Studies Employing Saccharomyces cerevisiae cpt1 and ept1 Null Mutants Implicate the CPT1 Gene in Coordinate Regulation of Phospholipid Biosynthesis*

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**The Saccharomyces cerevisiae CPT1 and EPT1 genes are structural genes encoding sn-1,2-diacylglycerol choline phosphotransferase and sn-1,2-diaclylglycerol choline/ethanolamine phosphotransferase, respectively. Incorporation of $^{32}$P into phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine in wild type and ept1 strains was decreased in the presence of exogenous inositol. In contrast, inositol did not affect $^{32}$P incorporation into phosphatidylcholine in ept1 or ept1cpt1 strains. In membranes isolated from wild type and ept1 strains grown in the presence of inositol or inositol/choline, the CPT1-derived cholinephosphotransferase activities were reduced 40-50 and 85%, respectively. Inositol-dependent reductions in CPT1-derived cholinephosphotransferase activity correlated with transcript levels in both wild type and ept backgrounds. The ethanolaminephosphotransferase activity of the EPT1 gene product in wild type cells was reduced 40% by exogenous inositol alone and 50% by inositol/choline. In the ept1 strain, however, the ethanolaminephosphotransferase activity was unaffected by exogenous inositol or inositol/choline. The inositol-dependent reduction of ethanolaminephosphotransferase activity observed in wild type cells correlated with reduced levels of EPT1 transcripts; in the ept1 strain, EPT1 transcript levels were not affected by inositol. These results indicate that 1) a functional CPT1 gene or gene product is required for inositol-dependent regulation of phospholipid synthesis; 2) the enzyme activities of both the CPT1 and EPT1 gene products are repressed by inositol and inositol/choline, and require an intact CPT1 gene; 3) inositol mediates its regulatory effects on phospholipid synthesis via a transcriptional mechanism.

Phosphatidylcholine (PC)$^1$ and phosphatidylethanolamine (PE) comprise greater than 50% of eukaryotic cell membrane phospholipids (1, 2) and thus play major roles in the maintenance of membrane structure and function. In mammalian cells PC and PE are synthesized from sn-1,2-diacylglycerol and CDP-choline or CDP-ethanolamine, respectively, in aminophospholipidphosphotransferase reactions commonly referred to as the Kennedy pathway (Fig. 1)(3). In certain cell types, most notably liver, PC can also be synthesized via methylation of PE (1). In the yeast Saccharomyces cerevisiae, the predominant route for PC synthesis is thought to be from phosphatidylserine (PS) (2). In the PS-dependent pathway, PS, synthesized from CDP-diacylglycerol and serine by PS synthase, is decarboxylated to PE, which, in turn, is methylated to PC (Fig. 1). However, the contributions of the Kennedy pathway to overall synthesis of PC and PE in yeast is not well understood. Recently, it has been shown that mutations in the Kennedy pathway of PC synthesis are able to bypass mutations in SEC14 (4), a gene encoding a Golgi-associated yeast PC/PI transfer protein (5). This observation, suggests the possibility that the Kennedy pathway of PC synthesis may play an important role in the coupling of phospholipid synthesis and the protein secretory pathway.

Although little is known on the coordinate regulation of phospholipid synthesis in mammalian cells, considerable understanding of the regulation of phospholipid biosynthesis in yeast has been achieved (reviewed in Ref. 6). This regulation is mediated by the availability of the water-soluble phospholipid precursors choline and inositol. A number of yeast phospholipid biosynthetic enzymes, including CDP-diacylglycerol synthase (7), PS synthase (8), PS decarboxylase (9, 10), the PE N-methyltransferases (11, 12), and choline kinase (13) are repressed in cells grown in the presence of inositol. Inositol-1-phosphate synthase, which catalyzes the first step in PI biosynthesis, is also repressed by inositol (14). Addition of choline to medium containing inositol results in a further repression of these activities. The structural genes for PS synthase (CHO1), choline kinase (CK1), inositol-1-phosphate synthase (INO1), and the PE N-methyltransferases (CHO2/PEM1) and OPZ3/PEM2 have been cloned, and the observed repression of these enzymes by inositol and inositol/choline was shown to occur at the level of mRNA abundance (13, 15, 16).

