Regulation of Phospholipid Biosynthesis in the Yeast
Saccharomyces cerevisiae*

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Phospholipids are key molecules that contribute to the structural definition of cells and that participate in the regulation of cellular processes. Phospholipid metabolism is a major activity that cells engage in throughout their growth. The yeast, Saccharomyces cerevisiae, serves as a model system for investigation of phospholipid synthesis and its regulation in eucaryotes. Its membranous organelles, the lipids that comprise these membranes, and the phospholipid biosynthetic pathways that generate these membranes typify eucaryotic cells (1, 2). Many of the structural genes encoding for the phospholipid biosynthetic enzymes have been cloned and characterized (Table I) (3–25). The characterization of the wild-type and mutant genes, as well as the gene products encoded by these alleles, has significantly advanced our understanding both of phospholipid biosynthesis and of its regulation. Results from these genetic, molecular, and biochemical studies have shown that the regulation of phospholipid synthesis is a complex, highly coordinated process. The mechanisms that govern this regulation mediate the mRNA and protein levels of the biosynthetic enzymes as well as their activity and localization (1, 2, 45). This review summarizes our current understanding of the regulation of phospholipid metabolism in S. cerevisiae with a particular focus on the regulation of the activity of the biosynthetic enzymes. For more comprehensive reviews, the reader is directed to recent articles by Paltauf et al. (2) and Greenberg and Lopes (45).

Phospholipid Composition of S. cerevisiae

The major phospholipids found in mitotically growing cells are PC, PE, PI, and PS (2). Phospholipid composition can vary dramatically when culture conditions are altered (2). Examples of this include inositol supplementation of wild-type cells (46, 47); inositol starvation of ino1 mutant cells (48–50); choline/ethanolamine starvation of cho1 mutant cells (51); fumonisin B1 supplementation of wild-type cells (52); and glucose starvation of wild-type and respiring cells (53, 54). Although the proportions of the individual phospholipids change with these growth conditions, the average charge of the membrane phospholipids remains relatively constant (2, 45). Therefore, the mechanisms exist in S. cerevisiae that compensate for changes in the levels of phospholipids of one charge by orchestrating parallel changes in the levels of phospholipids of the opposite charge. The mechanisms that mediate these processes and other aspects of phospholipid metabolism include genetic regulation and biochemical regulation of the phospholipid biosynthetic enzymes.

Phospholipid Biosynthetic Pathways

Phospholipid biosynthesis is a complex process that contains a number of branch points (Fig. 1). PS, PE, and PC are synthesized from inositol by the CDP-DG pathway (indicated in Fig. 1 by the color blue), while PE and PC are also synthesized by the Kennedy (CDP-choline and CDP-ethanolamine) pathway (indicated in Fig. 1 by the color red) (1, 2, 55, 56). CDP-DG is also used for the synthesis of other phospholipids, including inositol-containing lipids (phosphoinositides and sphingolipids) and CL. The CDP-DG pathway is used by wild-type cells for the synthesis of PE and PC when they are grown in the absence of ethanolamine or choline (1, 2, 57, 58). The Kennedy pathway assumes a critical role in PC synthesis when the enzymes in the CDP-DG pathway are defective or repressed (1, 2, 45). Mutants defective in the CDP-DG pathway require choline for growth and synthesize PC via CDP-choline (9–12, 26, 29, 59, 60). Mutants defective in PS synthase (26, 59) and PS decarboxylase (9, 60) can also synthesize PC if they are supplemented with ethanolamine. Under these conditions, PE is synthesized from CDP-ethanolamine. The PE may be subsequently methylated by the phospholipid N-methyltransferases to form PC (Fig. 1). It is not clear what the relative contributions of the CDP-DG and Kennedy pathways are to PE and PC synthesis when ethanolamine and/or choline is present in the growth media.

The utilization of the CDP-DG and Kennedy pathways is also regulated by the cellular levels of CTP (61). The elevation of cellular levels of CTP results in a 2-fold increase in the utilization of the Kennedy pathway for PC synthesis. This has been attributed to an increase in substrate availability for the choline-P cytidylyltransferase reaction in the Kennedy pathway and the inhibition of PS synthase activity by CTP in the CDP-DG pathway (61).

Regulation of Phospholipid Biosynthesis

A number of factors regulate phospholipid biosynthesis including inositol, choline, ethanolamine, lipids (e.g. PC and CDP-DG), nucleotides (e.g. ATP and CTP), and growth phase. The regulation of phospholipid biosynthetic enzymes by inositol has been the most extensively characterized (2, 45).

