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Review

Regulation of phospholipid synthesis in *Saccharomyces cerevisiae* by zinc depletion

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Abstract

The synthesis of phospholipids in the yeast *Saccharomyces cerevisiae* is regulated by zinc, an essential mineral required for growth and metabolism. Cells depleted of zinc contain increased levels of phosphatidylinositol and decreased levels of phosphatidylethanolamine. In addition to the major phospholipids, the levels of the minor phospholipids phosphatidate and diacylglycerol pyrophosphate decrease in the vacuole membrane of zinc-depleted cells. Alterations in phosphatidylinositol and phosphatidylethanolamine can be ascribed to an increase in *PIS1*-encoded phosphatidylinositol synthase activity and to decreases in the activities of CDP-diacylglycerol pathway enzymes including the *CHO1*-encoded phosphatidylserine synthase, respectively. Alterations in the minor vacuole membrane phospholipids are due to the induction of the *DPP1*-encoded diacylglycerol pyrophosphate phosphatase. These changes in the activities of phospholipid biosynthetic enzymes result from differential regulation of gene expression at the level of transcription. Under zinc-deplete conditions, the positive transcription factor Zap1p stimulates the expression of the *DPP1* and *PIS1* genes through the *cis*-acting element $\mathrm{UAS_{ZRE}}$. In contrast, the negative regulatory protein Opi1p, which is involved in inositol-mediated regulation of phospholipid synthesis, represses the expression of the *CHO1* gene through the *cis*-acting element $\mathrm{UAS_{INO}}$. Regulation of phospholipid synthesis may provide an important mechanism by which cells cope with the stress of zinc depletion, given the roles that phospholipids play in the structure and function of cellular membranes.

Keywords: Phospholipid synthesis; Phosphatidylinositol synthase; Phosphatidylserine synthase; Transcriptional regulation; Yeast; Zinc

1. Introduction

The yeast *Saccharomyces cerevisiae* serves as a eukaryotic model organism to study the regulation of phospholipid synthesis [1–6]. Almost all of the structural genes responsible for phospholipid synthesis have been identified in *S. cerevisiae* [1–8], and many of the phospholipid biosynthetic enzymes have been purified from the organism [1–6]. The characterization of these genes along with their encoded enzymes has significantly advanced the understanding of phospholipid synthesis and its regulation in eukaryotes.

Phospholipids play diverse roles that are essential for growth and metabolism. It is well known that phospholipids govern membrane-associated functions such as enzyme catalysis, receptor-mediated signaling, and solute transport [9,10]. In addition, phospholipids are precursors for the synthesis of macromolecules [11–15], serve as molecular chaperons [16,17], serve in protein modification for membrane association [18], and are reservoirs of second messengers [19]. Thus, as shown in *S. cerevisiae*, the activities of phospholipid biosynthetic enzymes are regulated to cope with a variety of stress conditions (e.g., nutrient depletion) [6,20–23,23,24]. In this review, we will discuss how phospholipid synthesis in *S. cerevisiae* is regulated in response to zinc depletion.

2. Phospholipid biosynthetic pathways in *S. cerevisiae*

The major phospholipids found in the membranes of *S. cerevisiae* include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS) [1–3,6,25]. Mitochondrial membranes also contain phosphatidylglycerol (PG) and cardiolipin (CL) [1–3,6,25]. The most common fatty acids esterified to the glycerophosphate backbone of the phospholipids include palmitic acid, palmitoleic acid, stearic acid, and oleic acid [25–27].

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PA, phosphatidate; CDP-DG, CDP-diacylglycerol; DGPP, diacylglycerol pyrophosphate; $\mathrm{UAS_{ZRE}}$, upstream activating sequence zinc-responsive element; $\mathrm{UAS_{INO}}$, upstream activating sequence inositol-responsive element

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Phospholipid synthesis in *S. cerevisiae* occurs by complementary pathways common to those found in mammalian cells (Fig. 1) [1,2,6,28,29]. One exception is that in yeast PS is synthesized from CDP-diacylglycerol (CDP-DG) and serine, whereas in mammalian cells, PS is synthesized from PE or PC by an exchange reaction with serine [1,2,6]. In yeast, PS, PE, and PC are synthesized from phosphatidate (PA) via the CDP-DG pathway (Fig. 1). The energy-rich liponucleotide CDP-DG is synthesized from PA and CTP by the *CDS1*-encoded CDP-DG synthase [30,31]. CDP-DG may then donate its phosphatidyl moiety to serine to form PS [32] in the reaction catalyzed by the *CHO1*-encoded PS synthase [33–35]. PS is dephosphorylated to PE by the *PSD1*- [36,37] and *PSD2*-encoded [38] PS decarboxylase enzymes. PE is then converted to PC by the three-step AdoMet-dependent methylation reactions [39]. The first methylation reaction is catalyzed by the *CHO2*-encoded PE methyltransferase [40,41] and the last two methylation reactions are catalyzed by the *OPI3*-encoded phospholipid methyltransferase [40,42].

