

Regulation of Phospholipid Synthesis in the Yeast *Saccharomyces cerevisiae*

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Abstract

The yeast *Saccharomyces cerevisiae*, with its full complement of organelles, synthesizes membrane phospholipids by pathways that are generally common to those found in higher eukaryotes. Phospholipid synthesis in yeast is regulated in response to a variety of growth conditions (e.g., inositol supplementation, zinc depletion, and growth stage) by a coordination of genetic (e.g., transcriptional activation and repression) and biochemical (e.g., activity modulation and localization) mechanisms. Phosphatidate (PA), whose cellular levels are controlled by the activities of key phospholipid synthesis enzymes, plays a central role in the transcriptional regulation of phospholipid synthesis genes. In addition to the regulation of gene expression, phosphorylation of key phospholipid synthesis catalytic and regulatory proteins controls the metabolism of phospholipid precursors and products.

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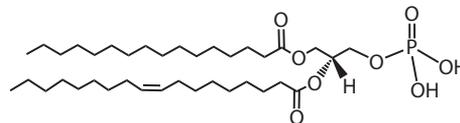


Figure 1

Basic phospholipid structure. The diagram shows the structure of phosphatidate (PA), the phospholipid precursor, with fatty acyl groups containing 16 carbon atoms (position 1) and 18 carbon atoms with one double bond (position 2).

full complement of organelles, phospholipids are synthesized via pathways that are generally common to those found in higher eukaryotic organisms (12, 13). 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 (13, 14). Moreover, the purification and characterization of key enzymes from the organism have led to an understanding of biochemical regulation in phospholipid synthesis (12, 15, 16). Phospholipid synthesis in yeast is a complex process that is regulated by genetic and biochemical mechanisms (12, 14–20), and its regulation is interrelated with the synthesis and regulation of other major lipid classes that include fatty acids, triacylglycerol (TAG), sterols, and sphingolipids (12, 21–25). In this review, the focus is on the interrelationships between the genetic and biochemical regulations of the synthesis of the major phospholipids in *S. cerevisiae*. The way that yeast gene and protein terms are named is described in the sidebar Yeast Gene/Protein Nomenclature.

PATHWAYS FOR THE SYNTHESIS OF THE MAJOR PHOSPHOLIPIDS

The major phospholipids found in the cellular membranes of *S. cerevisiae* include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS) (26–29). Phosphatidylglycerol (PG) and cardiolipin (CL) are also major phospholipids in mitochondrial membranes (26, 28, 29). Minor phospholipids include intermediates: phosphatidate

Phospholipid: a membrane component whose structure is based on a glycerol-3-phosphate backbone with fatty acids esterified to positions 1 and 2

PC: phosphatidylcholine

PE: phosphatidylethanolamine

PI: phosphatidylinositol

PS: phosphatidylserine

INTRODUCTION

Phospholipids are major structural components of cellular membranes and are essential for vital cellular processes (1, 2). Their structure is based on a glycerol-3-phosphate backbone in which fatty acyl groups are esterified to positions 1 and 2 (**Figure 1**). As amphipathic molecules, they form a bilayer in which integral and peripheral membrane proteins, as well as other complex components, associate (3). In addition, they are reservoirs of second messengers (4), provide precursors for the synthesis of macromolecules (5–9), serve in the modification of membrane association (10), and function as molecular chaperons (11). In the budding yeast, *Saccharomyces cerevisiae*, which contains a

(PA); CDP-diacylglycerol (CDP-DAG) phosphatidylmonomethylethanolamine and phosphatidyl dimethylethanolamine; the D-3, D-4, and D-5 polyphosphoinositides; lysophospholipids; and diacylglycerol (DAG) pyrophosphate (26, 30, 31). The most common fatty acids esterified to the glycerophosphate backbone of these lipids include palmitic acid, palmitoleic acid, stearic acid, and oleic acid (26, 27, 32–34). The relative amounts of the phospholipids vary with growth conditions (e.g., carbon source, nutrient availability, temperature, and growth phase) and with genetic variations (18, 26, 28, 29). Although the proportions of the individual phospholipids change, the average charge of the membrane phospholipids remains relatively constant (29, 35). Thus, regulatory mechanisms exist in *S. cerevisiae* to compensate for changes in the levels of phospholipids of one charge by coordinating parallel changes in the levels of phospholipids of the opposite charge.

The pathways for the synthesis of major phospholipids in *S. cerevisiae* are presented in **Figure 2**. The structural genes and enzymes involved in the pathways have been confirmed by the analysis of gene mutations and the biochemical characterization of purified enzymes (12, 15, 28, 36, 37). The synthesis of membrane phospholipids begins with the phospholipid PA, which is produced from glycerol-3-phosphate or dihydroxyacetone phosphate after fatty acyl coenzyme A (CoA)-dependent reactions that are catalyzed by the *SCT1*- and *GPT2*-encoded glycerol-3-phosphate acyltransferases and the *SLC1*- and *ALE1*-encoded lysophospholipid acyltransferases (38–44). In the de novo pathways, all membrane phospholipids are synthesized from PA via the liponucleotide intermediate CDP-DAG. The *CDS1*-encoded CDP-DAG synthase catalyzes the formation of CDP-DAG from PA using the nucleotide CTP for the donation of the CMP moiety (45, 46). CDP-DAG may then donate its phosphatidyl moiety to inositol to form PI (47) in the reaction catalyzed by the *PIS1*-encoded PI synthase (48, 49). The inositol used in this reaction is derived from glucose-6-phosphate via the reactions catalyzed

YEAST GENE/PROTEIN NOMENCLATURE

S. cerevisiae genes are designated by three uppercase italicized letters followed by a number (e.g., *PIS1* for phosphatidylinositol synthase 1), ideally describing the biochemical/molecular function of their protein products. Lowercase italicized letters designate a recessive mutation in the gene (e.g., *pis1*). Some yeast genes (e.g., *CHO1* and *OPI3*) are named after their mutant phenotypes (e.g., *cho1* mutants require choline and *opi3* mutants overproduce and excrete inositol, respectively) or other genetic phenotypes. For genes that have several names (e.g., the gene for PS synthase has two names: *CHO1* and *PSS1*) because of independent identification and naming, the standard name is the one generally described first, and the other names are designated as aliases (see the *Saccharomyces* Genome Database, <http://www.yeastgenome.org/>). The protein product of a yeast gene is designated by the gene acronym followed by the letter p (e.g., Pis1p, Cho1p, Opi3p).