Inositol Effects on the CDP-DG and Kennedy Pathways—The addition of inositol to the growth medium of wild-type cells alters phospholipid composition. The level of PI increases while the levels of PS, PE, and PC decrease (46, 47). These changes are due in part to repression mechanisms. These mechanisms regulate mRNA and protein levels and/or the activity of the phospholipid biosynthetic enzymes. For example, the activity and/or levels of the CDP-DG pathway enzymes (i.e. CDP-DG synthase [62, 63], PI synthase [46, 64–66], PS decarboxylase [67, 69], and the two phospholipid N-methyltransferases [46, 67, 70–73]) are reduced when wild-type cells are supplemented with inositol. In many instances, the repressive effects of inositol are enhanced by the inclusion of ethanolamine or choline in the growth medium. This regulation is absolutely dependent on inositol (1, 2, 45). Under these growth conditions, the exogenous ethanolamine and choline is used to synthesize PE and PC via the Kennedy pathway (1, 2). The coordinate regulation of the CDP-DG pathway enzymes by inositol requires ongoing PC synthesis (70, 74). Data from recent studies have shown that, even in the absence of exogenous ethanolamine and choline, the Kennedy pathway contributes to the synthesis of PC (61, 75, 76). Data suggest that the choline required is derived from the turnover of PC synthesized by the CDP-DG pathway (75, 76). This may indicate that the PC generated by each pathway has distinct as well as overlapping functions in cell physiology. The relative contributions of the Kennedy and CDP-DG pathways to phospholipid synthesis in the absence of exogenous ethanolamine or choline are not known. An apparent paradox in the regulation of

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The abbreviations used are: PC, phosphatidylcholine; PA, phosphatidate; PS, phosphatidylserine; PE, phosphatidylethanolamine; DG, diacylglycerol; TG, triacylglycerol; PI, phosphatidylinositol; PIP, PI 4-phosphate; PIP2, PI 4,5-bisphosphate; inositol phospholipidceramide; MIPC, mannosyl/inositol phospholipidceramide; M(IP)2C, mannosyl/inositol phospholipidceramide; PGP, phosphatidylglycerophosphate; CL, cardiolipin; DGPP, diacylglycerol pyrophosphate.

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Phospholipid biosynthetic pathways in \textit{S. cerevisiae}. The indicated reactions are catalyzed by the following enzymes: 1, glycerol-3-P acetyltransferase; 2, CDP-DG synthase; 3, PS synthase; 4, PS decarboxylase; 5, PE methyltransferase; 6 and 7, phospholipid methyltransferase; 8, PA phosphatase; 9, ethanolamine kinase; 10, ethanolamine-P cytidylyltransferase; 11, ethanolaminephosphotransferase; 12, choline kinase; 13, choline-P cytidylyltransferase; 14, cholinephosphotransferase; 15, DG acetyltransferase; 16, inositol-1-P synthase; 17, inositol-1-P phosphatase; 18, PI synthase; 19, PI 4-kinase; 20, PIP kinase; 21, PI 3-kinase; 22, IPC synthase; 23, PGP synthase; 24, PGP phosphatase; and 25, CL synthase. The CDP-DG pathway is indicated by the color blue and the Kennedy pathway is indicated by the color red. Etn, ethanolamine; Cho, choline; Pme, phosphatidymonomethylethanolamine; Pde, phosphatidylmethyllethanolamine; Pg, phosphatidylglycerol. The four major phospholipids (PC, PE, PI, and PS) are indicated by green boxes.

Fig. 1. Phospholipid biosynthetic pathways in \textit{S. cerevisiae}. The indicated reactions are catalyzed by the following enzymes: 1, glycerol-3-P acetyltransferase; 2, CDP-DG synthase; 3, PS synthase; 4, PS decarboxylase; 5, PE methyltransferase; 6 and 7, phospholipid methyltransferase; 8, PA phosphatase; 9, ethanolamine kinase; 10, ethanolamine-P cytidylyltransferase; 11, ethanolaminephosphotransferase; 12, choline kinase; 13, choline-P cytidylyltransferase; 14, cholinephosphotransferase; 15, DG acetyltransferase; 16, inositol-1-P synthase; 17, inositol-1-P phosphatase; 18, PI synthase; 19, PI 4-kinase; 20, PIP kinase; 21, PI 3-kinase; 22, IPC synthase; 23, PGP synthase; 24, PGP phosphatase; and 25, CL synthase. The CDP-DG pathway is indicated by the color blue and the Kennedy pathway is indicated by the color red. Etn, ethanolamine; Cho, choline; Pme, phosphatidymonomethylethanolamine; Pde, phosphatidylmethyllethanolamine; Pg, phosphatidylglycerol. The four major phospholipids (PC, PE, PI, and PS) are indicated by green boxes.

The rapid changes in the rates of phospholipid synthesis in response to inositol supplementation (47), choline/ethanolamine starvation of cho1 mutant cells (51), fumonisin B1 supplementation of wild-type cells (52), and glucose starvation of wild-type and respiratory deficient cells (53, 54) cannot be simply ascribed to genetic mechanisms. It is likely that the direct regulation of enzyme activities also mediates phospholipid synthesis. A number of the biosynthetic enzymes (e.g., CDP-DG synthase (34), PS synthase (35), PA phosphatase (36), PI synthase (37), and PI 4-kinase) have been purified to near homogeneity, and defined studies of their biochemical regulation have been conducted (Table II). This regulation will be discussed in the context of phospholipid synthesis.