PE and PC are also synthesized via the CDP-ethanolamine and CDP-choline branches of the Kennedy pathway (Fig. 1). Ethanolamine and choline are phosphorylated with ATP to form phosphoethanolamine and phosphocholine, respectively, by the *EKI1*-encoded ethanolamine kinase [43] and the *CKI1*-encoded choline kinase [44]. Phosphoethanolamine and phosphocholine are activated with CTP to form CDP-ethanolamine and CDP-choline, respectively, by the *ECT1*-encoded phosphoethanolamine cytidylyltransferase [45] and the *PCT1*-encoded phosphocholine cytidylyltransferase [46]. CDP-ethanolamine and CDP-choline react with diacylglycerol (DG) to form PE and PC, respectively, in the reaction catalyzed by the *EPT1*-encoded ethanolamine phosphotransferase [47,48] and the *CPT1*-encoded choline phosphotransferase [49,50].

The *PIS1*-encoded PI synthase [51,52] catalyzes the formation of PI by displacement of CMP from CDP-DG with inositol [53]. The inositol used in this reaction is derived from glucose-6-phosphate via the reactions catalyzed by the *INO1*-encoded inositol-3-phosphate synthase [54,55] and the *INM1*-encoded inositol-3-phosphate phosphatase [56]. In the CL pathway (not shown in Fig. 1), PGP is formed from CDP-DG by displacement of CMP with glycerol-3-phosphate in the reaction catalyzed by the *PGS1*-encoded phosphatidylglycerophosphate (PGP) synthase [57]. PGP is dephosphorylated to PG by a phosphatase. The *CRD1*-encoded CL synthase [58–60] catalyzes the reaction between PG and CDP-DG to generate CL. The CTP required for the synthesis of the activated, energy-rich intermediates (CDP-DG, CDP-ethanolamine, and CDP-choline) is derived from UTP by the *URA7*- [61] and *URA8*-encoded [62] CTP synthetase enzymes. The DG used for the synthesis of PE and PC via the Kennedy pathway is derived from PA by the *PAH1*-encoded Mg^{2+}-dependent PA phosphatase, and it is also used as a substrate for the synthesis of triacylglycerol [63].

The CDP-DG pathway is primarily used for the synthesis of PE and PC when cells are grown in the absence of ethanolamine and choline [1,2,6,64,65]. Yet, the Kennedy pathway contributes to the synthesis of PE and PC in this growth condition [5,43,66]. For example, the PC synthesized via the CDP-DG pathway is constantly hydrolyzed to choline and PA [66,67] by the *SPO14*-encoded [68,69] 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-DG and DG [2,3,6]. Analysis of mutants in *S. cerevisiae* [6,70,71] as well as in mammalian cells [72,73] indicates that the physiological roles of PC synthesized via the two pathways are different.

The Kennedy pathway plays a critical role in phospholipid synthesis when enzymes in the CDP-DG pathway are defective [1,2,4,6]. The *cho2 opi3* mutant deficient in the methylation of PE requires choline for growth and synthesizes PC via the CDP-choline branch of the Kennedy pathway [40–42,74]. The *cho1* and *psd1 psd2* mutants deficient in the synthesis of PS [75,76] and

