Regulation of phospholipid synthesis in yeast

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Abstract   Phospholipid synthesis in the yeast *Saccharomyces cerevisiae* is a complex process that involves regulation by both genetic and biochemical mechanisms. The activity levels of phospholipid synthesis enzymes are controlled by gene expression (e.g., transcription) and by factors (lipids, water-soluble phospholipid precursors and products, and covalent modification of phosphorylation) that modulate catalysis.▮▮ Phosphatidic acid, whose levels are controlled by the biochemical regulation of key phospholipid synthesis enzymes, plays a central role in the regulation of phospholipid synthesis gene expression.—Carman, G. M., and G-S. Han. **Regulation of phospholipid synthesis in yeast.** *J. Lipid Res.* 2009. S69–S73.

Supplementary key words   phospholipid synthesis • gene regulation • enzyme regulation • phosphatidic acid • yeast

The budding yeast *Saccharomyces cerevisiae*, with its full complement of organelles, synthesizes membrane phospholipids by pathways that are generally common to those found in higher eukaryotic organisms (**Fig. 1**) (1, 2). Its tractable genetics has facilitated the identification and characterization of nearly all of the structural and regulatory genes that are involved in de novo phospholipid synthesis (2, 3). Moreover, the purification and characterization of several key phospholipid synthesis enzymes have led to an understanding of the biochemical regulation of phospholipid synthesis (1, 4, 5). In *S. cerevisiae*, phospholipid synthesis is a complex process that is regulated by both genetic and biochemical mechanisms (1, 3–9). The expression of phospholipid synthesis genes is controlled at the levels of transcription and mRNA stability (1, 3, 10). The activities of key phospholipid synthesis enzymes are regulated by lipids, water-soluble phospholipid precursors and products, and by the covalent modification of phosphorylation (1, 4, 8). Moreover, the regulation of phospholipid synthesis is interrelated with the synthesis of other major lipid classes [e.g., fatty acids, triacylglycerol (TAG), sterols, sphin-

golipids] (1, 11–15). In this review, we focus on how genetic and biochemical mechanisms work together to regulate phospholipid synthesis.

PHOSPHOLIPID SYNTHETIC PATHWAYS

The de novo pathways for the synthesis of phospholipids in *S. cerevisiae* are shown in Fig. 1. All major phospholipids are derived from phosphatidate (PA), which is partitioned between CDP-diacylglycerol (CDP-DAG) and diacylglycerol (DAG). The major phospholipids phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS) are primarily synthesized from PA via CDP-DAG (i.e., CDP-DAG pathway) (1, 3). CDP-DAG is also used for the synthesis of phosphatidylglycerol and cardiolipin, phospholipids that are confined to mitochondrial membranes (16). As in higher eukaryotes, DAG is used for the synthesis of the storage lipid TAG and for the synthesis of PE and PC via the CDP-ethanolamine and CDP-choline branches, respectively, of the Kennedy pathway (1, 11). The ethanolamine or choline required for the Kennedy pathway is obtained from growth medium supplementation or from the phospholipase D-mediated turnover of the PE or PC synthesized via CDP-DAG (Fig. 1) (17–19).

Mutants (e.g., *cho1*, *psd1 psd2*, *cho2*, and *opi3*) defective in the CDP-DAG pathway can synthesize PE or PC if they are supplemented with ethanolamine, choline, lysoPE, lysoPC, or PC with short acyl chains (1, 20–23). Ethanolamine and choline are converted to PE and PC via the Kennedy pathway (Fig. 1). lysoPE and lysoPC are acylated to PE and PC, respectively, by the *ALE1*-encoded lysophospholipid acyltransferase (21, 22, 24, 25). Short acyl chain PC, which is not incorporated into membranes, is remolded with 16-

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Abbreviations: CDP-DAG, CDP-diacylglycerol; DAG, diacylglycerol; ER, endoplasmic reticulum; PA, phosphatidate; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; TAG, triacylglycerol; UAS_{INO}, upstream activating sequence inositol-responsive element; UAS_{ZRE}, upstream activating sequence zinc-responsive element.