by the *INO1*-encoded inositol-3-phosphate synthase (50, 51) and the *INM1*-encoded inositol-3-phosphate phosphatase (52). Alternatively, the inositol used in the reaction may be obtained exogenously via the *ITR1*- and *ITR2*-encoded inositol permeases (53). CDP-DAG may also donate its phosphatidyl moiety to glycerol-3-phosphate to form phosphatidylglycerophosphate (PGP) in the reaction catalyzed by the *PGS1*-encoded PGP synthase (54, 55). PGP is then dephosphorylated to PG by the *GEP4*-encoded PGP phosphatase (56). The *CRD1*-encoded CL synthase (57–59) catalyzes the reaction between PG and another molecule of CDP-DAG to generate CL. The final enzyme that utilizes CDP-DAG in the pathway is the *CHO1*-encoded PS synthase (60–62), which catalyzes the formation of PS by displacement of CMP from CDP-DAG with Ser (63). PS is then decarboxylated to PE by the *PSD1*- (64, 65) and *PSD2*-encoded (66) PS decarboxylase enzymes. PE is then converted to PC by the three-step *S*-adenosyl methionine (AdoMet)-dependent methylation reactions (67), whereby the first methylation reaction is catalyzed by the *CHO2*-encoded PE methyltransferase (68, 69) and the last two methylation

PG:
phosphatidylglycerol

CL: cardiolipin

Phosphatidate (PA):
a phospholipid precursor that stabilizes the interaction of Opi1p with Scs2p at the nuclear/ER membrane

CDP-DAG:
CDP-diacylglycerol

DAG: diacylglycerol

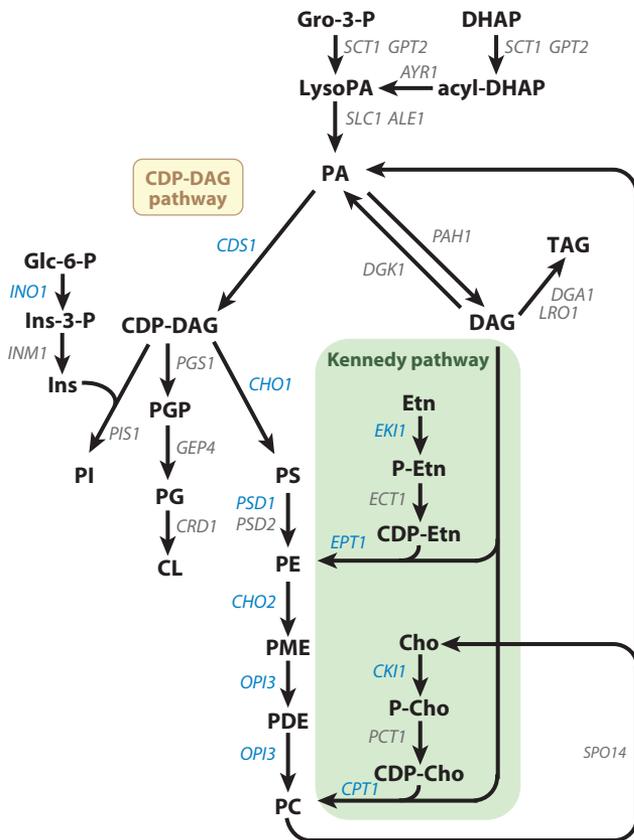


Figure 2

Phospholipid synthesis pathways in *S. cerevisiae*. The pathways shown for the synthesis of phospholipids include the relevant steps discussed in this review. 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 Ino2p-Ino4p activator complex and the Opi1p repressor are shown (blue). Abbreviations: CDP-DAG, CDP-diacylglycerol; Cho, choline; CL, cardiolipin; Gro, glycerol; DHAP, dihydroxyacetone phosphate; Etn, ethanolamine; Glc, glucose; Ins, inositol; PA, phosphatidate; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PGP, phosphatidylglycerophosphate; PDE, phosphatidylidimethylethanolamine; PI, phosphatidylinositol; PME, phosphatidylmonomethylethanolamine; PS, phosphatidylserine; TAG, triacylglycerol; UAS_{INO}, an inositol-responsive upstream activating sequence.

reactions are catalyzed by the *OPI3*-encoded phospholipid methyltransferase (68, 70).

PE and PC are also synthesized from exogenously supplied ethanolamine and choline [via the *HNMI*-encoded choline permease (71, 72)], respectively, by way of the CDP-ethanolamine and CDP-choline branches of

the Kennedy pathway (Figure 2). The *EKI1*-encoded ethanolamine kinase (73) and the *CKI1*-encoded choline kinase (74) enzymes phosphorylate ethanolamine and choline with ATP to form phosphoethanolamine and phosphocholine, respectively. These intermediates are then activated with CTP to form CDP-ethanolamine and CDP-choline, respectively, by the *ECT1*-encoded phosphoethanolamine cytidyltransferase (75) and the *PCT1*-encoded phosphocholine cytidyltransferase (76). Finally, CDP-ethanolamine and CDP-choline react with DAG to form PE and PC, respectively, in the reactions catalyzed by the *EPT1*-encoded ethanolamine phosphotransferase (77, 78) and the *CPT1*-encoded choline phosphotransferase (79, 80).

The CTP required for the synthesis of CDP-DAG, CDP-ethanolamine, and CDP-choline is derived from UTP by the *URA7*- (81) and *URA8*-encoded (82) CTP synthetase enzymes. The DAG used for the synthesis of PE and PC via the Kennedy pathway is derived from PA by the *PAH1*-encoded PA phosphatase (24).¹ The DAG generated in the PA phosphatase reaction may be converted back to PA by the *DGK1*-encoded DAG kinase² (83, 84) or used for the synthesis of the neutral lipid TAG (24) by acyltransferase enzymes encoded by *DGA1* and *LRO1* (85). The *ARE1*- and *ARE2*-encoded acyltransferase enzymes, which are primarily responsible for the synthesis of ergosterol esters, can also acylate DAG to form TAG (85).

¹PA phosphatase is distinguished in catalytic and physiological functions from the *DPPI1*- and *LPPI1*-encoded lipid phosphate phosphatase enzymes that dephosphorylate a broad spectrum of substrates (including PA, lysoPA, DAG pyrophosphate, sphingoid base phosphates, and isoprenoid phosphates) by a distinct catalytic mechanism that does not require divalent cations (16, 31, 213, 214). The *DPPI1*- and *LPPI1*-encoded lipid phosphate phosphatase enzymes are associated with the vacuole and Golgi membranes, respectively, and are thought to be involved with lipid signaling (16, 31).

²The yeast DAG kinase differs from the enzyme in animals, plants, worms, flies, and bacteria in that the yeast enzyme utilizes CTP instead of ATP as the phosphate donor in the reaction (83, 84).

The CDP-DAG pathway is primarily used for the synthesis of PE and PC when cells are grown in the absence of ethanolamine and choline (12, 27, 28, 86). Yet, the Kennedy pathway contributes to the synthesis of PE and PC when these precursors are not supplemented (37, 73, 87). For example, the PC synthesized by way of the CDP-DAG pathway is constantly hydrolyzed to choline and PA (87, 88) by the *SPO14*-encoded (89, 90) phospholipase D. The choline is incorporated back into PC via the CDP-choline branch of the Kennedy pathway, and the PA is converted to other phospholipids via the intermediates CDP-DAG and DAG (12, 15, 87). Choline may also be derived from PC through the phospholipase B and glycerophosphocholine phosphodiesterase activities encoded by *NTE1* (91) and *GDE1* (92), respectively (93–95). Analysis of mutants indicates that the physiological roles of PC synthesized by the two pathways may be different (93, 96, 97).