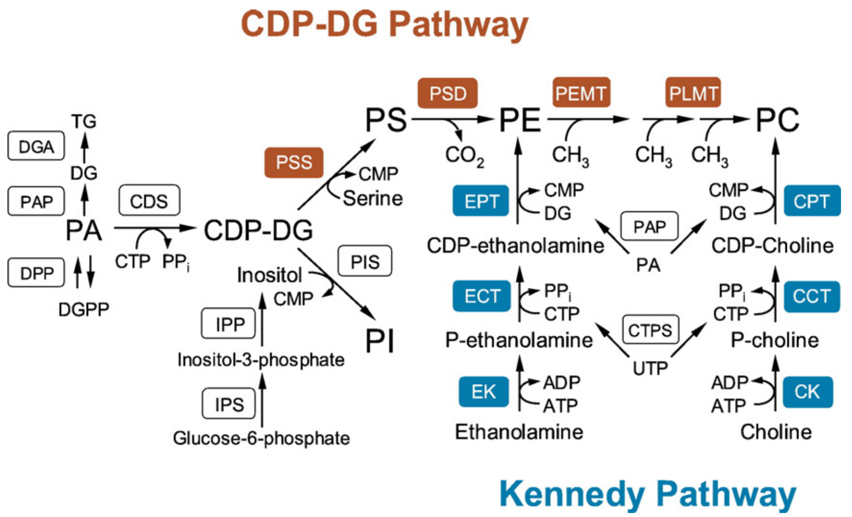

Fig. 1. Pathways for the synthesis of the major phospholipids in *S. cerevisiae*. The pathways shown for the synthesis of phospholipids include the relevant steps discussed in the review. The CDP-DG pathway enzymes (PSS, PS synthase; PSD, PS decarboxylase; PEMT, PE methyltransferase; and PLMT, phospholipid methyltransferase) are highlighted in red. The Kennedy pathway enzymes (EK, ethanolamine kinase; ECT, phosphoethanolamine cytidylyltransferase; EPT, ethanolaminephosphotransferase; CK, choline kinase; CCT, phosphocholine cytidylyltransferase; CPT, cholinephosphotransferase) are highlighted in blue. IPS, inositol phosphate synthase; IPP, inositol phosphate phosphatase; PIS, PI synthase; DPP, DGPP phosphatase; PAP, PA phosphatase; DGA, DG acyltransferase; CTPS, CTP synthetase.

PE [38,77], respectively, can synthesize PC when supplemented with ethanolamine. The ethanolamine is incorporated into PE via the CDP-ethanolamine branch of the Kennedy pathway, and the PE is subsequently methylated to form PC. The Kennedy pathway mutants (e.g., *cki1 eki1*, *cpt1 ept1*) defective in both the CDP-choline and CDP-ethanolamine branches can synthesize PC only via the CDP-DG pathway [43,78,79]. However, unlike the CDP-DG pathway mutants [38,40–42,74–77], the Kennedy pathway mutants do not exhibit any auxotrophic requirements [43,79]. These mutants have an essentially normal complement of phospholipids including PC [43,79].

3. Zinc-mediated regulation of the *DPP1*-encoded DGPP phosphatase

Zinc is an essential nutrient required for the growth and metabolism of *S. cerevisiae* and higher eukaryotes [80]. Zinc is a cofactor for key metabolic enzymes such as alcohol dehydrogenase, carbonic anhydrase, proteases, RNA polymerases, and superoxide dismutase [80]; it is also a structural component of a diverse set of proteins such as chaperons, lipid binding proteins, and transcription factors [81,82]. Accordingly, an insufficient amount of zinc is detrimental to organisms. Zinc deficiency in rats is associated with oxidative damage to DNA, lipids, and proteins [83]; in humans, it is manifested by defects in appetite, cognitive function, embryonic development, epithelial integrity, and immune function [84].

In the genome-wide analysis of gene expression in the *S. cerevisiae* cell depleted of zinc, *DPP1* was identified as one of the most highly induced genes, and this regulation is coordinated with the regulation of genes responsible for zinc transport [85] (see below). The *DPP1* gene encodes diacylglycerol pyrophosphate (DGPP) phosphatase that is associated with the vacuole membrane [24,86–88]. This enzyme catalyzes the removal of the β-phosphate from DGPP to form PA, followed by the dephosphorylation of PA to form DG [86]. Since DGPP is a preferred substrate to PA, the *DPP1*-encoded DGPP phosphatase does not dephosphorylate PA in the presence of relatively low levels of DGPP [86]. The DGPP molecule was originally identified in plants as the product of the PA kinase [89]. Research with plants under a variety of stress conditions indicates that DGPP may function as a signaling molecule [90,91,91–93]. The accumulation of DGPP is transient and coincides with a rise in the level of PA [91,93].