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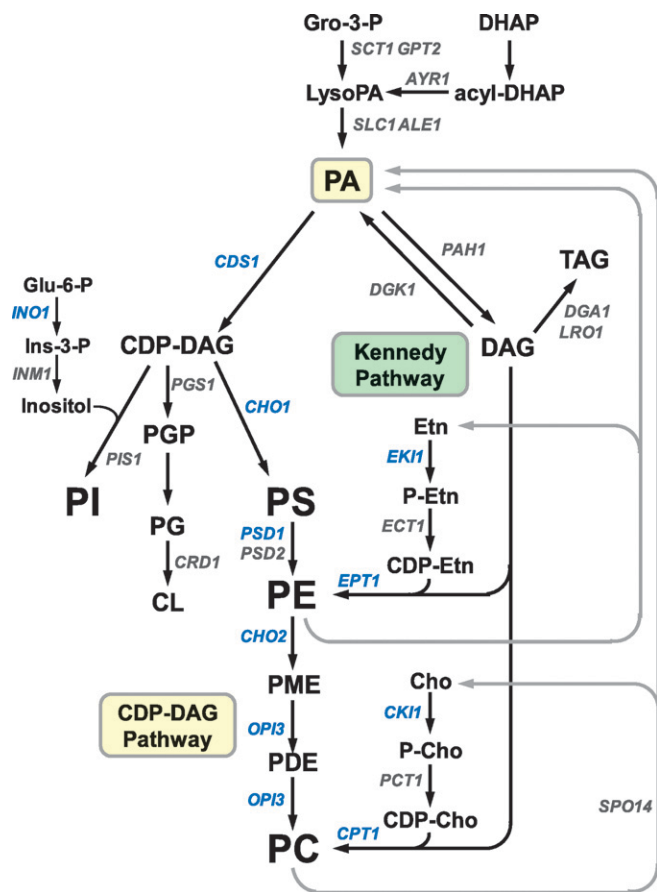   e-mail: carman@aesop.rutgers.edu

Fig. 1. Phospholipid synthesis pathways in *S. cerevisiae.* The pathways shown for the synthesis of phospholipids include the relevant steps discussed in this review. The synthesis of PE and PC from lysoPE and lysoPC, respectively, is not shown in the figure. The genes that are known to encode enzymes catalyzing individual steps in the lipid synthesis pathways are indicated. The UAS$_{INO}$-containing genes that are subject to regulation by the Ino2-Ino4 activation complex and the Opi1 repressor are blue. Gro, glycerol; DHAP, dihydroxyacetone phosphate; Glu, glucose; Ins, inositol; PME, phosphatidylmonomethylethanolamine; PDE, phosphatidyldimethylethanolamine; Etn, ethanolamine; Cho, choline.

and 18-carbon acyl chains (26) through the activities of phospholipase B and lysophospholipid acyltransferase (27–29). Kennedy pathway mutants (e.g., *cki1 eki1* and *cpt1 ept1*) defective in both the CDP-choline and CDP-ethanolamine branches synthesize PC only via the CDP-DAG pathway (19, 30, 31). These mutants, unlike those defective in the CDP-DAG pathway (1), do not exhibit any auxotrophic requirements (19, 31).

GENETIC AND BIOCHEMICAL MECHANISMS FOR THE REGULATION OF PHOSPHOLIPID SYNTHESIS

The synthesis of phospholipids is regulated by mechanisms that affect the expression of enzymes and the modulation of their activities. The expression of phospholipid synthesis genes is controlled by multiple factors, including nutrient availability, growth stage, pH, and temperature (1,

2, 9, 16, 28, 32). The mechanisms responsible for the regulation of gene expression include a number of *cis*- and *trans*-acting elements (7, 9). In this review, we focus on phospholipid synthesis genes that contain the inositol-responsive element (UAS$_{INO}$) and that are regulated by the transcription factors Ino2, Ino4, and Opi1. As will be discussed below, the transcriptional regulation of UAS$_{INO}$-containing genes is generally triggered by the biochemical regulation of phospholipid synthesis enzymes.

Genes encoding enzymes in both the CDP-DAG (*CDS1, CHO1, PSD1, CHO2, OPI3*) and Kennedy (*EKI1, EPT1, CKI1, CPT1*) pathways and for the synthesis of PI (*INO1*) contain a UAS$_{INO}$ element in the promoter. The UAS$_{INO}$ element is the binding site for the Ino2-Ino4 heterodimer complex that activates transcription (3, 7). Repression of UAS$_{INO}$-containing gene expression is controlled by Opi1, whose function is governed by its nuclear localization (3, 7, 33). Opi1 binds PA and the Scs2 protein at the nuclear/endoplasmic reticulum (ER) membrane, which blocks its repressor function in the nucleus (33, 34). When PA levels are reduced, Opi1 is released from the nuclear/ER membrane and enters into the nucleus, where it attenuates transcription by binding to Ino2 (3, 7). Thus, PA content at the nuclear/ER membrane is an important factor that controls the Opi1-mediated regulation of UAS$_{INO}$-containing gene expression (3).