The Kennedy pathway plays a critical role in the synthesis of PE and PC when the enzymes in the CDP-DAG pathway are defective (12, 36). For example, the *cho2 opi3* mutant deficient in the three-step methylation of PE requires choline supplementation for growth and synthesizes PC via the CDP-choline branch of the Kennedy pathway (68–70, 98). The *cho1* and *psd1 psd2* mutants deficient in the synthesis of PS (99, 100) and PE (66, 101), respectively, can synthesize PC if they are supplemented with ethanolamine or choline. The ethanolamine is incorporated into PE via the CDP-ethanolamine branch of the Kennedy pathway, and the PE is subsequently methylated to form PC. Mutants defective in the CDP-DAG pathway can also synthesize PE or PC when they are supplemented with lysoPE, lysoPC, or PC with short acyl chains. LysoPE and lysoPC transported into the cell are acylated to PE and PC, respectively, by the *ALE1*-encoded lysophospholipid acyltransferase, which also utilizes lysoPA as a substrate (41, 42, 102, 103). Short acyl chain PC, which is not incorporated directly into membranes, is remodeled with 16- and

18-carbon acyl chains (104) by the activities of phospholipase B and lysophospholipid acyltransferase (94, 95, 105). The Kennedy pathway mutants (i.e., *cki1 eki1* and *cpt1 ept1*) defective in both the CDP-choline and CDP-ethanolamine branches can synthesize PC only by the CDP-DAG pathway (73, 106, 107). However, unlike the CDP-DAG pathway mutants (66, 68–70, 98–101), the Kennedy pathway mutants do not exhibit any auxotrophic requirements and have an essentially normal complement of phospholipids (73, 107).

GENETIC AND BIOCHEMICAL MECHANISMS THAT CONTROL PHOSPHOLIPID SYNTHESIS

The synthesis of phospholipids is regulated by controlling the expression of enzymes and/or by modulating the catalytic activities. The expression of phospholipid synthesis genes is controlled by multiple factors, including carbon source, nutrient availability, growth stage, pH, and temperature (12, 13, 18, 20, 95, 108, 109). The mechanisms responsible for the regulation of gene expression include a number of *cis*- and *trans*-acting elements (14, 18, 20). Of these, the inositol-responsive *cis*-acting element (UAS_{INO}) and the corresponding *trans*-acting factors (Ino2p, Ino4p, Opi1p) have been well characterized for transcriptional regulation of phospholipid synthesis genes and are discussed here.

Genes encoding enzymes in the CDP-DAG (e.g., *CDS1*, *CHO1*, *PSD1*, *CHO2*, and *OPI3*) and Kennedy (e.g., *EK11*, *EPT1*, *CK11*, *CPT1*) pathways, and in the synthesis of PI (e.g., *INO1*), as well as the genes encoding the inositol (*ITR1*) and choline/ethanolamine (*HNMI*) permeases are coordinately regulated through a UAS_{INO} element in the promoter. The UAS_{INO} element binds the Ino2p-Ino4p heterodimer that activates transcription, and transcriptional activation is repressed when Opi1p binds to Ino2p (14, 18). Thus, the *opi1* mutant exhibits derepressed levels of the UAS_{INO}-containing genes, whereas the *ino2* and *ino4* mutants exhibit repressed levels of UAS_{INO}-containing genes

UAS_{INO}: an inositol-responsive *cis*-acting element found in the promoter of several phospholipid synthesis genes

Opi1p: a repressor protein that interacts with Ino2p to attenuate transcriptional activation by the Ino2p-Ino4p complex

Ino2p-Ino4p heterodimer: a complex of regulatory proteins that interact with the UAS_{INO} element in the promoter to activate transcription

Nuclear/endoplasmic reticulum (ER) membrane:

the outer nuclear membrane that is continuous with the endoplasmic reticulum membrane

(28, 29, 36, 37). Because of the constitutive derepression of *INO1* expression, *opi1* mutants produce excessive amounts of inositol and excrete it into the growth medium, but *ino2* and *ino4* mutants lacking transcriptional activation of *INO1* are auxotrophic for inositol (28, 29, 36, 37). These inositol-related phenotypes are commonly used as indicators of the misregulation of UAS_{INO}-containing genes (36, 37).

The repressor function of Opi1p is controlled by its cellular location (14, 18, 110). Opi1p, which lacks a membrane-spanning domain (111), is found at the nuclear/endoplasmic reticulum (ER) membrane and within the nucleus (110, 112, 113). It associates with the membrane through interaction with the integral membrane protein Scs2p, and its membrane association is stabilized by interaction with PA (110, 112). The involvement of Scs2p in the Opi1p-mediated regulation of phospholipid synthesis is evident because the *scs2* mutant is auxotrophic for inositol, a phenotype shown by the constitutive repression of *INO1* (114–116). This observation predicts a nuclear localization of Opi1p for its increased repressor function. Interestingly, the *scs2* mutant also shows an increase in PC synthesis via the Kennedy pathway, and a block in PC synthesis (e.g., *cki1 scs2*) restores normal *INO1* expression (116). As discussed above, PC synthesis via the Kennedy pathway consumes PA via DAG, and a block in the Kennedy pathway should result in PA accumulation. Thus, the suppression of inositol auxotrophy for the *scs2* mutant might be explained by a change in the localization of Opi1p to the nuclear/ER membrane through interaction with PA.

Genetic and biochemical data indicate that PA has a major effect on the localization and function of Opi1p (14). According to a recent model (**Figure 3**) (14, 18, 110), Opi1p is tethered to the nuclear/ER membrane via interactions with Scs2p and PA. When PA levels are reduced, Opi1p is released from the membrane and enters into the nucleus, where it attenuates transcription by binding to Ino2p. That PA governs the Opi1p-mediated regulation of UAS_{INO}-containing genes is supported by the

analyses of mutants defective in the CDP-DAG and Kennedy pathways (14). For example, mutants (e.g., *cho1*, *psd1*, *cho2*, and *opi3*) defective in any step of the CDP-DAG pathway exhibit increased levels of PA and excrete inositol owing to constitutive derepression of *INO1* expression (36, 37). However, the inositol excretion phenotype can be alleviated when the mutants are supplemented with a water-soluble precursor (e.g., ethanolamine and choline) that channels PA to phospholipid synthesis via the Kennedy pathway (12, 37). In combination with a mutation in the *SEC14*-encoded PI/PC transfer protein, CDP-choline pathway mutants (e.g., *cki1 sec14*, *pct1 sec14*, and *cpt1 sec14*) excrete inositol and choline, and this phenotype is dependent on the excess productions of PA and choline by the *SPO14*-encoded phospholipase D-mediated turnover of PC (87, 117).