The regulation of *DPP1* expression in zinc-depleted cells is mediated by the zinc-sensing and zinc-inducible transcription factor Zap1p [94–97], which binds to the *cis*-acting element UAS_{ZRE} (*z*inc-*r*esponsive *e*lement) in the *DPP1* promoter [24,85]. Analysis of mutants lacking zinc transporters in the plasma membrane and in the vacuole membrane indicates that *DPP1* expression is sensitive to the cytosolic level of zinc [24]. The regulation of DGPP phosphatase expression correlates with the metabolism of DGPP and PA in the vacuole membrane [98]. When cells are grown in zinc-rich conditions, DGPP and PA account for 0.6 mol% and 1.4 mol% of the total phospholipids in vacuole membranes. Under zinc-depleted conditions, however, the amounts of DGPP and PA are decreased to an undetectable level and 0.3 mol%, respectively [98].

The *DPP1* gene is not essential, and *dpp1*Δ mutant cells do not exhibit any distinct phenotypes under various growth conditions [87], including fluctuations in zinc supplementation [99]. Thus, the role of DGPP phosphatase during zinc depletion would have to complement other mechanisms that respond to this stress. Although the function of DGPP phosphatase in yeast is still unclear, we speculate that the enzyme controls the levels of DGPP and PA in vacuolar membranes, which in turn mediates the cellular functions occurring in response to zinc depletion.

4. Zinc-mediated regulation of phospholipid synthesis

Zinc depletion has more global effects on phospholipid synthesis in the cell. In addition to the changes in DGPP and PA, it results in a reduction in the level of PE and an increase in the level of PI in the vacuole membrane [98]. The cellular levels of PE and PI are also altered similarly in response to zinc depletion [23]. These changes in the major phospholipids are independent of the regulation of the *DPP1*-encoded DGPP phosphatase activity [100].

In zinc-depleted cells, the activity levels of all enzymes in the CDP-DG pathway (PS synthase, PS decarboxylase, PE methyltransferase, and phospholipid methyltransferase) are reduced, while the activity of PI synthase is elevated [23]. Thus, the decrease in the cellular PE content correlates with the decreases in the activities of PS synthase and PS decarboxylase, and the increase in the cellular PI content correlates with the increase in the activity of PI synthase. Although the activities of the phospholipid methyltransferase enzymes are also reduced in zinc-depleted cells, these changes do not have a major effect on PC content [23]. Stimulation of the Kennedy pathway for phospholipid synthesis appears to compensate for the decrease in activities of the CDP-DG pathway enzymes. Recent studies have shown that the *EKI1*-encoded ethanolamine kinase is induced in zinc-depleted cells, and this regulation is mediated in part by Zap1p [101].

The coordinate regulation of the PI synthase and PS synthase enzymes, which compete for CDP-DG (Fig. 1), is part of an overall mechanism by which the synthesis of PI is coordinately regulated with the synthesis of PC [1,2,4–6,102,103]. The induction of PI synthase expression may represent one mechanism by which cells cope with zinc depletion, given that PI is a precursor to inositol-containing lipid molecules (sphingolipids, phosphoinositides, and glycosylphosphatidylinositol anchors) that are essential to growth and metabolism [2,13,104–114]. The repression of PS synthase activity alleviates the competition both enzymes have for CDP-DG [115]. These important phospholipid biosynthetic enzymes are regulated differently at the level of transcription in zinc-depleted cells.

5. Zinc-mediated regulation of the *PIS1*-encoded PI synthase

The increase in the activity of PI synthase in zinc-depleted cells results from the regulation of the *PIS1* expression at the level of transcription, as shown by an increase in the levels of *PIS1* mRNA, its encoded protein Pis1p, and the β-galactosidase activity driven by a P_{PIS1}-*lac*Z reporter gene [116]. Like *DPP1*, the regulation of *PIS1* transcription is mediated by Zap1p [116]. A model for the transcriptional regulation of *PIS1* by Zap1p in response to zinc depletion is shown in Fig. 2 (*left*). The promoter of the *PIS1* gene contains a sequence that shares homology with a consensus UAS_{ZRE} (ACCTTNAAGGT) [116]. Electrophoretic mobility shift assays with DNA probes containing the putative UAS_{ZRE} sequence and purified recombinant Zap1p show that the sequence in the *PIS1* promoter is required for Zap1p binding *in vitro* [116]. Moreover, mutations in sequence to a nonconsensus UAS_{ZRE} abolish Zap1p-DNA interactions *in vitro* and the induction of *PIS1* gene expression in response to zinc depletion [116]. In contrast to *DPP1*, the *PIS1* gene was not identified in the genome-wide analysis of gene expression that revealed 46 direct Zap1p target genes that are highly induced by zinc depletion [85]. This is attributed to the relatively modest level of *PIS1* induction (~2-fold) when compared with the >10-fold inductions of other Zap1p target genes (e.g., *DPP1*) [24,85]. Notwithstanding, the 2-fold induction of the *PIS1* gene in response to zinc depletion correlates with the ~2-fold increase in the PI content of yeast cells depleted of zinc [23].