Conditions that trigger the regulation of UAS$_{INO}$-containing genes include nutrient availability (e.g., inositol or zinc) and growth stage (1, 7, 9). For example, the expression of UAS$_{INO}$-containing genes is activated when the essential nutrient zinc is supplemented to the growth medium (9, 35). By contrast, the gene expression is repressed by inositol supplementation and requires ongoing PC synthesis (1, 3, 7). In this regulation, ethanolamine or choline supplementation enhances the repressive effect on gene expression (1). Depletion of inositol or zinc from the growth medium has the opposite effect on gene expression (1, 3, 7, 9). The UAS$_{INO}$-containing genes are maximally expressed in the exponential phase of growth, whereas they are repressed in the stationary phase of growth (1, 3, 7). The repression of the UAS$_{INO}$-containing genes by zinc depletion, or when cells enter the stationary phase of growth, is independent of inositol supplementation (1, 9).

Regulation by inositol and zinc

The mechanisms that control PA content and expression of UAS$_{INO}$-containing genes are typified by the inositol- and zinc-mediated regulation of phospholipid synthesis. Upon inositol supplementation, the synthesis of PI is elevated through increased substrate availability for the *PIS1*-encoded PI synthase (36). In addition, inositol directly inhibits the activity of the *CHO1*-encoded PS synthase, which favors the utilization of CDP-DAG for PI synthesis (36). This biochemical regulation draws upon PA content through CDP-DAG and causes the translocation of Opi1 into the nucleus for repression of UAS$_{INO}$-containing genes (3, 33). Overall, the repression of UAS$_{INO}$-containing genes leads to a decrease in the synthesis of enzymes used in both the CDP-DAG and Kennedy pathways, and changes in phos-

pholipid composition that include an increase in PI and decreases in PA, PS, and PC (1, 36).

Regulation of UAS$_{INO}$-containing genes by zinc also involves the control of PA content through the activation of PI synthase. This regulation that occurs in the absence of inositol supplementation is mediated by the zinc-sensing and zinc-inducible transcriptional activator Zap1 and the zinc-responsive *cis*-acting element (UAS$_{ZRE}$) of phospholipid synthesis genes (9). Zinc depletion results in an increase in PI synthesis through increased expression of the *PIS1*-encoded PI synthase (35, 37) that is mediated by the interaction of Zap1 with a UAS$_{ZRE}$ in the *PIS1* promoter (35, 37). As indicated above, the increase in PI synthesis causes a decrease in PA content. The net result is the Opi1-mediated repression of UAS$_{INO}$-containing genes and a decrease in the activities of the CDP-DAG pathway enzymes (35). The major effects of zinc depletion on phospholipid composition include an increase in PI and a decrease in PE (35). Interestingly, the PC content is not significantly affected by zinc depletion, although enzyme activities in the CDP-DAG pathway are repressed (35). Maintenance of a normal PC content has been attributed to the Zap1-mediated activation of *CKI1*-encoded choline kinase expression for PC synthesis via the Kennedy pathway (38). Any effect that Opi1 would have on *CKI1* expression (because it contains a UAS$_{INO}$ element) is overcome by the derepression of *CKI1* by Zap1 (38).

Regulation by CTP and AdoHcy

CTP and AdoHcy are molecules that regulate UAS$_{INO}$-containing genes as well as the activities of phospholipid synthesis enzymes. CTP is essential for phospholipid synthesis; it is the direct precursor of the activated, energy-rich intermediates CDP-DAG, CDP-choline, and CDP-ethanolamine (6). CTP is also used as the phosphate donor for the synthesis of PA by the *DGK1*-encoded DAG kinase (39). Because the cellular levels of CTP are primarily controlled by product inhibition of CTP synthetase activity, expression of a mutant enzyme lacking this regulation results in elevated levels of CTP as well as an increased rate of PA synthesis and the derepression of UAS$_{INO}$-containing genes (6, 40). The increase in PA content and the inactivation of Opi1 repressor function (39) may result from the stimulation of DAG kinase activity by increased availability of its substrate CTP. CTP also favors an elevation of PA content by inhibiting *PAH1*-encoded PA phosphatase[2] activity (41).