The level of PA can be directly affected by the activities of lysoPA acyltransferase, CDP-DAG synthase, PA phosphatase, DAG kinase, and phospholipase D. Of these enzymes, PA phosphatase and DAG kinase have emerged as key regulators of PA in the expression of UAS_{INO}-containing genes. Cells lacking the *PAH1*-encoded PA phosphatase activity contain an elevated level of PA and exhibit the derepression of UAS_{INO}-containing phospholipid synthesis genes (e.g., *INO1*, *OPI3*, and *INO2*) (24, 108, 118). By contrast, the overexpression of PA phosphatase activity causes the loss of PA, the repression of *INO1* expression, and inositol auxotrophy (119). The lack of PA phosphatase activity also causes the abnormal expansion of the nuclear/ER membrane (108, 118), underscoring the importance of phospholipid synthesis to organelle synthesis and structure. The *DGK1*-encoded DAG kinase counteracts the role that the *PAH1*-encoded PA phosphatase plays in controlling PA content and the transcriptional regulation of UAS_{INO}-containing genes (83, 84). The overexpression of DAG kinase causes an increase in PA content, the derepression of UAS_{INO}-containing genes, and the abnormal nuclear/ER membrane expansion (83) as in those shown in the *pab1* mutant (108, 118). Yet, the overexpression of

DAG kinase activity complements the inositol auxotrophy caused by the overexpression of PA phosphatase activity (83), whereas the loss of DAG kinase activity (e.g., in the *dgk1* mutation) complements the phenotypes caused by the loss of PA phosphatase activity (e.g., in the *pab1* mutation) (83, 84).

The PA-mediated regulation of UAS_{INO}-containing genes is triggered by the availability of inositol, zinc, and nitrogen as well as the growth stage (12, 18, 20). For example, the supplementation of the essential nutrient zinc to the growth medium activates the expression of UAS_{INO}-containing genes (20, 120). By contrast, inositol supplementation represses the gene expression (12, 14, 18), and the repressive effect is enhanced by ethanolamine or choline supplementation (12). The UAS_{INO}-containing genes are maximally expressed in the exponen-

tial phase of growth, whereas they are repressed in the stationary phase of growth (12, 14, 18). In many cases, it is the biochemical regulation of a phospholipid synthesis enzyme that ultimately controls the cellular level of PA. The following examples typify this theme of regulation.

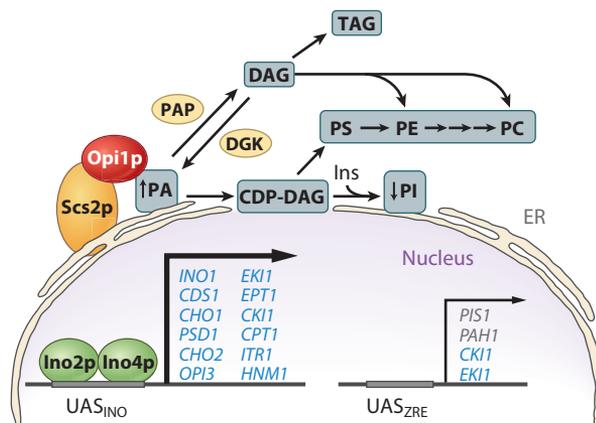
Inositol-Mediated Regulation

Inositol is an essential phospholipid precursor in yeast cells because it is incorporated, through the major phospholipid PI, into polyphosphoinositides (30), sphingolipids (121), and

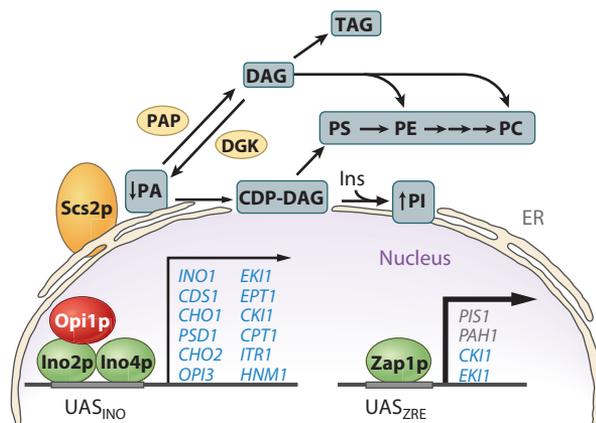
Figure 3

Model for the phosphatidate (PA)-mediated regulation of UAS_{INO}-containing phospholipid synthesis genes. (a) Under growth conditions whereby the levels of PA are increased, the Opi1p repressor is tethered to the nuclear/endoplasmic reticulum (ER) membrane via interactions with Scs2p and PA, allowing the maximal expression (*bold arrow*) of UAS_{INO}-containing genes (*blue*) by the Ino2p-Ino4p activator complex. (b) Under growth conditions whereby the levels of PA are reduced, Opi1p is dissociated from the nuclear/ER membrane and enters into the nucleus, where it binds to Ino2p and attenuates (*thin arrow*) the transcriptional activation by the Ino2p-Ino4p complex. The PA level in the cell is decreased by the stimulation of phosphatidylinositol (PI) synthesis in response to inositol (Ins) supplementation and by the Zap1p-mediated induction of *PIS1* that occurs in response to zinc depletion. The regulations that occur in response to zinc depletion and stationary phase occur in the absence of inositol supplementation. PA phosphatase (PAP) and DAG kinase (DGK) play major roles in the regulation of PA content and thereby in the transcriptional regulation of UAS_{INO}-containing genes. CDP-DAG, CDP-diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; TAG, triacylglycerol; UAS_{INO}, inositol-responsive element; UAS_{ZRE}, zinc-responsive element; Zap1p, a transcriptional activator protein that interacts with the UAS_{ZRE}.

a Inositol depletion, zinc supplementation, and exponential phase



b Inositol supplementation, zinc depletion, and stationary phase



Zap1p:

a transcriptional activator protein that interacts with the UAS_{ZRE}

UAS_{ZRE}:

a zinc-responsive *cis*-acting element found in the promoter of some phospholipid synthesis genes

glycosylphosphatidylinositol anchors (122). The syntheses and physiological roles of these inositol-containing membrane components are covered in recent review articles (30, 121, 122). As discussed above, inositol is synthesized in the cell, but when supplemented to the growth medium, it affects the *de novo* synthesis of inositol and membrane phospholipid synthesis (12, 13, 18, 28, 36). Inositol supplementation causes an increase in the synthesis of PI by a mechanism that involves increased substrate availability for PI synthase (123). In addition, inositol directly inhibits the activity of PS synthase, which favors the utilization of their common substrate CDP-DAG for PI synthesis (123). This biochemical regulation draws upon the PA content through CDP-DAG and causes the translocation of Opi1p into the nucleus for repression of UAS_{INO}-containing genes (14, 110). Overall, the repression of the UAS_{INO}-containing genes leads to a decrease in the synthesis of enzymes used in both the CDP-DAG and Kennedy pathways, and this repression causes changes, including an increase in PI and decreases in PA, PS, and PC, in the phospholipid composition (12, 123). Changes in membrane phospholipid composition are also the result of phospholipid turnover that is mediated by enzymes, such as the *NTE1*-encoded phospholipase B (94, 124–126). In fact, the activity of this phospholipase B, which does not have a direct effect on the metabolism of PA, attenuates the repressor function of Opi1p when PC synthesis via the Kennedy pathway is stimulated by choline supplementation at an elevated temperature (126). Inositol supplementation also has global effects on cell physiology, including the unfolded protein response and cell wall integrity pathways (13, 127, 128).