6. Zinc-mediated regulation of the *CHO1*-encoded PS synthase

The expression of *CHO1*-encoded PS synthase is also controlled at the level of transcription in zinc-depleted cells [23]. In contrast to PI synthase, zinc depletion results in the repression of *CHO1* expression, resulting in decreased levels of the PS synthase mRNA, protein, and activity [23]. The downregulation of PS synthase expression and the lack of a UAS_{ZRE} in the *CHO1* promoter indicate that the transcription factor Zap1p does not

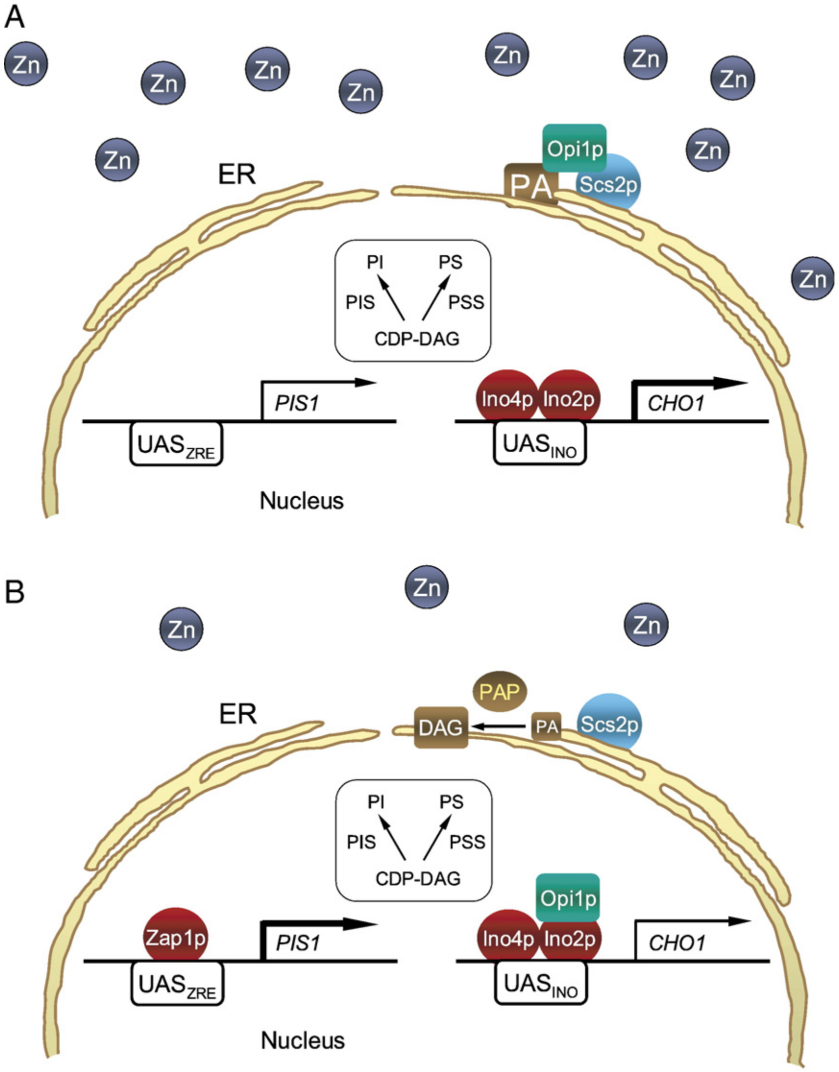

Fig. 2. Models for the transcriptional regulation of *PIS1* and *CHO1* by zinc depletion in the absence of inositol. (A) *PIS1* (*left*) and *CHO1* (*right*) are expressed at some level when cells are grown in a zinc-rich medium (depicted by numerous zinc atoms outside the nucleus). Maximum expression of *CHO1* (indicated by the bold arrow) is dependent on the interaction of the Ino2p–Ino4p complex with the UAS_{INO} element in the gene promoter. Under this growth condition, the repressor Opi1p is associated with the nuclear/ER membrane through interactions with PA and Scs2p. (B) when zinc is limiting (depicted by a reduced number of zinc atoms outside the nucleus), the Zap1p transcription factor is induced and binds to the UAS_{ZRE} in the *PIS1* gene promoter to increase transcription (indicated by the bold arrow). Transcription of *CHO1* is attenuated in zinc-depleted cells by the interaction of Opi1p with Ino2p (indicated by the thin arrow). Dissociation of Opi1p from the nuclear/ER membrane and its translocation into the nucleus are caused by a decrease in PA concentration. An increase in Mg^{2+}-dependent PA phosphatase (*PAP*) activity may be responsible for the decrease in PA concentration.

directly control the expression of PS synthase. Moreover, an indirect effect of Zap1p on the expression of PS synthase is ruled out because a *zap1*Δ mutation does not affect the zinc-mediated regulation of the enzyme [23]. Instead, the repression of PS synthase by zinc depletion is mediated through the UAS_{INO} element in the *CHO1* promoter and by the phospholipid synthesis regulatory proteins Opi1p, Ino2p, and Ino4p [23]. This conclusion is supported by the observations that mutations in the UAS_{INO} element abolish the zinc-mediated regulation of *CHO1* expression, and that regulation PS synthase expression by zinc depletion is lost in *ino2*Δ, *ino4*Δ, and *opi1*Δ mutants [23].