Two classes of regulatory mutants have also been identified. The ino2 and ino4 mutants (inositol auxotrophs) show constitutive repression of the activities that are regulated in response to inositol and choline in wild type cells (15-17). In contrast, op1 mutants (7, 8, 15, 16) constitutively express these same activities and display an inositol excretion phenotype. It has been observed that cells which are decreased in the synthesis of PC are not regulated by inositol and exhibit an op1 phenotype (6, 18), suggesting that ongoing PC synthesis is required in the coordinate regulation of phospholipid metabolism mediated by inositol.

Our laboratory has cloned two yeast structural genes for sn-1,2-diacylglycerolaminolcocholophosphotransferases: the CPT1 gene which encodes a cholinephosphotransferase (19) and the EPT1 gene whose product possesses both choline- and ethanolaminephosphotransferase activities (20). Strains bearing ept1 and ept1 null mutations as well as a ept1 ept1 double null mutant (21) were constructed by chromosomal disruption. In the present work, these strains were employed to investigate the roles of the CPT1 and EPT1 genes in yeast phospholipid synthesis, to evaluate the effect of exogenous inositol and choline on the expression of their gene products, and to explore the**

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$^1$The abbreviations used are: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; MOPS, 3-(N-morpholino)propanesulfonic acid; dC18:1, dioleoylglycerol; diC16:1, dipalmityloylglycerol; kb, kilobase; TLC, thin layer chromatography.
consequences of mutations in the Kennedy pathway on the changes in phospholipid metabolism induced by inositol.

**EXPERIMENTAL PROCEDURES**

**Materials—** 32P, [γ-32P]ATP, and [α-32P]dCTP were purchased from DuPont NEN. [32P]CDP-choline and [32P]CDP-ethanolamine were synthesized as described previously (19). Safety-Solv was from Research Products International. Amino acids, myo-inositol, phenol, salmon sperm DNA, phospholipase C (Bacillus cereus), bovine serum albumin, choline chloride, and MOPS were purchased from Sigma. Diphosphatidylcholine and dipalmitylophosphatidylcholine were obtained from Avanti Polar Lipids, Inc.; the corresponding diacylglycerols were prepared from them by phospholipase C digestion (22). Triton X-100 was obtained from Pierce. Oligo(dT)-cellulose was purchased from Collaborative Research, Inc. Zeta-probe cationic membranes, SDS, and agarose were from Bio-Rad. Restriction enzymes, RNA ladders, formamide, and DNA polymerase I (Klenow fragment) were obtained from Life Technologies, Inc. Materials for growth media were purchased from Difco.

**Strains, Media, and Growth Conditions—** The haploid S. cerevisiae strain DBY746 (a his3-Δ1 leu2-3 leu2-112 ura3-52 trpl-1 trp2-399 ept1::LEU2) was obtained from the Yeast Genetic Stock Center. Strain HJ001 (a his3-Δ1 leu2-3 leu2-112 ura3-52 trp1-1 trp2-399 ept1::LEU2) contains an insertional mutation in the CPT1 locus introduced into DBY746 by gene disruption (20). Strain HJ000 (a his3-Δ1 leu2-3 leu2-112 ura3-52 trp1-1 trp2-399 ept1::LEU2 ept1-1::URA3) was constructed by integrative transformation of the ept1::LEU2 insertional mutation into the chromosomal CPT1 locus of strain HJ001 (21). Both the ept1::LEU2 and the ept1::URA3 mutations were used to exhibit the effects of mutants in the corresponding gene products (19, 20).

