Phosphatidylcholine synthesis in yeast

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Abstract The two pathways for the biosynthesis of phosphatidylcholine, by way of phosphocholine and by methylation of phosphatidylethanolamine, in wild-type yeast (*Saccharomyces cerevisiae*) and in the yeast mutant GL7 have been compared. The mutant requires for growth a sterol, unsaturated fatty acids, and methionine. The uptake of labeled choline or labeled methionine and their conversion to phosphatidylcholine were determined in both cell types. The activities of the major enzymes for both pathways were assayed in vitro. We find that the methylation pathway is predominant in both wild-type and mutant cells though the overall activity of the choline pathway is lower in the yeast mutant. The methionine analogue ethionine inhibits the growth of the mutant more strongly than growth of wild-type yeast. Ethionine, while a powerful inhibitor of phosphatidylcholine synthesis by methylation, stimulates the choline pathway in both cell types. — **Chin, J., and K. Bloch.** Phosphatidylcholine synthesis in yeast. *J. Lipid Res.* 1988. **29:** 9–14.

Supplementary key words CDP-choline • phosphatidylethanolamine N-methylation • ethionine

Eukaryotic cells synthesize phosphatidylcholine (PC) by two independent routes. The pathway starting with choline and leading to phosphatidylcholine by way of CDP-choline was discovered by Kennedy and Weiss (1). In the alternative or methylation pathway, phosphatidylcholine arises by three successive transfers of methyl-groups from S-adenosylmethionine to phosphatidylethanolamine (PE), as shown first by Bremer and Greenberg (2). The relative contribution of the two pathways to PC synthesis varies among organisms and in higher eukaryotes from one tissue to another. In animal tissues, the pathway starting with choline predominates (3) whereas in yeast PC may be entirely derived from the methylation of PE (4).

We have recently shown that in the yeast mutant GL7, a strain that requires exogenous sterol for growth, certain reactions of phospholipid biosynthesis, e.g., of PC and phosphatidylinositol (PI), are stimulated by ergosterol (5). The question therefore arose whether one or both of the alternate routes to PC are sterol-controlled. We had already shown that in GL7 cells raised on ergosterol, PE–PC transmethylation occurs at a faster rate than in mutant cells supplied with cholesterol (6). Here we describe experiments comparing the rates of the two pathways for PC synthesis in the mutant with the corresponding rates in wild-type yeast.[2]

MATERIALS AND METHODS

Materials

The *Saccharomyces cerevisiae* strain GL7 requiring for growth methionine, oleic acid, and sterol (erg 12 heme 3), was originally obtained from D. B. Sprinson (7) and the parental wild-type was from H. Greer. Most of the chemicals, reagents, and lipids were from Sigma and the radioactive compounds were from New England Nuclear. The specific activities were 80 Ci/mmol for [^{3}H]choline and 200 mCi/mmol for [^{3}H]methionine.

Growth conditions

Yeasts, either wild-type or mutant, were grown at 30°C on a rotary shaker in liquid minimal media supplemented with 1 μg of ergosterol, 50 μg of oleic acid, and 20 μg of methionine per ml, as previously described (5, 6). Growth was monitored by measuring absorbance at 540 nm.

Methionine uptake and conversion

Yeast cells were grown to log phase (A_{540} = 0.6–0.8) and, after harvest, were washed with methionine-free medium, resuspended in fresh methionine-free medium, and incubated at 30°C for 1 hr. Then [^{3}H]methionine (1–2 μCi/ml) was added to a final concentration of 100–300 μM. After 15 and 30 min at 30°C, the cells were washed three times with 10 volumes of cold saline. Lipids were extracted and analyzed by thin-layer chromatography, as previously described (5, 6), while uptake was measured by counting the amount of labeled methionine associated with an aliquot of the washed whole-cell fraction (5, 8). The results presented are those from a typical experiment and the data given are averages of duplicate assays which differed by less than 6%. All experiments were done at least twice.

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; WT, wild-type yeast; GL7, mutant yeast; DZA, deazaadenosine.

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[2]Preliminary experiments indicate that the several activities of the choline pathway to PC are the same whether the cells are grown on ergosterol or cholesterol.