Zinc-Mediated Regulation

The synthesis of phospholipids is coordinately regulated with the expression of zinc transporters that control zinc homeostasis (20, 129). Cells grown in zinc-depleted medium exhibit the induced expression of zinc transporters (e.g., Zrt1p, Zrt2p, Fet4p, and Zrt3p)

to maintain the cytoplasmic levels of zinc (130, 131). At the same time, zinc depletion causes alterations in membrane phospholipid composition that are brought about by changes in the expression of phospholipid synthesis enzyme activities (20, 120, 132–134). The regulation of UAS_{INO}-containing genes by zinc involves the control of PA content through the activation of *PIS1*-encoded PI synthase and *PAH1*-encoded PA phosphatase activities. This regulation, which occurs in the absence of inositol supplementation, is mediated by the zinc-sensing and zinc-inducible transcriptional activator Zap1p and the zinc-responsive *cis*-acting element (UAS_{ZRE}) (129). Zinc depletion causes increased expressions of *PIS1* (133) and *PAH1* (A. Soto-Cardalda & G.M. Carman, unpublished data) by the interaction of Zap1p with a UAS_{ZRE} in the promoter. The net results are the Opi1p-mediated repression of UAS_{INO}-containing genes and a decrease in the activities of the CDP-DAG pathway enzymes (120). The major effects of zinc depletion on phospholipid composition include an increase in PI and a decrease in PE (120). Although enzyme activities in the CDP-DAG pathway are reduced by zinc depletion, the amount of PC is not significantly affected (120). Maintenance of a normal PC content is attributed to the Zap1p-mediated inductions of choline kinase and ethanolamine kinase for PC synthesis via the Kennedy pathway (132, 135). Like *PIS1* and *PAH1*, the *CKI1* and *EKI1* genes contain a UAS_{ZRE} in their promoters that interacts with Zap1p for gene activation (132, 135). Any effect that Opi1p would have on the expressions of *CKI1* and *EKI1* (because they contain UAS_{INO} elements) is overcome by the derepression by Zap1p (132, 135).

The fact that the zinc transporters are localized to cellular membranes raises the question as to whether zinc-mediated alterations in phospholipid composition might regulate transporter function. Several reports have shown that PE plays a major role in transporter function. For example, PE is required for amino acid transporter function in *S. cerevisiae* (136, 137), and its content in *Escherichia coli* is required for function of the α -aminobutyric

acid (138), lactose (139, 140), and phenylalanine (141) transporters. The availability of mutants (e.g., *eki1*, *psd1*, *psd2*) defective in PE synthesis should facilitate studies to address the importance of changing PE content for the zinc transport function in *S. cerevisiae*.

CTP-Mediated Regulation

In *S. cerevisiae*, the nucleotide CTP plays a critical role in phospholipid synthesis as the direct precursor of the high-energy intermediates CDP-DAG, CDP-choline, and CDP-ethanolamine that are used in the CDP-DAG and Kennedy pathways (Figure 2) (17). It is also used as the phosphate donor for the synthesis of PA by the DAG kinase (84). CTP synthetase (81, 82), which produces CTP, is allosterically inhibited by the product (142, 143), and this regulation ultimately determines the intracellular concentration of CTP (142, 144). CTP inhibits the CTP synthetase activity by increasing the positive cooperativity of the enzyme for UTP and by simultaneously decreasing the enzyme's affinity for UTP (142, 143). However, CTP synthetases containing an E161K mutation are less sensitive to CTP product inhibition (145), and cells expressing the mutant enzymes exhibit a 6- to 15-fold increase in their CTP level (145). They also show alterations in the synthesis of membrane phospholipids, which include a decrease in the synthesis of PS and an increase in the synthesis of PC, PE, and PA (145). The decrease in the synthesis of PS results from direct inhibition of PS synthase activity by CTP (144), and this inhibition favors the synthesis of phospholipids by the Kennedy pathway. The increase in PC synthesis is ascribed to a higher utilization of the CDP-choline branch of the Kennedy pathway owing to the stimulation of phosphocholine cytidyltransferase activity (144, 145) by the increased substrate availability of CTP (144, 146). Likewise, the increase in PE synthesis could be attributed to stimulation of phosphoethanolamine cytidyltransferase activity. The increase in PA content may result from the stimulation of DAG kinase activity

by increased availability of its substrate CTP (84). The cells expressing the E161K mutant enzyme excrete inositol (145), a characteristic phenotype that typifies the misregulation of UAS_{INO}-containing phospholipid synthesis genes when cells accumulate an excess of PA (14). It is unclear whether UAS_{INO}-containing genes in the CDP-DAG and Kennedy pathways are derepressed in CTP overproducing cells, but the overriding regulation that governs the utilization of the two pathways appears to be biochemical in nature.

CDP-Diacylglycerol-Mediated Regulation

The PA phosphatase and DAG kinase enzymes are differentially regulated by CDP-DAG. This liponucleotide intermediate stimulates PA phosphatase activity (147) but inhibits DAG kinase activity (84). However, the opposing regulations of PA metabolic enzymes favor a decrease in PA content and the Opi1p-mediated repression of UAS_{INO}-containing genes. One of the UAS_{INO}-containing genes that are repressed by Opi1p is *CDS1* (148), which codes for CDP-DAG synthase (46). Thus, the regulation of its own expression provides a mechanism for controlling the synthesis of CDP-DAG from PA and the CDP-DAG-dependent synthesis of phospholipids. This regulation is supported by the genetic evidence that a conditional *cds1* mutant defective in CDP-DAG synthase activity exhibits an elevated PA content and the derepression of UAS_{INO}-containing genes (149, 150). 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 neutral lipid TAG.

S-Adenosyl-L-Homocysteine-Mediated Regulation

S-Adenosyl-L-homocysteine (AdoHcy) is a product of the AdoMet-dependent methylation reactions that are catalyzed by the *CHO2*-encoded PE methyltransferase and

Phosphorylation: a protein modification that regulates the functions of Opi1p and key phospholipid synthesis enzymes

OPI3-encoded phospholipid methyltransferase in the CDP-DAG pathway (**Figure 2**). AdoHcy, which is removed by the *SAHI*-encoded AdoHcy hydrolase (23), is a competitive inhibitor of the methyltransferase enzymes (151). 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 (23). Although the effects of AdoHcy on phospholipid composition have not been addressed, its accumulation causes an increase in TAG synthesis and lipid droplet content (23).