**Isolation of Membranes—** Yeast cultures (500 ml) were grown in minimal medium in the absence or presence of 1 mg choline or 50 μM inositol or both to mid-logarithmic phase and harvested as described previously (19). Following glass bead disruption, the homogenate was removed, and the membranes were rinsed three times with 0.5 ml of GME with 0.2 ml: 50 mM MOPS-NaOH, pH 7.5, 20 mM MgCl2, 0.45% Triton X-100 (6.5 mM), 10 mol % dioleyl-PC, 10-20 μg of membrane protein, and either 5 mol % dioleoylglycerol and 100 μM [32P]CDP-ethanolamine (20 μCi/mmole) for ethanolaminophosphotransferase activity or 5 mol % dipalmitoylglycerol and 250 μM [32P]CDP-choline (20 μCi/mmole) for cholinephosphotransferase activity.

**Effect of Mutations in CPT1 and EPT1 on Phospholipid Metabolism**

FIG. 1. **Pathways of glycerophospholipid biosynthesis in eukaryotic organisms.** The biosynthetic pathways for synthesis of the major phospholipids PC, PE, PS, and PI are shown. The enzymes catalyzing these reactions are: 1, choline kinase; 2, CTP:phosphocholine cytidylyltransferase; 3, CDP-choline:1,2-DAG cholinephospho-transferase; 4, phospatidic acid phosphohydrolase; 5, ethanolamine kinase; 6, CTP:phosphocholine cytidylyltransferase; 7, CDP-ethanolamine:1,2-DAG ethanolaminophosphotransferase; 8, PS synthase; 9, PS decarboxylase; 10, PE N-methyltransferase; 11, phosphatidylethanolamine synthase; 12, CDP-DAG synthase; 13, inositol-1-phosphate synthase; 14, PI synthase. These pathways are similar in mammalian cells and yeast with the exception that mammalian cells lack PS synthase and synthesize PS by a base-exchange reaction between phosphatidylethanolamine and an existing phospholipid.

![Pathways of glycerophospholipid biosynthesis in eukaryotic organisms](image-url)
**Effect of Mutations in CPT1 and EPT1 on Phospholipid Metabolism**

**Table I**

| Strain          | Growth condition | PtdCho | PtdSer | PtdIns | PtdEtn | Other* |
|-----------------|------------------|--------|--------|--------|--------|--------|
| Wild type       | No addition      | 37.4±4.4 | 10.7±5.0 | 11.6±5.7 | 20.5±4.4 | 21.7±9.5 |
| (DBY746)        | + choline        | 35.7±4.8 | 9.9±0.6  | 21.4±1.2 | 19.1±1.9 | 17.8±5.4 |
|                 | + inositol       | 32.6±4.9 | 9.1±1.2  | 21.4±1.4 | 24.3±1.2 | 12.3±2.4 |
|                 | + choline/inositol| 33.2±2.4 | 9.0±1.8  | 21.2±1.4 | 23.2±3.4 | 16.4±3.4 |
|                 | No addition      | 36.2±4.9 | 10.1±1.9 | 13.7±1.5 | 23.2±3.4 | 16.4±3.4 |
| cpt1            | (HJO01)          |        |        |        |        |        |
|                 | + choline        | 36.0±4.4 | 11.6±0.1 | 11.9±5.6 | 23.0±3.2 | 17.4±1.1 |
|                 | + inositol       | 28.7±5.8 | 4.6±4.4  | 21.6±3.7 | 23.4±5.6 | 23.6±9.6 |
|                 | + choline/inositol| 29.2±4.0 | 4.4±4.0  | 27.2±3.6 | 21.5±2.7 | 17.5±2.8 |
|                 | No addition      | 42.0±2.2 | 11.8±1.7 | 11.6±3.0 | 19.7±1.7 | 17.7±4.3 |
| ept1            | (HJO51)          |        |        |        |        |        |
|                 | + choline        | 44.0±5.3 | 9.7±3.8  | 9.5±3.7  | 19.0±1.6 | 20.5±2.6 |
|                 | + inositol       | 34.7±3.0 | 9.2±2.1  | 21.6±3.3 | 18.8±3.3 | 15.6±6.7 |
|                 | + choline/inositol| 34.3±1.5 | 9.8±0.5  | 23.1±2.7 | 20.5±0.6 | 12.3±3.1 |
|                 | No addition      | 38.3±3.9 | 12.0±1.0 | 15.0±0.0 | 19.4±4.0 | 15.2±4.4 |
| cptleptl        | (HJO00)          |        |        |        |        |        |
|                 | + choline        | 35.5±8.6 | 10.2±1.1 | 13.6±1.4 | 20.4±5.9 | 14.6±1.9 |
|                 | + inositol       | 32.1±3.2 | 8.5±1.6  | 26.5±5.7 | 20.4±2.3 | 12.7±3.6 |
|                 | + choline/inositol| 31.7±4.1 | 8.3±4.1  | 20.0±1.2 | 20.9±3.4 | 19.1±9.4 |