Choline uptake and conversion

The methods were identical to those used for determining methionine uptake and conversion except that incubations were for 5 and 10 min. The label was [^{3}H]choline (1-2 μCi/ml) at a final concentration of 100-300 μM. The organic phase was assayed for conversion of labeled choline to PC while the aqueous phase was analyzed for the water-soluble intermediates of the choline pathway (8-10). The activities of the partial reactions of the choline pathway were assayed according to published procedures: choline kinase (11), CTP: choline phosphate cytidylyltransferase (12), and CDP-choline:1,2-diacylglycerol choline phosphotransferase (13).

Ethionine inhibition of the methylation pathway

The methionine analog ethionine (14, 15) was added to the growth medium at the following final concentrations: 1, 2, 5, and 10 mM, all in the presence of 20 μg of methionine per ml of medium. Cells were washed with methionine-free media containing ethionine and used for measuring [^{3}H]choline or [^{3}H]methionine uptake and conversion, as described above except that ethionine was also present.

RESULTS AND DISCUSSION

To assess the relative significance of the choline-nucleotide pathway and PE-methylation for PC synthesis, we first determined the uptake of [^{3}H]choline and [^{3}H]methionine by whole yeast cells, mutant as well as wild-type. It should be noted that GL7, apart from requiring sterol and oleic acid, is also auxotrophic for methionine, but not for choline (7). As shown in **Table 1,** choline uptake on a molar basis was about half in mutant as compared to wild-type cells, while methionine uptake was somewhat greater in the mutant than in wild-type yeast, reflecting perhaps the absence of methionine synthesis in GL7. When the same set of cells was analyzed for conversion of labeled precursor to phospholipid, the specific activity of the methylation pathway to PC in the mutant was more than 20 times greater than for PC formation from choline, while in wild-type cells the utilization of methionine exceeded that of choline about fourfold. These results are consistent with the data showing a greater uptake of methionine than choline both in mutant and wild-type cells. In order to identify potentially rate-limiting reactions in the choline conversion to PC, we next assayed relevant enzyme activities in the various cell fractions of both mutant and wild-type cells (**Table 2**). High choline kinase activity was found in the cytosol of both cell types, and was actually somewhat greater in mutant than normal cells. CTP: choline phosphate cytidylyltransferase, thought to be the rate-determining enzyme of the choline pathway in rat liver (3), had an activity almost fourfold lower in the mutant than in wild-type cells. In fact the mutant

TABLE 1. Choline and methionine uptake and conversion to phospholipid in mutant and wild-type cells

	GL7	WT
	nmol · 10 min^{-1} · mg^{-1}	
[^{3}H]Choline uptake	1.34	2.48
Chloroform-methanol extract	0.40	1.40
Conversion to PC	0.09	0.38
[^{3}H]Methionine uptake	32.39	24.53
Chloroform-methanol extract	9.10	9.20
Conversion to PC	2.55	1.71

Cells were incubated with either [^{3}H]choline for 5 and 10 min or [^{3}H]methionine for 15 and 30 min, each at a final concentration of 150μM, harvested, and extracted as described in Materials and Methods. Uptake of labeled substrate was linear during the indicated incubation periods. The results presented are those from a typical experiment and the data given are averages of duplicate assays which differed by less than 6%.

TABLE 2. Enzyme activities of the choline pathway and of the methylation pathway

Enzyme Activity	GL7	WT	WT / GL7
	nmol · min^{-1} · mg^{-1}		
Choline pathway			
Choline kinase	1.33	0.81	1.1-1.6 (3)
CTP:choline phosphate cytidylyltransferase	0.15 ± 0.01	0.61 ± 0.08	2.7-4.0 (3)
CTP-choline:1,2-diacylcholine phosphatransferase	0.27	0.45	1.2-1.6 (2)
Methylation pathway			
Phosphatidylethanolamine N-methyltransferase	0.28	0.35	1.1-1.3 (3)

Cells were grown to log phase, washed, and broken with glass beads as previously described (5, 6). The high-speed supernatant was assayed for choline kinase (11) and the high-speed pellet was assayed for all other enzyme activities (6, 12, 13). The results given above for all activities except for cytidylyltransferase are averages of duplicates differing by less than 6%. The cytidylyltransferase was assayed in triplicate and the data presented are the means ± standard error. The last column, the ratios of activities for WT and GL7, summarizes the results for yeast cultures grown at different times. In parentheses are the numbers of individual experiments.

cytidylyltransferase was the least active of the three component enzymes. Cytidylyltransferase, rather than choline uptake, therefore appears to be rate-controlling for PC formation in the yeast mutant GL7 as it is in animal tissues (3). It is not clear from our data, however, which enzyme is rate-controlling in wild-type yeast.