Ino2p, Ino4p, and Opi1p play an important role in the inositol-mediated regulation of *CHO1* and other UAS_{INO}-containing genes involved in phospholipid synthesis [4–6,117–119]. Inositol is an essential nutrient that can be synthesized in *S. cerevisiae* by the *INO1*-encoded inositol-3-phosphate synthase (Fig. 1). The essential nature of inositol stems from the fact that it is the water-soluble precursor for the synthesis of PI and other inositol-containing lipids [2,13,107,110–114]. Ino2p [120] and Ino4p [121] are positive regulatory proteins, whereas Opi1p [122] is a negative regulatory protein. The UAS_{INO} element contains a consensus-binding site (CANNTG) for an Ino2p-Ino4p heterodimer, which is required for maximum expression of the co-regulated UAS_{INO}-containing genes [4–6,123–125]. The *CHO1* and other UAS_{INO}-containing genes are maximally expressed when inositol is absent from the growth medium, but these genes are repressed when inositol is supplemented to the growth medium. The coordinate repression of UAS_{INO}-containing genes by inositol requires the ongoing synthesis of PC [79,126], and is enhanced by the inclusion of choline in the growth medium [1,2,4–6]. According to the model for this regulation, which is based on a recent paper by Loewen et al. [127], Opi1p is associated with the nuclear/endoplasmic reticulum (ER) membrane through interactions with the integral membrane protein Scs2p (a VAP homolog) [128] and with PA [127] when cells are grown without inositol. Upon inositol supplementation, the level of PA reduces due to the utilization of CDP-DG and increased synthesis of PI. The decrease in the PA level results in loss of Opi1p association with the nuclear/ER membrane, followed by its translocation into the nucleus [127]. In the nucleus, Opi1p mediates repression of the co-regulated phospholipid biosynthetic genes through the UAS_{INO} element [129], but not by direct interaction [130]. Instead, Opi1p inhibits transcriptional activation by binding to DNA-bound Ino2p [131]. Interestingly, the repression of *CHO1* by zinc depletion occurs through this regulatory circuit in the absence of inositol supplementation. A model for the zinc-mediated regulation of *CHO1* transcription is shown in Fig. 2 (*right*).

