc3p, and Vtc4p also contain an SPX domain, and their functions are related with phosphate metabolism (29, 30). It was reported that VTC2, VTC3, and VTC4 as well as YPL110c expression is regulated by phosphate (29). Accordingly, using a tagged allele of YPL110c, we detected by Western blotting a significant increase in YPL110c expression when yeast cells were grown under low phosphate conditions (data not shown). We also presented substantial in vivo evidence indicating that the YPL110c ORF has a major role in the utilization of GroPCho as a source of phosphate. Growth of ypl110cΔ cells on GroPCho was barely detectable, whereas growth on GroPIns was reduced considerably, in comparison with wild type cells grown on these sources of phosphate. Since growth was indistinguishable from wild type on PO4, this indicates severely impaired metabolism of GroPCho and reduced metabolism of GroPIns in ypl110cΔ cells. This is supported by our in vivo analysis of intracellular GroPCho metabolism that clearly indicated that the YPL110c gene product functions as the prominent mediator of GroPCho to PtdCho metabolism. The rate of radioactivity transfer from GroPCho to PtdCho observed at 25 °C in the presence of Cho for ypl110cΔ strain was very low compared with that observed for wild type cells.

The YPL206c ORF codes for a 37-kDa integral membrane protein with a short hydrophobic C-terminal tail appended to the glycerophosphodiester phosphodiesterase domain that may function as a membrane anchor (31, 32). The evidence presented here also suggests that the yeast YPL206c ORF product exhibits glycerophosphodiester phosphodiesterase activity in vivo, although its contribution to the metabolism of GroPCho and GroPIns appears to be far less substantial than that of YPL110c. Analogous to the ypl110cΔ strain, ypl206cΔ cells exhibited a peculiar profile of radioactivity transfer from GroPCho to PtdCho when they were chased in the presence of Cho that indicated a reduced rate of GroPCho consumption compared with wild type cells. Although YPL206cΔ cells grew on GroPIns or GroPCho with rates similar to those of wild type cells, the double mutant ypl110cΔ ypl206cΔ strain grew slower than the single mutant ypl110cΔ. In agreement with the intracellular GroPCho metabolism data, these results indicate that, under the particular conditions analyzed, the YPL110c gene product performs as the major intracellular glycerophosphodiester phosphodiesterase activity, but in its absence a minor role of YPL206c gene product becomes apparent.

Our data also revealed that yeast cells can utilize exogenous GroPCho as a source of phosphate. Git1p has been characterized as a GroPIns transporter and allows for the use of GroPIns as the sole source of phosphate or inositol. Previously, GroPCho was demonstrated to be a poor competitor of GroPIns uptake (24), and in agreement with these results, yeast cells barely grew on 75 μM GroPCho as a source of phosphate, whereas the identical concentration of GroPIns supported robust growth. However, increasing GroPCho concentrations lead to higher growth rates and consequent higher biomass production. The absence of the Git1p transporter completely prevented growth on GroPIns with very marginal growth observed on GroPCho. Taken together, these results indicate that Git1p is a major GroPCho transporter.

In summary, our data demonstrate that intracellular GroPCho is not an inert metabolite, but it is susceptible to further transformations. We observed that under low CDP-Chol-mediated PtdCho biosynthesis, the Cho moiety of GroPCho is reused for PtdCho biosynthesis and that YPL110c and YPL206c are differentially involved in intracellular GroPCho consumption, and accordingly, both genes have different effects on the utilization of GroPIns and GroPCho as alternate phosphate sources.

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