### Biochemical Regulation of Phospholipid Biosynthetic Enzymes

Regulation of DG/CDP-DG Synthesis—A major branch point in phospholipid synthesis involves the enzymes PA phosphatase and CDP-DG synthase. These enzymes utilize PA as a substrate (Fig. 1). The partitioning of PA at this step in the pathway would influence the levels of individual phospholipids and would also alter the proportions of the phospholipids and the neutral lipids, DG and TG. Based on the relative \( K_m \) values for PA, the 45- and 104-kDa forms of PA phosphatase have a greater affinity for PA than does CDP-DG synthase (34, 36, 37). This suggests that the partitioning of PA between CDP-DG and DG may be primarily governed by the regulation of PA phosphatase activity. The 45- and 104-kDa PA phosphatase activities are each inhibited by sphingoid bases (i.e., sphinganine and phytosphingosine) (93) and AMP (54). However, they are regulated differentially by phosphorylation (94), cAMP-dependent protein kinase phosphorylates and activates the 45-kDa enzyme but has no effect on the 104-kDa PA phosphatase (94). The regulation of PA phosphatase activity by sphingoid bases, ATP, and phosphorylation correlates with observed changes in the synthesis of phospholipids and TG (52, 54, 92, 94–96). Both PA phosphatase activities are activated by CL, CDP-DG, and PI (97). Since the activation constants for these phospholipids are within the range of their cellular concentrations (97), this activation may be physiologically relevant. In contrast to the PA phosphatases, CDP-DG synthase activity is not regulated by phosphorylation, nucleotides, sphingoid bases, or phospholipids (52, 98, 99).
The inhibition of PS synthase activity by inositol (47), sphingoid bases (52) and by cAMP-dependent protein kinase phosphorylation (47). PS synthase activity is also inhibited by sphingoid bases and that this inhibition correlates with a decrease in sphingolipid synthesis (52).

Table II

| Enzyme                 | Regulatory by        | Effect |
|------------------------|----------------------|--------|
| 45-kDa PA phosphatase  | cAMP-dependent protein kinase phosphorylation | Activation 94 |
|                        | CL, CDP-DG, PI, DGPP | Activation 44, 97 |
|                        | Sphingoid bases      | Inhibition 93 |
|                        | Nucleotides          | Inhibition 54 |
| 104-kDa PA phosphatase | CL, CDP-DG, PI, DGPP | Activation 44, 97 |
|                        | Sphingoid bases      | Inhibition 93 |
|                        | Nucleotides          | Inhibition 54 |
| PS synthase            | cAMP-dependent protein kinase phosphorylation | Activation 94 |
|                        | PA                   | Activation 101 |
|                        | CL, DG               | Inhibition 101 |
|                        | Sphingoid bases      | Inhibition 52 |
|                        | Inositol             | Inhibition 47 |
| 45-kDa PI              | CDP-DG, PG           | Inhibition 50 |
|                        | Nucleotides          | Inhibition 102 |
| 55-kDa PI 4-kinase     | Nucleotides          | Inhibition 102 |
| IPC synthase           | Sphingoid bases      | Inhibition 52 |

DGPP phosphatase is a membrane-associated enzyme recently identified in S. cerevisiae (44). This enzyme catalyzes the dephosphorylation of DGPP to generate PA. DGPP is a novel metabolite that contains a pyrophosphate group attached to DG (105). This metabolite accounts for 0.18 mol % of the total phospholipid content of S. cerevisiae (44). When DGPP is supplied as a substrate in vitro, the enzyme removes the β-phosphate of DGPP to generate PA and then removes the α-phosphate to generate DG (44).

DGPP phosphatase activity is not significantly altered by PC, PE, PI, PS, or DG (44). In addition, PA does not alter DGPP phosphatase activity (44). However, DGPP does competitively inhibit the PA phosphatase activity of the DGPP phosphatase enzyme (44). In contrast, DGPP stimulates the activities of the 45- and 104-kDa PA phosphatases (44). Moreover, these PA phosphatase enzymes do not utilize DGPP as a substrate (44). These data indicate that the activities of the DGPP phosphatase enzyme and of the DGPP phospholipid may influence PA levels in vivo. Since PA plays a major role in phospholipid metabolism, it is likely that the activities of the DGPP phosphatase enzyme will influence these processes.

Another enzyme activity that has been recently identified in S. cerevisiae is PA kinase (44). This enzyme catalyzes the phosphorylation of PA to generate DGPP. The PA kinase from yeast has not been purified, and its response to various lipid regulators is not known. Since the activity of PA kinase and DGPP phosphatase will contribute to the levels of DGPP, PA, and DG, the enzymes likely participate in a novel cycle for the regulation of the levels of these lipids.

Concluding Comments

Research on phospholipid synthesis in S. cerevisiae has significantly advanced our understanding of this process. It is clear from studies of phospholipid enzymes and their genes that the mechanisms that govern this metabolism are intricate and are integrated with other aspects of cell physiology. Investigators are using a combination of approaches (genetic, molecular, and biochemical) to unravel the complexity of this process. This effort requires the discovery of those genes that encode enzymes that have been identified as well as the purification and characterization of the products of these genes. In addition, as the recent discovery of DGPP phosphatase and PA kinase illustrates, there may be other components of phospholipid metabolism yet to be identified.

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