*Includes phosphatidylglycerol, phosphatidic acid and polar lipids remaining at or near the origin.

**Isolation and Northern Analysis of RNA**—Yeast cultures (500 ml) were grown to mid-logarithmic phase as described above. Total cellular RNA was isolated by glass bead disruption followed by hot phenol extraction as described by McAlister and Finkelstein (26). Poly(A)* RNA was isolated by oligo(dT)-cellulose chromatography according to the manufacturer's directions. RNA was quantitated by measuring absorbances at 260 nm, using an extinction coefficient of 40 μg/ml (27), and stored in water at −70 °C.

Poly(A)* RNA (3−6 μg) was electrophoresed through a 1% agarose, 7% formaldehyde denaturing gel containing 20 mM MOPS, pH 7.4, 5 mM sodium acetate, and 1 mM EDTA (27). RNA was transferred to a Zeta-probe membrane by electrophoretic transfer following the manufacturer’s suggested protocol using a Trans-Blot (Bio-Rad) electrophoretic transfer system and UV cross-linked using Stratagene's UV Stratalinker.

DNA probes were radiolabeled to high specific activity (10⁵ counts/min/ng) with ³²P-dCTP by the oligolabeling method (28, 29) and used at 2−5 x 10⁵ counts/min/ng of hybridization solution. Prehybridization and hybridization of radiolabeled probes were performed at 42 °C in 50% formamide, 6 x SSC (0.9 M NaCl, 0.09 M trisodium citrate), 5 x Denhardt's (0.1% bovine serum albumin, 0.1% polyvinylpyrrolidone, 0.1% Ficoll), 0.5% SDS, and 0.4 μg/ml of salmon sperm DNA (27). Following hybridization, membranes were washed for 15 min each with 2 x SSC, 0.1% SDS and 0.5 x SSC, 0.1% SDS at room temperature and with 0.1 x SSC, 0.1% SDS at 65 °C. Washed membranes were exposed to Kodak X-Omat AR film at −70 °C with an intensifying screen. Membranes were stripped prior to rehybridization by heating at 95 °C in an excess of 0.1 x SSC, 0.5% SDS for 20 min. RNA was quantitated using a LKB UltraScan XL laser densitometer. Only measurements which were within the linear limits of the film and densitometer were used.

**RESULTS AND DISCUSSION**

**Equilibrium-labeling Studies**—To investigate the roles of the CPT1 and EPT1 gene products in phospholipid metabolism, the growth characteristics and phospholipid composition of mutant strains bearing null mutations in the CPT1 and EPT1 loci were compared to the parental wild type strain. The response of cpt1 and ept1 mutants to the presence of choline (1 mM) and inositol (50 μM) in the growth medium was also assessed. The growth characteristics of all strains were similar (doubling time of 3 h) and were unaffected by addition of choline or inositol.

Phospholipid compositions were determined by labeling to constant specific activity with ³²P (0.4 μCi/mm;mol) for a minimum of five generations. Under these conditions, the specific activity of [³²P]phospholipid was constant at 88±30 counts/min/mmol phospholipid phosphate for all strains regardless of media additions. These results indicate mutations in cpt1 and ept1 have no effect on overall cell growth or amount of total phospholipid.

To assess the effect of cpt1 and ept1 mutations on phospholipid class distribution, phospholipids were separated by two-dimensional thin layer chromatography and the percentage of label in each phospholipid determined (Table I). No major differences in phospholipid compositions were observed between wild type and mutant strains, indicating any perturbations in phospholipid synthesis resulting from mutations in the CPT1 and EPT1 loci are largely compensated in the steady-state. However, some significant, albeit small changes, in phospholipid composition were observed between strains (Table I).