REGULATION OF PHOSPHOLIPID SYNTHESIS BY PHOSPHORYLATION

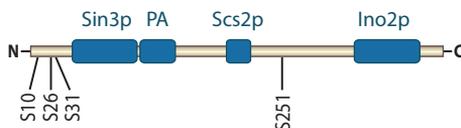
Phosphorylation is a major covalent posttranslational modification by which the activity of an enzyme or a transcription factor is regulated (152–157). Enzyme phosphorylation can affect catalytic activity and/or subcellular localization. Phosphorylation of a regulatory protein can control its localization, stability, and interaction with DNA or other proteins. Data indicate that phospholipid synthesis in yeast is regulated by phosphorylation at Ser and Thr residues. The protein kinases known to regulate the function of catalytic and regulatory proteins in phospholipid synthesis include protein kinase A, protein kinase C, casein kinase II, and cyclin-dependent kinase. Protein kinase A is the principal mediator of signals transmitted through the *RAS*/cAMP pathway (158, 159). Its activity is required for proper regulation of growth, progression through the cell cycle, and development in response to various nutrients (158, 159). Protein kinase A consists of two catalytic subunits (encoded by *TPK1*, *TPK2*, and *TPK3*) and two regulatory subunits (encoded by *BCY1*). Elevated cAMP levels, which are controlled by adenylate cyclase (encoded by *CYR1*) via the *RAS*-cAMP pathway, promote dissociation of the regulatory subunits from the catalytic subunits and thus allow the catalytic subunits to phosphorylate a variety of

substrates (158, 159). Protein kinase C (encoded by *PKC1*) is essential for the progression of the cell cycle (160) and plays a role in cell wall formation (161). Casein kinase II is essential for cell viability (162–164), and the enzyme is composed of two catalytic (encoded by *CKA1* and *CKA2*) and two regulatory (*CKB1* and *CKB2*) subunits (165–168). The *CDC28* (*CDK1*)-encoded cyclin-dependent kinase is a master regulator of cell-cycle transitions whose activity is governed by interactions with various G1 and B-type cyclins (169). Phospholipid synthesis enzymes, which are regulated by phosphorylation, include PS synthase, CTP synthetase, choline kinase, and PA phosphatase. The transcriptional repressor Opi1p is also regulated by phosphorylation.

Opi1p Phosphorylation

The Opi1p repressor plays a negative regulatory role in the expression of UAS_{INO}-containing genes involved in the synthesis of membrane phospholipids (111, 170). In vivo labeling studies have shown that Opi1p is phosphorylated at multiple Ser residues (171, 172). In vitro studies indicate that protein kinase A (172), protein kinase C (171), and casein kinase II (173) play major roles in the phosphorylation. The major sites of Opi1p phosphorylation include Ser¹⁰ (for casein kinase II), Ser²⁶ (for protein kinase C), and Ser³¹ and Ser²⁵¹ (for protein kinase A) (**Figure 4**) (171–173). The analysis of *opi1* cells expressing phosphorylation-deficient (e.g., Ser → Ala mutations) forms of Opi1p indicates that protein kinases A and C are responsible for ~50% of the total phosphorylation that occurs in vivo (171, 172). By contrast, phosphorylation by casein kinase II does not have a major effect on the extent of Opi1p phosphorylation in vivo (173). In vitro the mutation (S26A) in the protein kinase C target site reduces the phosphorylation of Opi1p by protein kinase A. Likewise, the mutations (S31A and S251A) in protein kinase A target sites reduce the phosphorylation by protein kinase C. By contrast, the mutation (S10A) in the casein kinase II target site does not affect the in vitro

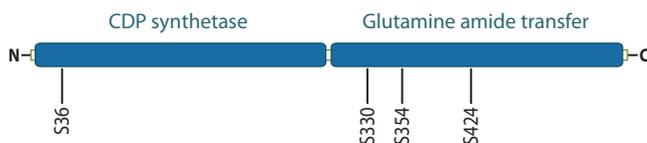
Opi1p



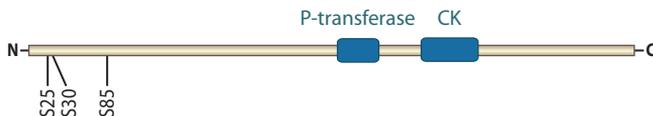
PS synthase



CTP synthetase



Choline kinase



PA phosphatase

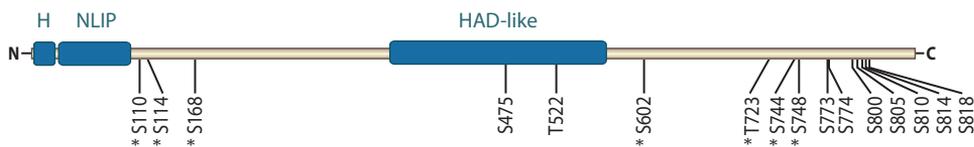


Figure 4

Domain structures and phosphorylation sites of Opi1p and phospholipid synthesis enzymes. Opi1p (with 404 amino acids) contains domains for interactions with Sin3p, phosphatidate (PA), Scs2p, and Ino2p. It also contains Ser (S) residues that are sites for phosphorylation by protein kinases A and C and casein kinase II. Phosphatidylserine (PS) synthase (with 277 amino acids) contains a CDP-alcohol phosphotransferase (P-transferase) domain and Ser residues for phosphorylation by protein kinase A. CTP synthetase (with 579 amino acids) contains the CTP synthetase and glutamine amide transfer domains as well as the Ser residues for phosphorylation by protein kinases A and C. Choline kinase (with 582 amino acids) contains the phosphotransferase and choline kinase (CK) domains as well as the Ser residues for phosphorylation by protein kinases A and C. PA phosphatase (with 862 amino acids) contains an amphipathic helix (H), NLIP and haloacid dehalogenase (HAD)-like domains, and 16 Ser/Thr residues for phosphorylation. The seven sites denoted with an asterisk are within the minimal Ser/Thr-Pro motif that is a target of cyclin-dependent kinases.

phosphorylation by either protein kinase A or protein kinase C. Furthermore, the mutations in the protein kinase A or protein kinase C target sites do not affect the phosphorylation of Opi1p by casein kinase II. These results indicate that phosphorylation by protein kinase A stimulates phosphorylation by protein kinase C and vice versa and that the phosphorylations by these kinases are independent of the phosphorylation by casein kinase II. The hierarchical phosphorylations by protein kinases A and C provide an explanation as to why the protein kinase A and protein kinase C phosphorylation site mutations affect the overall phosphorylation state of Opi1p in vivo and as to why the casein kinase II site mutation does not have a major effect on the overall phosphorylation state of the protein.