AdoHcy is a product of the AdoMet-dependent methylation reactions that are catalyzed by the *CHO2*-encoded PE methyltransferase and *OPI3*-encoded phospholipid methyltransferase in the CDP-DAG pathway (Fig. 1). AdoHcy,

which is removed by the *SAH1*-encoded AdoHcy hydrolase (13), is a competitive inhibitor of the methyltransferase enzymes (42). Thus, downregulation of the AdoHcy hydrolase causes the accumulation of AdoHcy and the inhibition of PC synthesis, which leads to an increase in PA content and the derepression of UAS$_{INO}$-containing genes (13). Although the effects of AdoHcy on phospholipid composition have not been addressed, its accumulation causes an increase in TAG synthesis and lipid droplet content (13).

ROLES OF PA PHOSPHATASE AND DAG KINASE IN CONTROLLING PA CONTENT

Among the enzymes (e.g., lysoPA acyltransferase, CDP-DAG synthase, PA phosphatase, DAG kinase, phospholipase D) that contribute to the metabolism of PA, the *PAH1*-encoded PA phosphatase has been identified as a key regulator of PA content. Cells lacking the enzyme activity contain an elevated PA content and exhibit the derepression of UAS$_{INO}$-containing phospholipid synthesis genes (14, 32, 43). In addition, elevated PA content stimulates PS synthase activity (44), which would favor the synthesis of PC via the CDP-DAG pathway. Moreover,
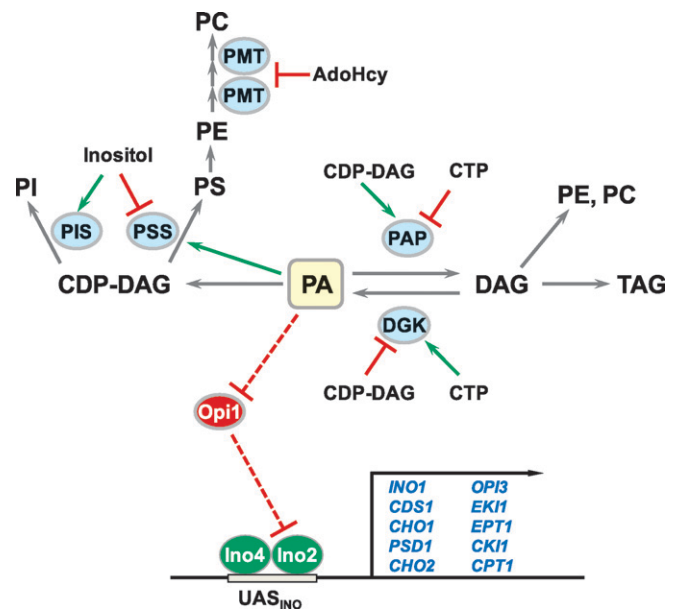

Fig. 2. Model for the biochemical regulation of phospholipid synthesis. The upper portion of the diagram shows the major steps in the synthesis of phospholipids. The key enzymes (*PMT*, *CHO2*-encoded PE methyltransferase and *OPI3*-encoded phospholipid methyltransferase; *PIS*, *PIS1*-encoded PI synthase; *PSS*, *CHO1*-encoded PS synthase; *PAP*, *PAH1*-encoded PA phosphatase; *DGK*, *DGK1*-encoded DAG kinase) that are biochemically regulated by phospholipid precursors and products are highlighted by the blue ellipses. The bottom portion of the diagram shows the transcriptional activation of UAS$_{INO}$-containing genes (blue) by the Ino2-Ino4 complex. Elevated PA (highlighted in yellow) content prevents the translocation of Opi1 into the nucleus, and thus its repressor function of UAS$_{INO}$-containing phospholipid synthesis genes. The color green designates stimulation, whereas the color red designates inhibition.