In addition to determining the uptake and conversion of [^{3}H]choline to PC and assaying the respective enzyme activities, we also measured the concentrations of the various choline-derived water-soluble intermediates (**Fig. 1**). Choline phosphate accumulation is comparable to that or somewhat higher in mutant than wild-type cells (Fig. 1, A and B), consistent with the respective choline kinase activities (Table 2). In the mutant essentially no CDP-choline accumulates in contrast to the substantial amounts of this intermediate in wild type cells (Fig. 1, C and D). Since the mutant contains measurable if low cytidylyltransferase activity (Table 2), the failure of product to accumulate can be attributed to its rapid conversion to PC by CDP-choline:1,2-diacylglycerol choline phosphotransferase. The overall result, a fourfold lower production of PC in the mutant than in wild-type cells (Fig. 1, E and F), is then attributable to a diminished cytidylyltransferase activity.

We next investigated how critically the mutant depends on the operation of the methylation pathway for PC synthesis. The S-adenosylmethionine analog deazaadenosine (DZA), an inhibitor of PE methylation in higher eukaryotes (16), had no effect on the growth of either mutant or wild-type cells even at high concentrations (250 μM) nor was it an inhibitor of in vitro PE–PC methyltransferase activity (data not shown). Recently one other example of a DZA-insensitive methylation of PE has been described (17). On the other hand, as shown in **Fig. 2** the methionine analog ethionine (14, 15), at 10 mM, substantially slowed the growth of GL7 (75%) whereas the growth of wild-type yeast was only slightly affected (17%). In the presence of methionine this inhibition lasts for about 20 hr, whereas after 25 hr the ethionine-treated mutant cells appear to recover, ultimately reaching the same cell density as untreated cells. Cells grown for 20 hr in the presence or absence of ethionine were harvested, washed, and incubated with [^{3}H]methionine in the presence or absence of ethionine. As expected, the methionine antagonist had a profound effect on PC synthesis. Ethionine-treated GL7 cells produced only about 5% as much labeled PC as untreated cells (**Fig. 3A**) while in wild-type cells methionine incorporation into PC was lowered to 10% of the value in control cells. In addition, the uptake of methionine was decreased about sixfold from 28.2 to 4.7 nmol · min^{-1} · mg^{-1} in GL7 and about fourfold from 22.0 to 5.5 nmol · min^{-1} · mg^{-1} in WT. From the effects of ethionine on cell growth and phospholipid synthesis it seems clear that wild-type yeast withstands interference with the

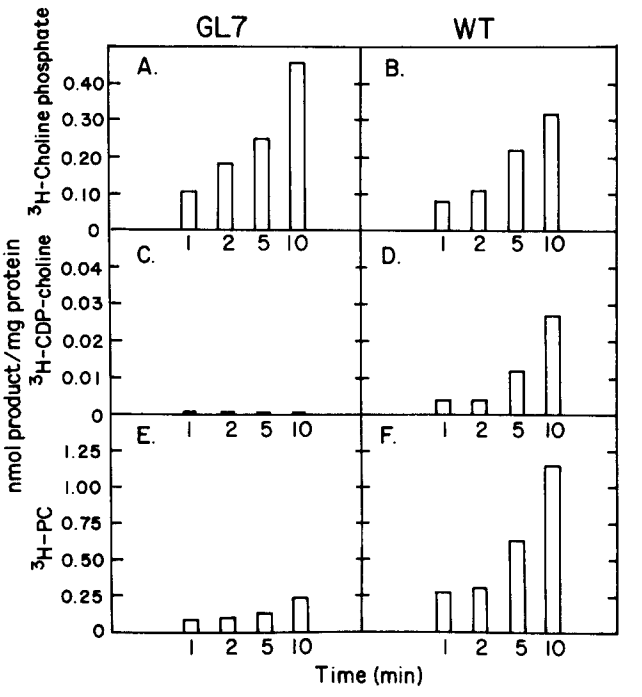

Fig. 1. Formation of phosphatidylcholine and intermediates from [^{3}H]choline by mutant and wild-type yeast. Log phase cells (A_{540} = 0.6–0.8), were washed with and resuspended in 10% of the original culture volume with methionine-free media. After incubation at 30°C for 1 hr, [^{3}H]choline (1–2 μCi/ml) was added to a final concentration of 300 μM and duplicate samples were incubated for the times indicated. The cell suspensions were diluted and washed three times with cold saline and then extracted with chloroform–methanol as described in Materials and Methods.

methylation pathway to PC much better than the mutant GL7 which, as pointed out, is a methionine auxotroph. Conceivably, wild-type yeast, unlike the mutant, can compensate for ethionine inhibition by overproducing methionine. The data in Fig. 3 also indicate that normally, i.e., in the absence of inhibitor, PC synthesis by PE methylation occurs somewhat more rapidly in the mutant than in wild-type yeast.

