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Review

Diacylglycerol pyrophosphate phosphatase in *Saccharomyces cerevisiae*

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Abstract

Diacylglycerol pyrophosphate (DGPP) phosphatase in the yeast *Saccharomyces cerevisiae* is a Mg^{2+}-independent and *N*-ethylmaleimide-insensitive 34-kDa vacuolar membrane-associated enzyme. It catalyzes the dephosphorylation of DGPP to form phosphatidate (PA) and then removes the phosphate from PA to form diacylglycerol (DAG). The enzyme is a member of the lipid phosphate phosphatase superfamily that contains a three-domain lipid phosphatase motif required for catalytic activity. Expression of the *DPP1* gene, which encodes DGPP phosphatase, is induced by zinc depletion, by inositol supplementation, and when cells enter the stationary phase. Induction by zinc depletion is mediated by the transcription factor Zap1p, which binds to a zinc-responsive element in the *DPP1* promoter. Repression of *DPP1* expression is mediated by the transcription factor Gis1p, which binds to three post-diauxic shift elements in the promoter. Regulation of *DPP1* correlates with the expression of DGPP phosphatase activity and the cellular levels of DGPP and PA.

Keywords: Diacylglycerol pyrophosphate phosphatase; Phosphatidate; Zinc; Growth phase; Inositol; Yeast

1. Discovery of DGPP and DGPP phosphatase in *Saccharomyces cerevisiae*

Diacylglycerol pyrophosphate (DGPP) phosphatase is a membrane-associated enzyme that was first discovered in the yeast *S. cerevisiae* by Wu et al. [1]. The enzyme catalyzes the removal of the β-phosphate from DGPP to form phosphatidate (PA), and it then removes the phosphate from PA to form diacylglycerol (DAG) [1] (Fig. 1). However, DGPP phosphatase will only utilize PA as a substrate in the absence of DGPP [1]. The yeast DGPP phosphatase enzyme is a member of the lipid phosphate phosphatase superfamily [2,3], which contains a three-domain lipid phosphatase motif [4] that is required for catalytic activity [5]. In addition to yeast, DGPP phosphatase activity has been identified in *Catharanthus roseus*, *Escherichia coli*, rat liver, pig liver, pig brain, and bovine brain [6]. The discovery of DGPP phosphatase in such a wide range of organisms suggests that it may play an important role in cell physiology.

The enzyme substrate DGPP is a novel glycerophospholipid metabolite originally identified from the plant *C. roseus* [7]. DGPP contains a pyrophosphate group attached to DAG (Fig. 1). This compound was previously observed by several workers [8–10] as an unidentified glycerophospholipid product of a lipid kinase reaction in plants [7]. It is now known that DGPP is synthesized from PA and ATP through the reaction catalyzed by the enzyme PA kinase [1,7]. The level of DGPP in *S. cerevisiae* is very low; depending on growth conditions, DGPP accounts for 0.2–0.4 mol% of the total glycerophospholipids [1,11]. The low abundance of this glycerophospholipid is reminiscent of lipid signaling molecules such as the inositol-containing glycerophospholipids [12–16]. Research with plants suggests that DGPP functions as a signal molecule under stress conditions. DGPP accumulates upon G-protein activation [17], hyperosmotic stress [18], dehydration [18], *Rhizobium*-secreted nodulation factors [19], and general elicitors such as xylanase [20]. DGPP accumulation is transient and coincides with a rise in PA levels [18,20]. As PA itself is a potent signaling molecule [21–23], it has been suggested that DGPP serves as the precursor for a specific pool of PA [1]. Alternatively, DGPP may be synthesized as a means to attenuate PA-mediated

Abbreviations: DGPP, diacylglycerol pyrophosphate; PA, phosphatidate; DAG, diacylglycerol; PI, phosphatidylinositol; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine