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[2] The *PAH1*-encoded PA phosphatase is a Mg^{2+}-dependent enzyme that is involved in *de novo* lipid synthesis. It is distinct from the *DPP1*- and *LPP1*-encoded lipid phosphate phosphatase enzymes that dephosphorylate PA and a host of lipid phosphate molecules by a catalytic mechanism that does not require Mg^{2+} (5). The *DPP1*- and *LPP1*-encoded enzymes play specific roles in controlling lipid phosphate metabolism confined to the vacuole and Golgi membranes, respectively (5).

the elevation of PA content triggers the anomalous expansion of the nuclear/ER membrane (32, 43), underscoring the importance of phospholipid synthesis to organelle synthesis/structure. In contrast to the loss of *PAH1*-encoded PA phosphatase activity, overexpression of the enzyme activity causes the repression of *INO1* expression and inositol auxotrophy (45).

The *DGK1*-encoded DAG kinase has recently been identified as an enzyme that counteracts the role that *PAH1*-encoded PA phosphatase plays in controlling PA content and the transcriptional regulation of UAS$_{INO}$-containing genes (39, 46). The overexpression of the enzyme activity causes an increase in PA content, the derepression of UAS$_{INO}$-containing genes, and the anomalous nuclear/ER membrane expansion (46) like those shown in the *pah1*Δ mutant (32, 43). In addition, the overexpression of *DGK1*-encoded DAG kinase activity bypasses the inositol auxotrophy (e.g., repression of *INO1* and other UAS$_{INO}$-containing genes) caused by the overexpression of *PAH1*-encoded PA phosphatase activity (46). Moreover, the *dgk1*Δ mutation bypasses the phenotypes caused by the *pah1*Δ mutation (39, 46).

PAH1-encoded PA phosphatase and *DGK1*-encoded DAG kinase are biochemically regulated by CDP-DAG. CDP-DAG stimulates PA phosphatase activity (47) but inhibits DAG kinase activity (39). Thus, the regulation of these PA metabolic enzymes by an elevated level of CDP-DAG favors a decrease in PA content and the Opi1-mediated repression of UAS$_{INO}$-containing genes. One of the UAS$_{INO}$-containing genes that is repressed by Opi1 is *CDS1* (48), which encodes CDP-DAG synthase (49). Thus, the regulation of its expression provides a mechanism for controlling the synthesis of CDP-DAG from PA and the CDP-DAG-dependent synthesis of phospholipids. This notion is supported by genetic evidence that a *cds1* mutant defective in CDP-DAG synthase activity exhibits an elevated PA content and the derepression of UAS$_{INO}$-containing genes (50, 51). The increased DAG levels caused by the CDP-DAG-mediated regulation of PA phosphatase and DAG kinase activities would be channeled to phospholipids via the Kennedy pathway or to the storage lipid TAG.

SUMMARY AND PERSPECTIVES

A major theme of this review is that the biochemical regulation of phospholipid synthesis enzymes ultimately controls the cellular content of PA, a lipid precursor and signaling molecule that triggers the transcriptional regulation of UAS$_{INO}$-containing phospholipid synthesis genes. The model shown in **Fig. 2** incorporates this theme and highlights the central role that the *PAH1*-encoded PA phosphatase and *DGK1*-encoded DAG kinase play in controlling PA content. Interestingly, the effector molecules inositol, CTP, and CDP-DAG play important roles in the regulation of phospholipid synthesis by stimulating or inhibiting key phospholipid synthesis enzymes (Fig. 2).

As discussed above, the repression of UAS$_{INO}$-containing genes in response to inositol supplementation or zinc depletion is attributed to the regulation of PI synthase activity or *PIS1* gene expression, respectively (36, 37). Previous studies have shown that the levels of PA phosphatase activity are elevated in inositol-supplemented (52) and zinc-depleted (53) cells as well as in stationary phase cells (54). Thus, elevated PA phosphatase activity provides another mechanism for controlling PA content under these growth conditions. While it is clear that *PAH1*-encoded PA phosphatase plays a major role in controlling PA content, its role in the regulation of UAS$_{INO}$-containing gene expression under the growth conditions discussed above has yet to be established.

While specifically not discussed in this review, the activities of phospholipid synthesis enzymes (e.g., PA phosphatase, PS synthase, choline kinase, and CTP synthetase) are also regulated by phosphorylation, affecting the cellular concentrations of phospholipid precursors and products (8, 45). The protein kinases that mediate this regulation include protein kinase A, protein kinase C, and cyclin-dependent protein kinase (8, 32, 45). However, additional studies are needed to make physiological connections between the phosphorylation-mediated regulation of enzyme activities and the transcriptional regulation of UAS$_{INO}$-containing phospholipid synthesis genes.

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