In comparable experiments with [^{3}H]choline as a PC precursor, the effect of ethionine was the reverse of that observed with [^{3}H]methionine (**Fig. 4**). Ethionine exposure during growth for 20 hr in methionine-containing media stimulated choline uptake about fivefold, from 1.2 to 6.2 nmol · min^{-1} · mg^{-1}, in GL7 and about fourfold, from 2.6 to 10.4 nmol · min^{-1} · mg^{-1}, in WT, and conversion to PC in mutant cells fourfold and by about twofold in wild-type cells. Clearly the yeast cells respond to the selective inhibition of one of the two PC biosynthetic pathways by stimulating the other. This suggests—but does not prove—that the PC species produced by the two independent pathways are functionally identical.

Inspection of the data in Figs. 3 and 4 shows that, on a molar basis and adjusted for a 10-min incubation period, methionine uptake and incorporation into PC is

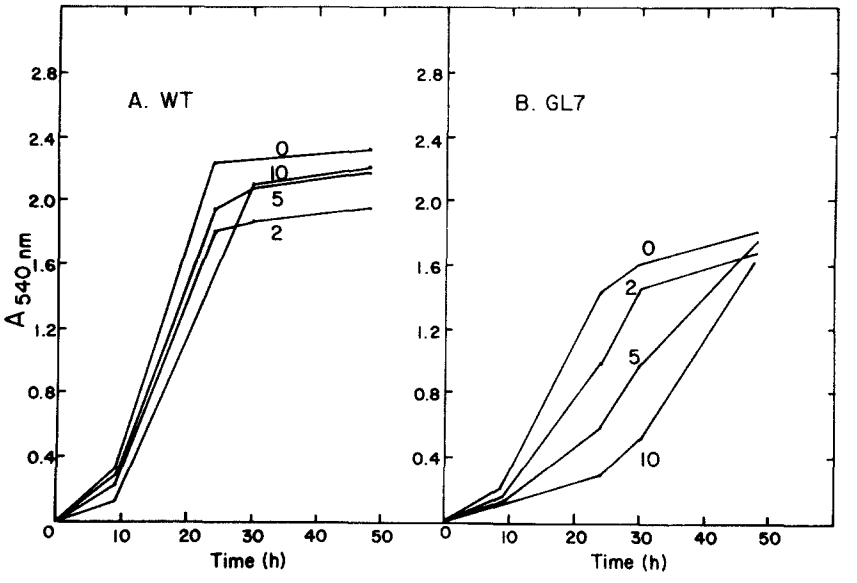

Fig. 2. Growth of wild-type and mutant yeast in media containing methionine and in the presence or absence of ethionine (2, 5, or 10 mM). To monitor growth, duplicate aliquots were removed at the indicated time points; averages which differed by less than 6% are presented.

substantially greater than choline utilization for PC synthesis, by a factor of 25-fold for the mutant and 4-fold for wild-type cells. The same conclusion had been reached earlier in the discussion of the data shown in Table 1. Thus, while PE methylation is the major source of PC in yeast, its share is significantly greater in the methionine auxotroph GL7 than in normal cells. Conceivably, this methionine requirement of GL7 may serve to suppress the choline pathway, as suggested by the present finding that the methionine antagonist, ethionine, appears to derepress choline utilization for PC synthesis.

The mode of regulation of the methylation pathway to PC and its ramification has attracted wide attention (3, 16–19). Thus, coordinate control of several phospholipid biosynthetic reactions in yeast is indicated by the finding that in yeast mutants PE methylation is inhibited by choline (20) and more effectively by choline and inositol (20–22).

Reciprocally, there is evidence for the regulation of the choline pathway by methionine in animal tissues (3, 23). For example, in rat liver and hepatocytes the methylation inhibitor, DZA, stimulates PC synthesis by the choline pathway (24).