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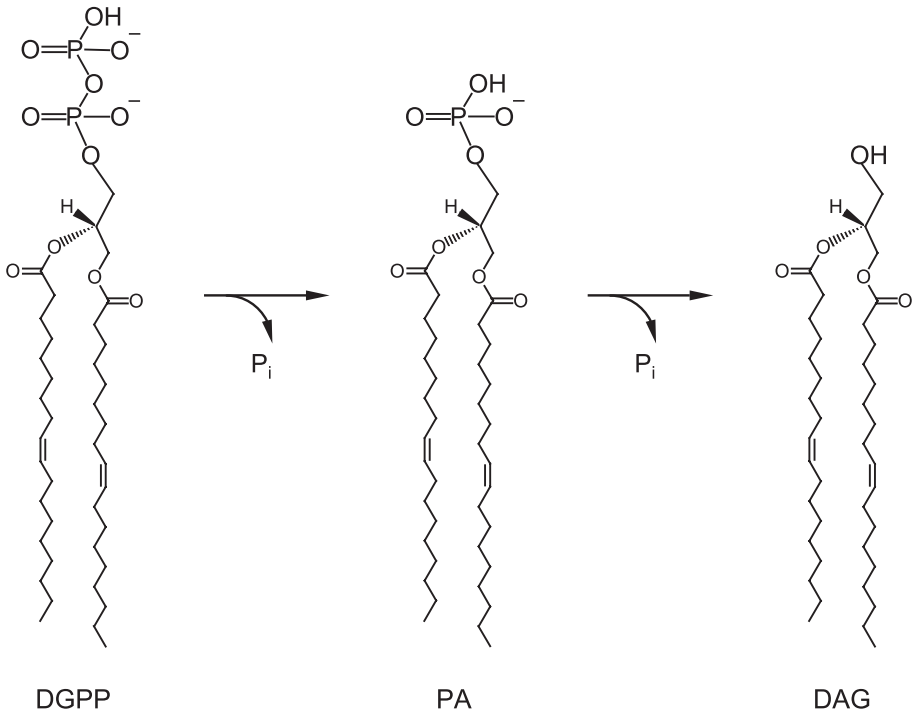

Fig. 1. Reactions catalyzed by DGPP phosphatase. DGPP, diacylglycerol pyrophosphate; PA, phosphatidate; DAG, diacylglycerol.

signals [17]. Although DGPP has not been identified in mammalian cells [17], it has been shown to function as a signaling molecule in mammalian cells [24]. DGPP potently activates mouse macrophages for enhanced secretion of arachidonic acid metabolites, an important event in the immunoinflammatory response of leukocytes [24]. It has been suggested that DGPP in the membranes of an invading organism may help macrophages target foreign cells [24]. Owing to the reactions catalyzed by DGPP phosphatase, the enzyme may function to regulate specific cellular pools of DGPP, PA, or DAG [25].

2. Purification and properties of DGPP phosphatase from *S. cerevisiae*

DGPP phosphatase activity is encoded by the *DPP1* [26] and *LPP1* [27] genes in *S. cerevisiae*. The *DPP1*-encoded DGPP phosphatase is more abundant than the *LPP1*-encoded enzyme, and it accounts for nearly all of the DGPP phosphatase activity in wild-type cells [26,27]. The *DPP1*-encoded enzyme has been purified 33,333-fold to apparent homogeneity and characterized with respect to its basic enzymological and kinetic properties [1]. The purification scheme includes the solubilization of membranes with Triton X-100, followed by chromatography with DE53 (DEAE-cellulose), Affi-Gel Blue, hydroxylapatite, and Mono Q [1]. The pure enzyme has a subunit molecular mass of 34 kDa [1]. It is not known whether oligomeric forms of the enzyme exist. DGPP phosphatase has a pH optimum of 5.0–6.0 [26] and does not have a divalent cation requirement for activity [1]. The enzyme is inhibited by Mn^{2+}, Cu^{2+}, Zn^{2+}, NaF, pyrophosphate, and CDP-DAG [1,11,28], but it is not affected by *N*-ethylmaleimide [1]. DGPP phosphatase utilizes several lipid phosphate substrates in vitro, including lysoPA [29], sphingoid base phosphates [30], isoprenoid phosphates [31], and phosphatidylglycerophosphate [29].