The phosphorylation of Opi1p at Ser¹⁰, Ser³¹, and Ser²⁵¹ stimulates its repressor function (172), whereas phosphorylation at Ser²⁶ attenuates its repressor function (171). The regulation of Opi1p function via phosphorylation by protein kinases A and C occurs in cells grown in the absence and presence of inositol (171, 172), whereas the regulation via phosphorylation by casein kinase II occurs only when cells are grown in the absence of inositol (173). Opi1p possesses binding domains for PA, Scs2p, Ino2p, and Sin3p (Figure 4) (110, 112, 174, 175), which affect its localization and function. Whether the phosphorylation of Opi1p by protein kinases A and C, as well as by casein kinase II, influences the interaction of Opi1p with its binding partners warrants further investigation.

Phosphatidylserine Synthase Phosphorylation

The *CHO1*-encoded PS synthase is one of the most highly regulated enzymes for the synthesis of phospholipids in *S. cerevisiae* (12, 15, 176). This ER-associated enzyme catalyzes the formation of PS in the Mn²⁺-dependent sequential reaction that displaces CMP from CDP-DAG with Ser (60–62, 177, 178). PS synthase possesses a CDP-alcohol phosphotransferase domain that is shared by other phospholipid

synthesis enzymes (e.g., PI synthase and PGP synthase) catalyzing similar types of reactions (Figure 4) (179). PS synthase exists in two forms that differ in the electrophoretic mobility (30 kDa and 27 kDa). The 30-kDa form of PS synthase is produced from the 27-kDa form by protein kinase A-mediated phosphorylation at Ser⁴⁶ and Ser⁴⁷ (180). The abundance of the two forms is dependent on the cell growth phase but not on the regulated expression of *CHO1* by inositol supplementation or respiratory deficiency (180). The 30-kDa and 27-kDa forms are present in exponential phase cells, whereas the 27-kDa form is primarily present in the stationary phase cells.

Phosphorylation of PS synthase by protein kinase A inhibits its catalytic activity (181), and the inhibitory effect of phosphorylation is abolished by S46A/S47A mutations (180). The expression of phosphorylation-deficient PS synthase shows that its cellular level is about twofold lower than that of the wild-type enzyme, resulting in a reduction of the total PS synthase activity. The lower PS synthase activity in cells expressing the S46A/S47A mutant enzyme correlates with a reduction in PS relative to PI and a decrease in PS synthesis in vivo (180). These observations support the conclusion that protein kinase A phosphorylation has dual roles in the regulation of PS synthase. On the one hand, phosphorylation inhibits PS synthase activity, but on the other hand, it stabilizes the abundance of the enzyme. The dual regulation of PS synthase results in a net increase in cellular PS synthase activity, which must be important to the optimal function of PS synthase during the exponential phase of cell growth (182). When the need for phospholipid synthesis is reduced in the stationary phase (183), the total amount of PS synthase is reduced because of a lack of phosphorylation and, at the same time, because of reduced gene repression (184).

CTP Synthetase Phosphorylation

CTP synthetase that is encoded by *URA7* and *URA8* is an essential cytosolic enzyme that

catalyzes a committed step in the synthesis of membrane phospholipids in *S. cerevisiae* (81, 82). The enzyme contains conserved CTP synthetase and glutamine amide transfer domains that are involved in catalysis (**Figure 4**) (17). CTP synthetase catalyzes a complex set of reactions, including the ATP-dependent transfer of the amide nitrogen from glutamine (i.e., the glutaminase reaction) to the C-4 position of UTP to generate CTP (185, 186). GTP stimulates the glutaminase reaction by accelerating the formation of a covalent glutaminy enzyme intermediate (185, 186). The *URA7*-encoded CTP synthetase is phosphorylated by protein kinase A and by protein kinase C (187, 188). The phosphorylations by these protein kinases stimulate CTP synthetase activity by two- to threefold (187, 188). Kinetic analyses show that the mechanisms for stimulation of CTP synthetase activity by these protein kinases are the same (187, 188). Phosphorylated CTP synthetase shows an increase in the V_{\max} with respect to the substrates UTP and ATP, a decrease in the K_m value for ATP, and a decrease in the positive cooperativity of the enzyme for ATP (187, 188). Moreover, the phosphorylation of CTP synthetase by protein kinases A and C attenuates the regulation of its activity by CTP product inhibition (187, 188).

The effects of phosphorylation on the regulation of CTP synthetase activity involve the oligomerization of the enzyme (142, 189). CTP synthetase exists as a dimer in the absence of ATP and UTP, but the enzyme forms a tetramer in the presence of saturating concentrations of the substrates (142, 189). The kinetics of enzyme tetramerization correlates with the kinetics of enzyme activity. The product CTP does not inhibit the ATP/UTP-dependent tetramerization of the enzyme (142, 189). Phosphorylation of native CTP synthetase with protein kinases A and C facilitates the nucleotide-dependent tetramerization, whereas dephosphorylation of native CTP synthetase prevents its nucleotide-dependent tetramerization (189). This regulation

correlates with the inactivation of CTP synthetase activity (189). The rephosphorylation of the enzyme with protein kinases A and C results in a partial restoration of the nucleotide-dependent tetramerization of the enzyme, and this correlates with the partial restoration of CTP synthetase activity (189).

Ser⁴²⁴ is a target site for both protein kinase A and protein kinase C (190, 191). The phosphorylation of this site is required to maintain optimum CTP synthetase activity *in vivo* (190, 191). Protein kinase C also phosphorylates the enzyme at Ser³⁶, Ser³³⁰, Ser³⁵⁴, and Ser⁴⁵⁴ (192). Biochemical and physiological analyses of Ser→Ala mutations have shown that phosphorylations at the Ser residues, except Ser³³⁰, result in the stimulation of CTP synthetase activity (191, 192). The phosphorylation at Ser³³⁰ results in the inhibition of the enzyme activity (192). Moreover, *in vivo* studies using these mutants have shown that the regulatory effects of the phosphorylations at specific sites have an impact on the pathways by which membrane phospholipids are synthesized. Phosphorylations at Ser³⁶, Ser³⁵⁴, and Ser⁴⁵⁴ correlate with an increase in PC synthesis via the Kennedy pathway (191, 192). In contrast, phosphorylation at Ser³³⁰ correlates with a decrease in the utilization of the Kennedy pathway (192).

Choline Kinase Phosphorylation

The *CKII*-encoded choline kinase (74, 193) is a cytosolic enzyme that plays a regulatory role in the synthesis of PC via the CDP-choline branch of the Kennedy pathway (12, 86, 194). The enzyme catalyzes the phosphorylation of choline with ATP to form phosphocholine and ADP (195). Choline kinase contains conserved phosphotransferase and choline kinase motifs (196–198) that are involved in its catalytic function (**Figure 4**) (198–200). Choline kinase is phosphorylated at multiple Ser residues *in vivo*, and the phosphorylations at some of these sites are mediated by protein kinases A (201) and C (202).