PC levels were higher in the ept1 mutant compared to other strains but were similar to wild-type in the presence of inositol. This increase was not observed in the double null mutant in which the synthesis of PC was also compromised, suggesting higher levels of PC in the ept1 strain may result from an increase in availability for the CPT1 gene product of the common substrate, sn-1,2-diacylglycerol. Consistent with this observation is the recent findings of this laboratory that have determined that the CPT1 gene product contributes to 95% of PC synthesized via the CDP-choline pathway, while the EPT1 gene product constitutes the remaining 5% (30). Inositol supplementation resulted in increased levels of PI and decreased PC in all strains. PS levels were appreciably reduced in the ept1 strain in the presence of inositol and to a lesser extent in the cpt1 ept1 strain, suggesting alterations in lipid metabolism induced by mutations in ept1 are alleviated by simultaneous mutation in ept1.

**Pulse-labeling Studies**—While the effect of ept1 and ept1 mutations on phospholipid distribution in the steady-state were small, differences observed in the presence of exogenous inositol suggested some alterations in phospholipid regulation in these strains. Pulse-labeling studies with ³²P were undertaken to determine the rate of synthesis of phospholipids in each strain in the absence and presence of the water-soluble precursors, choline and inositol (Fig. 2). In the absence of exogenous precursors or in the presence of choline alone, no differences were observed in the incorporation of ³²P in wild type and ept1 strains during a 1-h labeling period. Under the same conditions, incorporation of label into phospholipids in the cpt1 and cpt1 ept1 mutants was increased slightly over wild type.
presence of exogenous inositol, the total incorporation of label into phospholipids was reduced 2.5-fold in wild type and cpl1 strains. In contrast, label incorporation was not reduced by inositol in either strain bearing a cpl1 null mutation, suggesting that a functional CPT1 gene or gene product is essential to observe this effect of inositol on phospholipid metabolism.

Analysis of individual phospholipids revealed that reduced incorporation of radiolabeled phosphorus in wild type and cpl1 strains in the presence of inositol was primarily attributable to decreased synthesis of PC (Fig. 3). PC synthesis was decreased 65-75% in wild type and cpl1 strains in the presence of inositol. The synthesis of PS and PE were also reduced in these strains in the presence of inositol while the synthesis of PI was relatively unchanged. Exogenous choline stimulated the synthesis of PC in wild type and cpl1 mutants, most likely due to increased flux through the Kennedy pathway of synthesis.

In both strains bearing a cpl1 null mutation, PC synthesis was lower than wild type or cpl1 strains in the absence of exogenous precursors. If PC is synthesized solely by the PS-dependent pathway in the absence of exogenous choline, then no difference between wild type and cpl1 strains would be expected. The observed differences suggest that the Kennedy pathway may contribute significantly to PC synthesis in the absence of exogenous precursors, presumably by utilization of choline derived from turnover of PC synthesized by the PS-dependent pathway. Alternatively, mutations in the CPT1 locus may have pleiotropic effects on the enzymes of the PS-dependent pathway of PC synthesis.

In the cpl1 and cpl1 cpl1p1 strains, neither PE nor PS synthesis was reduced by growth in inositol as was observed for strains possessing an intact CPT1 locus. Inositol supplementation increased PI synthesis 3-fold in the cpl1 strain and 1.7-fold in cpl1 cpl1p1 strain which exhibited higher levels of synthesis in the absence of exogenous inositol. These findings were in contrast to strains lacking a cpl1 mutation where PI synthesis remained constant under the growth conditions examined. The cpl1 cpl1p1 double null strain exhibited higher levels of PS and PI synthesis compared to the other strains under all growth conditions. This increase may reflect a shift in phosphatidic acid metabolism from diacylglycerol synthesis in the absence of functional aminoalcoholphosphotransferases and toward CDP-diacylglycerol synthesis, the common precursor of both PS and PI (Fig. 1).