The DGPP phosphatase and PA phosphatase activities of the *DPP1*-encoded DGPP phosphatase enzyme are Mg^{2+}-independent and *N*-ethylmaleimide-insensitive [1]. The PA phosphatase activity of the DGPP phosphatase enzyme is distinct from the conventional PA phosphatase enzymes (104-, 75-, and 45-kDa forms) that are presumably responsible for the synthesis of glycerophospholipids and triacylglycerols in *S. cerevisiae* [32]. The conventional PA phosphatase enzymes have a Mg^{2+} ion requirement and are sensitive to inhibition by *N*-ethylmaleimide [32]. The 45- and 104-kDa Mg^{2+}-dependent PA phosphatases do not utilize DGPP as a substrate [1]. In fact, 104-kDa PA phosphatase activity is stimulated by DGPP [1].

3. Structure, localization, and topography of DGPP phosphatase

The protein product of the *DPP1* gene is 289 amino acids in length, has a subunit mass of 33.5 kDa, and is predicted to have six transmembrane-spanning regions distributed over the entire polypeptide sequence (Fig. 2)

[26]. As indicated above, the enzyme has been purified from the membrane fraction, and the pure enzyme migrates on SDS-polyacrylamide gels as a 34-kDa protein [1]. Studies with well-characterized subcellular fractions and indirect immunofluorescence microscopy of whole cells have shown that the DGPP phosphatase enzyme is localized to the vacuolar membrane [28]. The DGPP phosphatase protein contains a novel phosphatase sequence motif (Fig. 2) shared by a superfamily of phosphatase enzymes [4,33,34]. This motif consists of three domains with the consensus sequences KXXXXXXRP (domain 1)—PSGH (domain 2)—SRXXXXXHXXXD (domain 3) where X is any amino acid [4]. Segments ranging from 12 to 54 amino acids separate each of the three domains [4]. Site-directed mutagenesis of the *DPP1*-encoded enzyme has shown that the conserved Arg^{125}, His^{169}, and His^{223} residues within domains 1, 2, and 3, respectively, play important roles in the DGPP phosphatase and PA phosphatase reactions catalyzed by the enzyme [5]. For example, the DGPP phosphatase activities of the R125A, H169A, and H223A mutant enzymes are 0.05%, 9%, and 0.03%, respectively, of the wild-type enzyme. Moreover, enzymes with mutations in more than one domain of the phosphatase sequence motif have no measurable DGPP phosphatase activity [5]. Based on limited proteolysis and cysteine labeling studies, the phosphatase motif of DGPP phosphatase is located on the cytosolic side of the vacuolar membrane (Fig. 2) (G.-S. Han and G.M. Carman, unpublished data). This active site orientation may have implications for the cellular function(s) of DGPP and/or PA.

4. *DPP1* and *LPP1* genes encoding DGPP phosphatase activities

The gene encoding DGPP phosphatase, *DPP1*, was identified and isolated based on amino acid sequence information derived from the purified DGPP phosphatase enzyme [26]. The *LPP1* gene was identified and isolated on the basis that its deduced protein product shows homology to the *DPP1*-encoded DGPP phosphatase [26] and to the mouse Mg^{2+}-independent PA phosphatase [35]. The homology between these proteins lies in the lipid phosphatase sequence motif [4]. The *LPP1*-encoded enzyme has not been purified from yeast. However, a membrane fraction derived from Sf-9 insect cells that overexpress the yeast *LPP1*–encoded enzyme has been used to characterize the properties of the enzyme [36]. Table 1 summarizes the enzymological properties of the *DPP1*- and *LPP1*-encoded enzymes. Like the *DPP1*-encoded DGPP phosphatase enzyme, the *LPP1*-encoded enzyme has broad substrate specificity [36]. In contrast to the *DPP1*-encoded enzyme, the *LPP1*-encoded enzyme prefers PA as a substrate