Protein kinase A phosphorylates choline kinase at Ser³⁰ and Ser⁸⁵, with the former site having the major effect on enzyme regulation (203). Protein kinase C phosphorylates Ser²⁵ as well as Ser³⁰ (202). Because protein kinases A and C phosphorylate Ser³⁰, phosphorylation of choline kinase by one protein kinase reduces phosphorylation of the enzyme at the same site by the other protein kinase (202). Phosphorylation of choline kinase at Ser²⁵ by protein kinase C does not affect phosphorylation by protein kinase A (202). Analysis of *cki1 eki1* cells expressing S25A and S30A mutant forms of choline kinase indicates that the phosphorylations at Ser²⁵ and Ser³⁰ by protein kinases A and C stimulate activity (~twofold) and that these phosphorylations cause the concomitant increase in PC synthesis via the Kennedy pathway (202, 203).

Phosphatidate Phosphatase Phosphorylation

The *PAH1*-encoded PA phosphatase catalyzes the dephosphorylation of PA to yield DAG and P_i (24, 204). This reaction is specific for PA, with a cofactor requirement of Mg²⁺ ions, and is based on a catalytic motif within a haloacid dehalogenase-like domain of the enzyme (**Figure 4**) (16, 24, 118). PA phosphatase activity is associated with the cytosolic and membrane fractions of the cell, and its membrane association is peripheral in nature (24). The regulation of PA phosphatase activity governs the synthesis of TAG, the pathways by which phospholipids are synthesized, PA signaling and transcriptional regulation of UAS_{INO}-containing genes, and the growth of the nuclear/ER membrane (205).

PA phosphatase is phosphorylated at multiple sites in vivo (119). Mass spectrometry analysis of purified PA phosphatase, in combination with immunoblot analysis using an antiphospho Ser/Thr-Pro antibody, has identified 16 sites of phosphorylation (**Figure 4**); seven of these sites are located within the minimal Ser/Thr-Pro motif that is a target for cell cycle-regulated protein kinases (e.g.,

Cdc28p and Pho85p) (119). Proteome-wide in vitro phosphorylation analyses have shown that the enzyme is a target for protein kinases, including those encoded by *CDC28* (*CDK1*) (206), *PHO85* (207, 208), and *DBF2* (209). That PA phosphatase is a target for Cdc28p in vivo is supported by the observations that the electrophoretic mobility of PA phosphatase increases in a temperature-sensitive *cdc28-4* mutant defective in cyclin-dependent kinase activity and in a cyclin *clb3 clb4* mutant, whereas PA phosphatase electrophoretic mobility decreases when cells enter the mitotic phase of growth (108). Moreover, the slower-migrating PA phosphatase protein is recognized by the antiphospho Ser/Thr-Pro antibody that is specific for cell cycle-regulated phosphoepitopes having the minimal Ser/Thr-Pro motif (108, 210), and the seven sites of phosphorylation identified in PA phosphatase have this motif (119).

Phosphorylation of PA phosphatase has an inhibitory effect on the enzyme function in vivo. The Nem1p-Spo7p phosphatase complex is responsible for the dephosphorylation of PA phosphatase, and yeast lacking the phosphatase complex exhibits the phenotypes characteristic of the *pab1* mutant (e.g., derepression of phospholipid synthesis genes and aberrant nuclear/ER membrane expansion) defective in PA phosphatase activity (108). In contrast to phosphorylation, the dephosphorylation of PA phosphatase has a stimulatory effect on enzyme function in vivo. Yeast overexpressing PA phosphatase with simultaneous mutations of the seven sites within the Ser/Thr-Pro motif to nonphosphorylatable Ala residues exhibits inositol auxotrophy by exacerbating the Opi1p-mediated repression of *INO1* expression (presumably owing to reduced PA content) (119). In addition, the phosphorylation-deficient septuple mutant PA phosphatase exhibits elevated (1.8-fold) activity in vitro (119).

Genetic and biochemical data indicate that the association of PA phosphatase with the membrane, where its substrate PA resides, is essential to the enzyme's function in vivo (108, 119). The fact that the Nem1p-Spo7p

complex is associated with the nuclear/ER membrane (108, 211) indicates that phosphorylated PA phosphatase is recruited to the membrane for its dephosphorylation. In vivo and in vitro studies have shown that the interaction of PA phosphatase with the membrane is dependent on an amphipathic helix found at the N-terminal region of the enzyme (212) and that the interaction through the amphipathic helix is dependent on dephosphorylation by the Nem1p-Spo7p phosphatase complex (212).

CONCLUSIONS

The regulation of phospholipid synthesis in *S. cerevisiae* is a complex coordinated process that is governed by genetic and biochemical mechanisms, which are interrelated. Gene expression in *S. cerevisiae* is largely controlled by transcriptional regulation that is triggered by PA content through biochemical modulation of phospholipid synthesis enzymes. The phospholipid precursors, products, and metabolites, as well as phosphorylation, play important roles in this regulation.

SUMMARY POINTS

1. Phospholipid synthesis genes containing a UAS_{INO} element are transcriptionally activated by the Ino2p-Ino4p heterodimer, which is repressed by Opi1p.
2. The nuclear localization and repressor function of Opi1p is regulated by its interaction with Scs2p and PA at the nuclear/ER membrane.
3. PA is a phospholipid precursor and also plays a major role as a signaling molecule in the regulation of phospholipid synthesis gene expression.
4. PA phosphatase and DAG kinase play major roles in regulating PA levels.
5. Genetic and biochemical mechanisms are interrelated to control membrane phospholipid synthesis.
6. Phosphorylation regulates, either positively or negatively, the function of Opi1p and the activity and localization of key phospholipid synthesis enzymes.

FUTURE ISSUES

1. Structures of phospholipid synthesis enzymes and regulatory proteins need to be solved to elucidate the molecular mechanisms of catalytic function, membrane association, and gene expression.
2. Phosphorylation regulates the functions of Opi1p and phospholipid synthesis enzymes. The molecular mechanisms for these regulations need further examination. Information on the physiological conditions that stimulate and repress phosphorylation/dephosphorylation by specific protein kinases and phosphatases is needed.
3. Data from the global analyses of gene and protein expressions, protein modifications, and metabolites (e.g., lipidomics) need to be evaluated and integrated for designing novel research approaches to better understand the regulation of phospholipid synthesis and its relationship with other metabolic pathways.

4. Some reactions in phospholipid synthesis are catalyzed by more than one enzyme (e.g., glycerol-3-phosphate and lysoPA acyltransferases, PS decarboxylase, CTP synthetase, and PA phosphatase). In the case of PS decarboxylase, enzyme activity is required for functions in different cellular compartments. For enzymes (e.g., acyltransferases and CTP synthetase) localized in the same cellular compartment, the reason for redundancy is not obvious and needs to be addressed.
5. Some enzymes participate in multiple biosynthetic pathways. For example, the product (DAG) of the PA phosphatase reaction is used for the synthesis of phospholipids and TAG, whereas the product (CTP) of the CTP synthetase reaction is used for the synthesis of phospholipids and nucleic acids. The mechanisms that control the utilization of the products for different metabolic processes warrant further investigations.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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