Results of the pulse-labeling studies indicated dramatic effects of mutations in the CPT1 locus on both the class distribution of phospholipid synthesis and changes in the pattern of synthesis induced by inositol. Taken together, these observations suggest the participation of the CPT1 gene product in PC synthesis independent of the availability of exogenous choline and that a functional CPT1 gene or gene product is required for inositol dependent regulation of phospholipid synthesis.

**Fig. 2. Incorporation of $^{32}P$ into total phospholipid of wild type and mutant strains.** Cells were grown to mid-logarithmic phase in minimal media containing no addition (○), 1 mm choline (□), 50 μM inositol (●), or 1 mM choline and 50 μM inositol (■). Cells were pulse-labeled with $^{32}P$ (0.4 mCi/mmol) for the indicated times and extracted as described under “Experimental Procedures.” Label incorporated into phospholipid was normalized to nanomoles of phospholipid phosphate. Results shown are from one experiment. Analogous results were obtained in two additional experiments.
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FIG. 3. Incorporation of $^{32}$P into individual phospholipids of wild type and mutant strains. Subsequent to a 1-h labeling of cells with $^{32}$P, total phospholipid was separated by TLC and plates were scanned as described under "Experimental Procedures." The percent distribution of label was used to calculate the counts/minute for each phospholipid from the total lipid extractable phosphate. Growth media conditions were as follows: no addition, solid bars; 1 mM choline, shaded bars; 50 μM inositol, hatched bars; 1 mM choline, 50 μM inositol, stippled bars.

In wild type cells, the 1.4-kb EPT1-derived transcript (Fig. 4) was reduced 70% in the presence of inositol and 80% in inositol plus choline compared to the no addition media. Significantly, this reduction in EPT1 transcript was not observed in the cpt1 strain. These results strongly suggest that the reduction of ethanolaminephosphotransferase activity in wild type cells grown in the presence of inositol is a consequence of transcriptional regulation of the EPT1 gene and that this regulation requires an intact CPT1 gene.

In wild type and ept1 strains, a 1.4-kb CPT1-derived transcript was expressed regardless of growth supplementation (Fig. 5A). The levels of this transcript were unaffected by the addition of choline but were decreased in the presence of inositol or inositol/choline (Fig. 5B) (30). The changes in transcript levels in response to choline and inositol supplementation were consistent with the changes in CPT1-derived cholinephosphotransferase enzyme activities (Table II). A second 1.7-kb CPT1-derived transcript was induced in the presence of inositol and inositol/choline (Fig. 5A). The nature of this induced transcript or its role in phospholipid metabolism is unknown. However, since the CPT1-dependent cholinephosphotransferase activity is reduced in wild type and ept1 strains grown in the presence...
of inositol (Table II), the induced 1.7-kb transcript is unlikely to support the translation of an enzymatically functional CPT1 gene product. Detailed analysis of the precise origin of the induced transcript and its putative role in the regulation of phospholipid metabolism will be required to definitively address this issue.

Concluding Discussion—The PS-dependent and Kennedy pathways in yeast have traditionally been regarded as alternative routes for synthesis of PC and PE and have been referred to as the primary and auxiliary pathways, respectively (6, 18). The present studies provide new information which requires reconsideration of existing conceptions regarding the relationship between these two pathways. Reduced synthesis of PC and PE in the cpl1 and cpl1 strains compared to wild type (Fig. 3) suggests significant synthesis of these phospholipids via the Kennedy pathway in the absence of exogenous choline or ethanolamine. While endogenous ethanolamine is known to derive from sphingolipid metabolism (31), there are no known endogenous sources of de novo choline synthesis in yeast. Thus, it is possible that synthesis of PC via the Kennedy pathway utilizes choline derived from PC turnover in the absence of exogenous choline (32). The role of phospholipid turnover in generating substrate pools for phospholipid resynthesis and the regulation of this process has not been studied. It seems likely that phospholipid turnover may also be an important effect in the regulation of phospholipid synthesis.