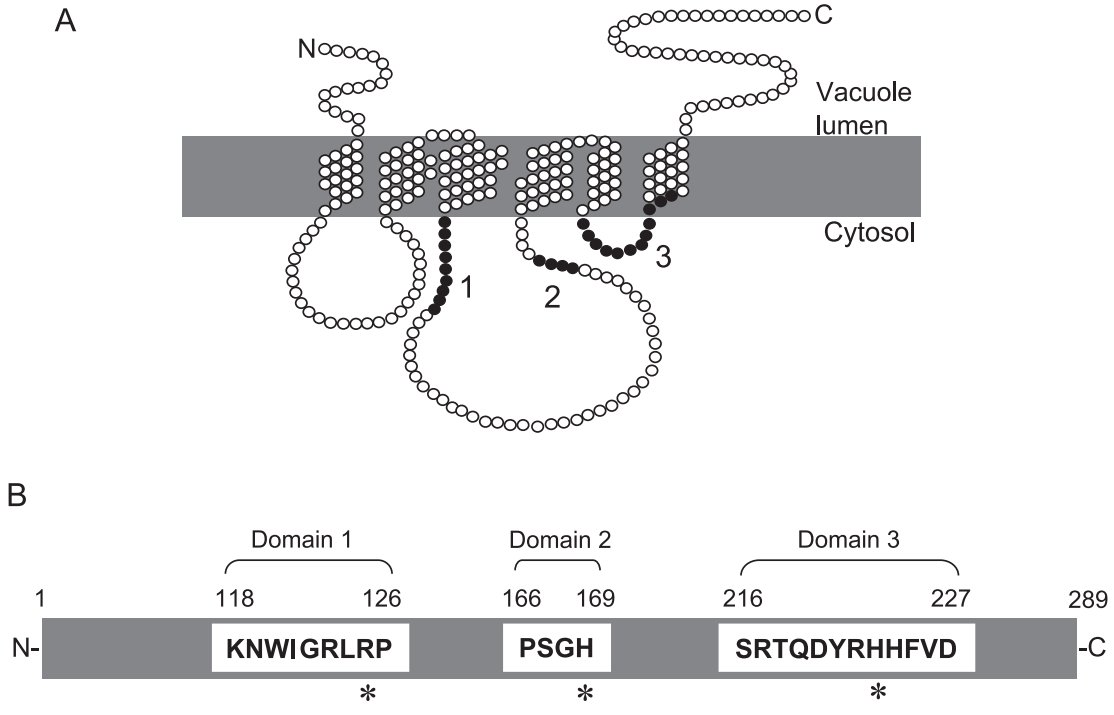

Fig. 2. Topography of the phosphatase sequence motif of the *DPP1*-encoded DGPP phosphatase. Panel A: the figure shows a model of DGPP phosphatase in the vacuolar membrane with the phosphatase motif (solid circles) facing the cytosol. The Arabic numerals indicate the regions that comprise the three domains of the phosphatase sequence motif. Panel B: the amino acid sequences that comprise the three domains of the phosphatase sequence motif within the DGPP phosphatase protein are indicated in the figure. The numbers on top of the diagram indicate the amino acid positions for each domain of the phosphatase sequence motif within the DGPP phosphatase protein. The conserved amino acid within each domain that is required for catalytic activity is indicated by the asterisk.

Table 1
Enzymological properties of the *DPP1*- and *LPP1*-encoded lipid phosphate phosphatases[a]

Property	Lipid phosphate phosphatase	
	DPP1-encoded enzyme	*LPP1*-encoded enzyme
Substrate preference	DGPP>LysoPA>PA	PA>DGPP>LysoPA
pH optimum with DGPP as substrate	5–6	7
Ionic requirements	none	none
Inhibition by PA with DGPP as substrate	not inhibited	K_i of PA>K_m of DGPP
Inhibition by DGPP with PA as substrate	K_i of DGPP≪K_m of PA	K_i of DGPP>K_m of PA
Inhibition by thioreactive agents	not inhibited	inhibited by *N*-ethylmaleimide, *p*-chloromercuriphenylsulfonic acid
Inhibition by phenylglyoxal	not inhibited	inhibited
Cation inhibitors	Mn^{2+}, Cu^{2+}, Zn^{2+}	Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}

[a] Data are taken from Refs. [1,28,30,36].

when compared with DGPP [36]. Moreover, the enzymological properties of the *LPP1*-encoded enzyme differ significantly from those of the *DPP1*-encoded DGPP phosphatase. For example, the *LPP1*-encoded enzyme is potently inhibited by *N*-ethylmaleimide [36].