The zinc-mediated repression of *CHO1* may be explained if the *INO2* gene is repressed and/or the *OPI1* gene is induced by zinc depletion. However, data indicate that the expression of the *INO2* and *OPI1* genes is not significantly affected by zinc depletion [132]. Moreover, Ino2p and Ino4p are not zinc-containing proteins, and thus a reduction in their function is not likely a direct consequence of zinc depletion. The key aspect common to the repression of *CHO1* (and other UAS_{INO}-containing genes) by inositol supplementation [1–6] and by zinc depletion [23] is the negative regulatory protein Opi1p. The concentration of PA in the nuclear/ER membrane plays a central role in the localization and regulation of Opi1p repressor function [127] (Fig. 2, *right*). PA levels in *S. cerevisiae* are governed by its *de novo* synthesis from glycerol-3-phosphate, its utilization by the CDP-DG synthase (CDP-DG pathway) and Mg^{2+}-dependent PA phosphatase (Kennedy pathway) enzymes required for phospholipid synthesis, and the phospholipase D-mediated hydrolysis of PC [6]. As indicated above, the decrease in PA concentration and resultant translocation of Opi1p from the nuclear/ER membrane to the nucleus in response to inositol supplementation is attributed to an increase in PI synthesis and utilization of CDP-DG [127]. The increase in PI synthase expression and PI synthesis in response to zinc depletion, which occurs in the absence of inositol supplementation [23,116], is consistent with this model. It is unknown what effect zinc depletion has on the cellular concentration of inositol, which might affect the synthesis of PI. It is known however, that inositol supplementation results in the induction of Mg^{2+}-dependent PA phosphatase activity [133], and preliminary studies have shown that this activity is also induced in response to zinc depletion [132]. These observations suggest that regulation of Mg^{2+}-dependent PA phosphatase also contributes to the reduction of PA in the nuclear/ER membrane, the translocation of Opi1p into the nucleus, and repression of *CHO1* and other UAS_{INO}-containing genes. The availability of the *pah1*Δ mutant defective in Mg^{2+}-dependent PA phosphatase [63] will permit studies to examine this question.

7. Perspectives

The zinc-mediated regulation of phospholipid synthesis clearly impacts on membrane phospholipid composition (i.e., changes in the levels of PE and PI) [23,98]. Clues for the physiological relevance of this regulation might stem from the roles that specific phospholipids play in the structure and function of cellular membranes. Both PE and PI play a role in the modification of proteins for attachment to membranes. For example, PE is used directly for covalent modification and membrane attachment of Apg8p, a protein essential to the process of autophagy occurring in response to nutrient limitation [134–137], and indeed zinc depletion results in an elevation of Apg8p-PE [23]. PE is also used for the glycosylphosphatidylinositol modification of proteins for membrane attachment [12]. The glycosylphosphatidylinositol anchor is attached to proteins through the amine group of phosphoethanolamine that is derived from PE [12]. Interestingly, zinc depletion [85] induces the expression of *MCD4* that encodes one of the enzymes responsible for the transfer of the phosphoethanolamine moiety of PE to make the anchor [138]. The importance of PE for Apg8p modification and for glycosylphosphatidylinositol anchor synthesis in response to zinc depletion warrants further examination. As indicated above, PI is also used for the synthesis of glycosylphosphatidylinositol anchor and for the synthesis of polyphosphoinositides and sphingolipids. The increase in PI content may be important for the synthesis of these molecules. Additional work is necessary to address these questions.

It is also noteworthy that the zinc-mediated regulation of phospholipid synthesis occurs in a coordinate manner with the control of zinc homeostasis. In *S. cerevisiae*, the cellular levels of zinc are controlled by zinc transporters in the plasma membrane (Zrt1p, Zrt2p, Fet4p) [139–141] and in the membranes of the vacuole (Zrt3p, Cot1p, Zrc1p) [142–145], endoplasmic reticulum (Msc2p, Zrg17p) [82,146], and mitochondria (Mrs3p, Mrs4p) [147]. The expression of these transporters is largely regulated at the transcriptional level to maintain zinc homeostasis [148]. For example, the expression of the high affinity zinc transporter Zrt1p is induced for increased zinc uptake when the cellular level of zinc is limiting, whereas the expression of Zrt1p is repressed to attenuate zinc uptake when the cellular level of zinc is high [139]. Like *DPP1* and *PIS1*, the activation of *ZRT1* expression is dependent on the transcription factor Zap1p and the *cis*-acting element $\mathrm{UAS_{ZRE}}$ [94,95,139]. The fact that the zinc transporters are located within the phospholipid bilayer of cellular membranes raises the question as to whether changes in phospholipid composition in response to zinc depletion might regulate their 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* [149,150], and PE content in *Escherichia coli* is required for function of the γ-aminobutyric acid [151], lactose [16,152], and phenylalanine [153] 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 zinc transport function in *S. cerevisiae*.

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