The central role of inositol in the complex regulation of yeast phospholipid synthesis is well documented and suggests that PC and inositol metabolism are coordinated (6, 18). The synergistic effect of choline on the repression of enzyme activities by inositol in the PS-dependent pathway of PC synthesis has been interpreted to represent a response to utilization of these precursors in the Kennedy pathway (6, 18). The finding that activities of the CPT1 and EPT1 gene products were regulated by exogenous inositol and inositol/choline and that the direction and magnitude of this regulation is similar to that observed for enzymes of PS-dependent PC and PE synthesis does not support a model of alternate pathways. Thus, the PS-dependent and Kennedy pathways of PC and PE synthesis appear to represent complementary routes of synthesis. The overall regulatory effect of inositol and inositol/choline is to coordinately repress the biosynthetic enzymes for PS, PE, and PC. PI synthase (8) is unaffected by exogenous inositol, however, its substrates CDP-diaclyglycerol (7) and inositol (14) are regulated by inositol. The only enzyme known to show increased activity in the presence of inositol is phosphatidic acid phosphatase which dephosphorylates phosphatidic acid to produce diacylglycerol (33). Taken together, these observations suggest that the regulatory effect of inositol is to coordinately decrease phospholipid synthesis and increase diacylglycerol synthesis. The increased diacylglycerol may be used for the synthesis of triacylglycerol as occurs when cells enter the stationary phase of cell growth (34).

The EPT1 and CPT1 genes are clearly shown in these studies to constitute a new addition to the group of genes subject to inositol repression. The regulatory effects of inositol are mediated through a 9-base pair repeated element (consensus 5'-AGTGTAAGA-3') that is present in the promoter regions of many phospholipid biosynthetic genes (including CPT1 and EPT1) and requires the activities encoded by the INO2, INO4, and OPI1 regulatory genes (6, 18, 35, 36). It has been hypothesized that the regulatory system requires ongoing PC synthesis on the basis of misregulation of inositol-1-phosphate synthase expression by inositol in mutants in the PS-dependent
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pathway. This misregulation is alleviated by the provision of precursors capable of being assimilated via the Kennedy pathway. In this model, a metabolic signal derived from the end-product PC is envisioned being assimilated via the Kennedy pathway. This misregulation is alleviated by the provision of precursors capable of being assimilated via the Kennedy pathway. In this model, a metabolic signal derived from the end-product PC is envisioned being assimilated via the Kennedy pathway.

Fig. 4. Northern analysis and quantitation of EPT1 mRNA levels in wild type and cptl strains. Cells were grown to mid-logarithmic phase in minimal media with no addition or supplemented with 1 mM choline or 50 μM inositol or both. Poly(A)+ RNA was isolated and Northern analysis performed as described under "Experimental Procedures." A, autoradiogram of poly(A)+ RNA (4 μg/lane) probed with a 1.1-kb HindIII fragment of the EPT1 gene which encompasses 80% of the EPT1 gene product reading frame (20). Following autoradiography, membranes were stripped and rehybridized to a 1.1-kb URA3 fragment as a control for the amount of RNA present. B, mRNA levels were quantitated by laser densitometry as described under "Experimental Procedures." The values reported are the ratio of EPT1 mRNA signal to URA3 mRNA signal. These values are also expressed as a percentage relative to those obtained for cells grown in the no addition media (parenthesis).

Fig. 5. Northern analysis and quantitation of CPT1 mRNA in wild type and eptl strains. A, cell growth, RNA isolation, and Northern analysis were performed as described under "Experimental Procedures" and in the legend to Fig. 4. Autoradiograph of poly(A)+ RNA (3–4 μg) from wild type and eptl cells probed with a 1.1-kb Ncol fragment of the CPT1 gene which encompasses 80% of the CPT1 gene product reading frame (19). Membranes were stripped and reprobed with a 1.1-kb HindIII fragment of URA3 as a control for the amount of RNA loaded. B, mRNA levels were quantitated by laser densitometry as described under "Experimental Procedures." The values reported are the ratio of CPT1 mRNA signal to URA3 mRNA signal. These values are also expressed as a percentage relative to those obtained for cells grown in the no addition media (parenthesis).
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