Enzymatic analyses of *dpp1*Δ, *lpp1*Δ, and *dpp1*Δ *lpp1*Δ double mutants have shown that the *DPP1* and *LPP1* gene products encode all of the DGPP phosphatase activity in *S. cerevisiae* [26,27]. These gene products also account for nearly all of the Mg^{2+}-independent PA phosphatase, lysoPA phosphatase, and most of the isoprenoid phosphate phosphatase activities in cell extracts [26,27,31]. Glycerophospholipid composition analyses of *dpp1*Δ, *lpp1*Δ, and *dpp1*Δ *lpp1*Δ mutant cells reveal that the *DPP1* and *LPP1* gene products control the cellular levels of DGPP and PA and influence the levels of phosphatidylinositol (PI) [27]. DGPP and PA levels are elevated in the mutant cells, whereas the levels of PI are reduced [27]. The fact that the *dpp1*Δ mutant exhibits elevated levels of DGPP and PA provides evidence that they are physiological substrates for the *DPP1*-encoded DGPP phosphatase. The products of the *LCB3/LBP1/YSR2* [37–39] and the *CWH8* [40] genes are responsible for the dephosphorylation of sphingoid base phosphates and isoprenoid phosphates, respectively.

DPP1 and *LPP1* are not essential genes under standard laboratory conditions [26,27]. Neither gene is required for cell growth, mating, or sporulation [26,27]. The *dpp1*Δ and *lpp1*Δ strains grow equally well as wild type at different temperatures and using different carbon sources [26,27]. These mutants do not show any gross morphological differences compared to wild-type cells [26], and they do not excrete or require inositol for growth [26], a phenotype often associated with specific glycerophospholipid biosynthetic mutants [41]. The *dpp1*Δ *lpp1*Δ double mutant is viable, and its growth properties are comparable to the single mutants and to wild-type cells [27].

5. Regulation of *DPP1*-encoded DGPP phosphatase

The *DPP1*-encoded DGPP phosphatase is regulated by the stress conditions of zinc depletion, stationary phase, and by inositol supplementation.

5.1. Regulation by zinc

Zinc limitation results in the induction of *DPP1* expression and the DGPP phosphatase enzyme [28]. In fact, *DPP1* is one of the most highly regulated genes that respond to zinc limitation in the *S. cerevisiae* genome [42,43]. The regulation of *DPP1* expression in zinc-limited cells is dependent on the transcription factor Zap1p and binding to a *cis*-acting element, UAS_{ZRE} (*z*inc-*r*esponsive *e*lement) [28]. Analysis of mutants defective in plasma membrane and vacuolar membrane zinc transporters indicates that *DPP1* expression is sensitive to the cytoplasmic levels of zinc [28]. The regulation of DGPP phosphatase expression correlates with the metabolism of DGPP and PA in the vacuolar membrane (C.N. Johnston and G.M. Carman, unpublished data). When grown in the presence of zinc, DGPP and PA account for 0.6 mol% and 1.4 mol% of the total glycerophospholipids in vacuolar membranes. Depletion of zinc from the growth medium results in a decrease in DGPP to an undetectable level and a decrease in PA to 0.3 mol%.

The expression of the *DPP1*-encoded DGPP phosphatase is coordinately regulated with mechanisms that control zinc homeostasis [28] (Fig. 3). Zinc is an essential mineral; it is a cofactor for over 300 enzymes and serves as a structural component for many proteins [44,45]. Notwithstanding its essential nature, zinc can be toxic when accumulated in excess amounts [44]. The cytoplasmic levels of zinc are controlled by a variety of mechanisms, including cellular influx [46], efflux [47,48], and chelation by metallothioneins [49]. The cytoplasmic levels of zinc are largely controlled by high affinity (Zrt1p) and low affinity (Zrt2p) plasma membrane zinc transporters [50,51]. These transporters are induced when the extracellular concentration of zinc is low [50,51]. Cytoplasmic levels of zinc are controlled further by the vacuolar membrane efflux transporter (Zrt3p). The regulation of these transporters is dependent on Zap1p and the UAS_{ZRE} [50–54].