Multiple pathways for the synthesis of a given phospholipid in the same organism are not uncommon but have so far remained without a physiological rationale. Benefits could conceivably accrue to the cell if one or the other pathway generated essential intermediates for the production of cell constituents not available from the alternate pathway. For phosphatidylcholine synthesis this explanation seems to be ruled out. None of the intermediates in the choline pathway serve purposes other than to provide precursors for PC. In the alternate route, neither monomethyl- nor dimethyl-derivatives of PE are known to be diverted into different routes. Perhaps evolutionary diver-

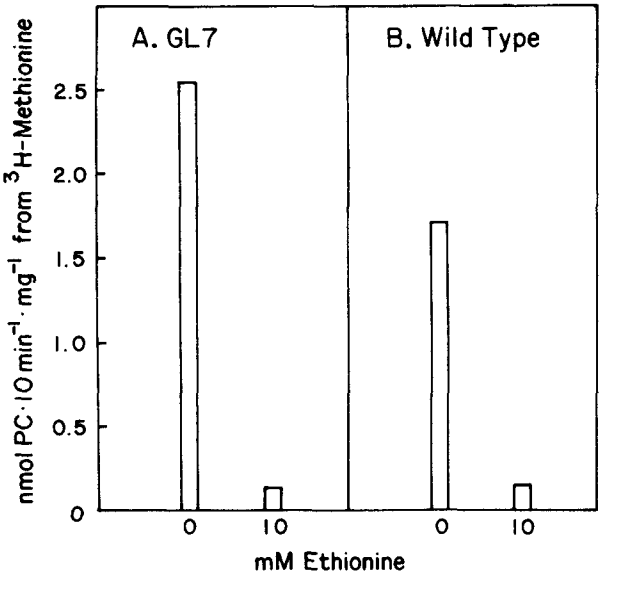

Fig. 3. Effect of ethionine on the utilization of [^{3}H]methionine for [^{3}H]phosphatidylcholine synthesis. As in Fig. 2, cells were grown in the presence or absence of ethionine (10 mM) for 20 hr and then washed with fresh methionine-free medium. These cells were first preincubated at 30°C for 1 hr in methionine-free medium and then incubated with [^{3}H]methionine (150 μM) for 15 and 30 min. Cells were harvested and analyzed in duplicate for ^{3}H-labeled PC formation.

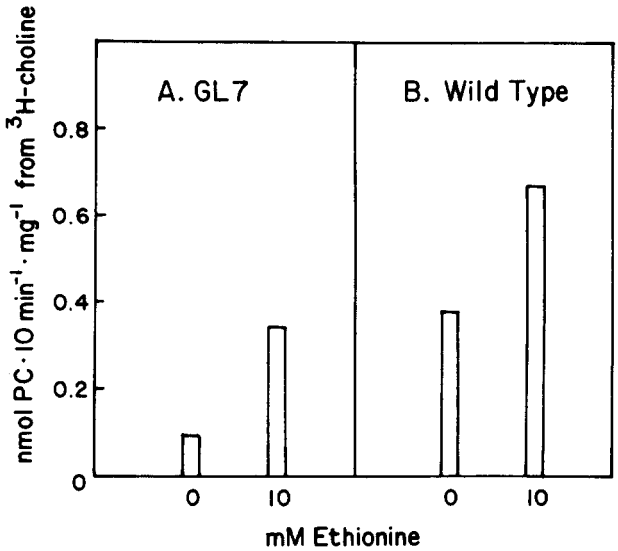

Fig. 4. Conversion of [^{3}H]choline to [^{3}H]phosphatidylcholine in the presence or absence of ethionine. The experimental conditions were the same as described in Fig. 3 except that [^{3}H]choline (150 μM) was the labeled substrate and incubation times were either 5 or 10 min. The specific activities shown are for 10-min periods.

sity provides a more plausible explanation. According to surveys of prokaryotic phospholipid biochemistry, phosphatidylcholine occurs only rarely in bacterial species (25), primarily in organisms that have intracellular lamellae and vesicular membranes (26). Whenever this is the case, monomethyl PE and dimethyl PE also accumulate, usually in amounts excceding those of PC. Also, a number of bacteria contain only the mono- and dimethyl derivatives of PE but no PC at all, in contrast to eukaryotic cells (26).

It therefore appears that the PE-PC methylation pathway evolved in steps and proceeded to completion only in a few bacterial species. The existence in bacteria of the alternate pathway by way of phosphocholine has not been reported, perhaps because it has not been systematically investigated. The possibility therefore exists that PE-PC transmethylation was the earlier of the two pathways to have evolved.

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