It is unclear why the expression of DGPP phosphatase is regulated in a coordinate manner with zinc transporters whose main function is to regulate zinc homeostasis. As indicated above, the *DPP1* gene is not essential, and *dpp1*Δ mutants do not exhibit any dramatic phenotypes under a variety of growth conditions [26], including

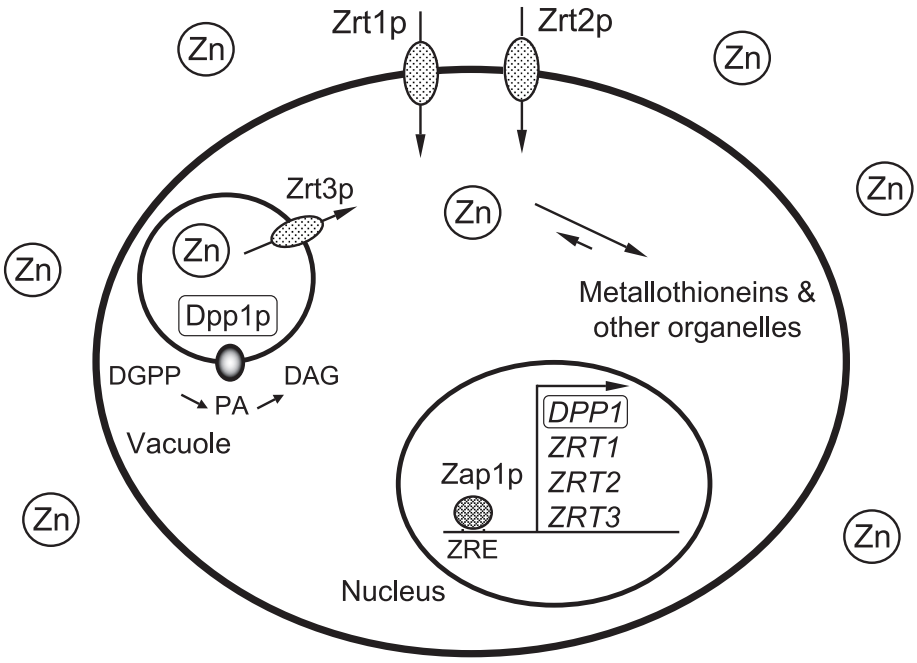

Fig. 3. Coordinate regulation of DGPP phosphatase with zinc transporters in response to zinc limitation. The expression of the *DPP1* gene is coordinately regulated with the expression of genes coding for the high affinity (Zrt1p) and low affinity (Zrt2p) plasma membrane zinc transporters and the vacuolar membrane efflux zinc transporter (Zrt3p). Under conditions of low zinc, the transcription factor Zap1p is induced and binds to the UAS_{ZRE} in the promoters of *ZRT1*, *ZRT2*, *ZRT3*, and *DPP1*. The induction by zinc limitation results in increased expression of the zinc transporters to increase the cytosolic levels of zinc. At the same time, there is an increase in expression of DGPP phosphatase (Dpp1p) resulting in decreased levels of DGPP and PA.

fluctuations in zinc supplementation [43]. Thus, the role of DGPP phosphatase during zinc limitation would have to complement other mechanisms that respond to this stress. Although the function of DGPP in yeast is still unclear, it is tempting to speculate that it functions to chelate a specific pool of zinc ions at the surface of the vacuolar membrane. As indicated above, DGPP phosphatase activity is inhibited by zinc ions [28]. The mechanism of inhibition involves the formation of a DGPP–zinc complex, which prevents the enzyme from removing the phosphate from the substrate [28]. Formation of the DGPP–zinc complex would be eliminated by the dephosphorylation of free DGPP by DGPP phosphatase, especially under zinc-limiting conditions. An alternative role for the DGPP phosphatase enzyme may be to control the levels of DGPP and PA in vacuolar membranes, which in turn mediate other cellular functions.

5.2. Regulation by inositol and growth phase

Inositol supplementation and growth phase have a major impact on the expression of many glycerophospholipid biosynthetic enzymes [55–59]. DGPP phosphatase is also regulated by these growth conditions. The addition of inositol to the growth medium results in the elevation of DGPP phosphatase activity in both the exponential and stationary phases of growth [11]. DGPP phosphatase activity is higher in stationary phase cells when compared with exponential phase cells. Moreover, the regulation of the enzyme by inositol and growth phase is additive. Analyses of DGPP phosphatase mRNA abundance and protein levels, as well as the expression of β-galactosidase activity driven by a P_{DPP1}-*lacZ* reporter gene, have shown that a transcriptional induction is responsible for this regulation [11].

Interestingly, the effects of inositol and growth phase on DGPP phosphatase expression are opposite to those of most glycerophospholipid biosynthetic enzymes. For example, expression of genes coding for enzymes responsible for the synthesis of PI (e.g., *INO1*) and phosphatidylcholine (PC) (e.g., *CDS1*, *CHO1/PSS1*, *PSD1*, *CHO2/PEM1*, *OPI3/PEM2*, *CKI1*, and *CPT1*) (Fig. 4) is regulated by inositol [55–60]. These genes are maximally expressed when inositol is absent from the growth medium and repressed when inositol is supplemented to the growth medium. Inositol-mediated regulation involves the transcriptional regulatory proteins Ino2p, Ino4p, and Opi1p [55–59]. Ino2p [61] and Ino4p [62] are positive transcription factors whereas Opi1p [63] is a negative transcription factor. Regulation of glycerophospholipid synthesis by inositol is mediated by a UAS_{INO} element [55,64–67] present in these genes. The UAS_{INO} element contains the binding site for an Ino2p-Ino4p heterodimer, which is required for maximum expression of the co-regulated UAS_{INO}-containing genes [57–59,68–70]. Repression of the co-regulated glycerophospholipid synthesis genes depends on Opi1p [41,63]. In addition, the UAS_{INO}-

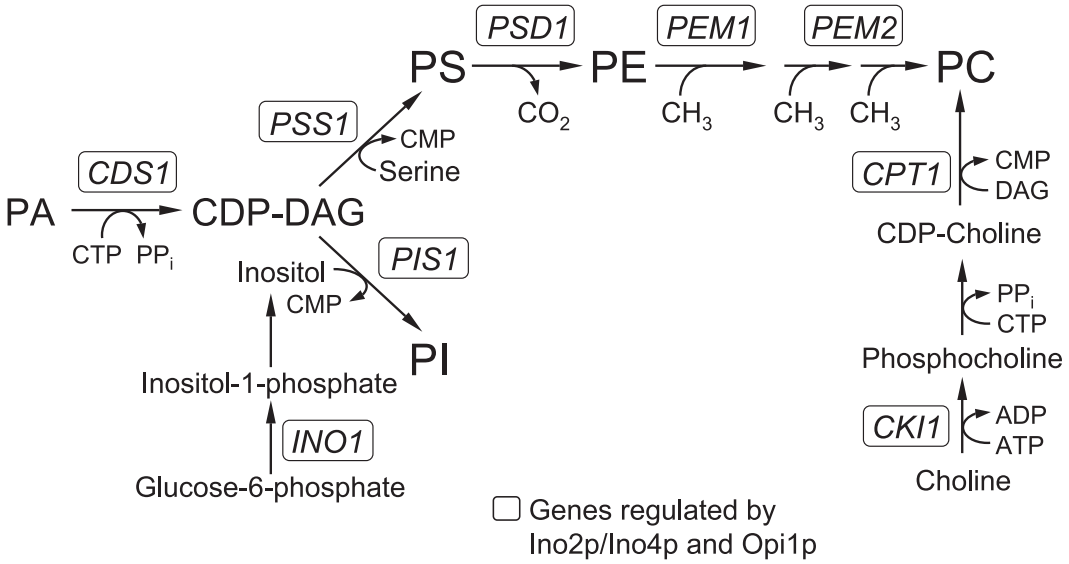

Fig. 4. Glycerophospholipid synthesis in *S. cerevisiae*. The pathways shown for the synthesis of glycerophospholipids include the relevant steps discussed in the text. The UAS_{INO}-containing genes that are regulated by the positive transcription factors Ino2p and Ino4p and the negative transcription factor Opi1p are indicated in the figure. PA, phosphatidate; CDP-DAG, CDP-diacylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; DAG, diacylglycerol.

containing genes are maximally expressed in exponential phase and are repressed in stationary phase in the absence of inositol [57–59]. The promoter of the *DPP1* gene does not contain a UAS_{INO} element. Yet, analyses of *ino2*Δ, *ino4*Δ, and *opi1*Δ mutants indicate that regulation of *DPP1* expression by inositol may be indirectly affected by transcription factors Ino2p, Ino4p, and Opi1p [11]. The elements responsible for *DPP1* regulation by inositol supplementation and growth phase have yet to be identified.

CDP-DAG, whose levels are also regulated by growth phase [71,72], inhibits DGPP phosphatase activity [11]. CDP-DAG is a branch point intermediate for the synthesis of glycerophospholipids and is a common substrate for phosphatidylserine (PS) synthase and PI synthase (Fig. 4) [25,55,59]. The inhibitor constant for CDP-DAG (K_i = 5 mol%) is within the range of its cellular concentration in exponential phase cells, and thus regulation of DGPP phosphatase activity by CDP-DAG should be physiologically relevant [11]. Stationary phase regulation by CDP-DAG may not occur, since the K_i value for CDP-DAG is 12-fold higher than its cellular concentration [11]. CDP-DAG regulates DGPP phosphatase activity; this in turn influences the utilization of CDP-DAG by the PS synthase and PI synthase enzymes. DGPP stimulates PS synthase activity with an activation constant ($A_{0.5}$ = 0.13 mol%) that is within the range of its cellular concentration in both exponential [1,27] and stationary [11] phase cells. Thus, regulation of PS synthase activity by DGPP may occur in vivo during both phases of growth. Stimulation of PS synthase by DGPP would favor the synthesis of PS at the expense of PI, as DGPP does not affect the activity of PI synthase [11]. Indeed, the major impact of the *dpp1*Δ mutation in exponential phase cells that are not supplemented with inositol [27] and stationary phase cells supplemented with inositol [11] is a decrease in PI content when compared with wild-type cells.

5.3. Regulation by transcription factor Gis1p

The expression of *DPP1* is negatively regulated by the transcription factor Gis1p [73]. Depending on the gene in question, Gis1p can act as a positive [74,75] or negative [76] regulator of gene expression. *DPP1* expression is elevated in a *gis1*Δ mutant, resulting in a 2.5-fold increase in DGPP phosphatase activity [73]. *DPP1* contains three URS_{PDS} (*p*ost-*d*iauxic *s*hift) elements, to which Gis1p binds [73]. All three elements play a role in regulating *DPP1* expression [73]. Glycerophospholipid composition is altered in a *gis1*Δ mutant [73]. As expected, the increase in DGPP phosphatase activity leads to a decrease (78%) in DGPP content. The amounts of the major glycerophospholipids phosphatidylethanolamine (PE) and PC are also altered in a *gis1*Δ mutant, with a 39% decrease and a 24% increase, respectively [73]. The changes in these glycerophospholipids may be a consequence of altered DGPP levels. Alternatively, Gis1p may directly affect the expression of other genes involved in glycerophospholipid metabolism [73]. Regulation of *DPP1* expression by Gis1p is most evident in stationary phase cells grown in the presence of inositol. These growth conditions [11], as well as zinc depletion [28], are stressful states whereby DGPP phosphatase is induced. Gis1p may function to attenuate the induction of DGPP phosphatase to maintain some steady-state level of DGPP and/or PA within the cell.

6. Unresolved issues and future developments

The DGPP phosphatase enzyme is induced under stressful growth conditions. This suggests that the level of DGPP and/or PA plays some role in a stress response. However, this role is not essential for growth under standard laboratory conditions. Instead, we believe that DGPP and/or PA plays a specialized role in vacuolar membrane function. That DGPP phosphatase expression is coordinately regulated with transporters controlling zinc homeostasis is an exciting discovery. Additional studies are needed to examine the physiological relevance of DGPP–zinc complexes and whether the DGPP and/or PA molecules play a cellular role (e.g., vesicular trafficking) in response to zinc depletion. The levels of DGPP and PA must also be controlled by the PA kinase enzyme. Little is known about the regulation of PA kinase activity in yeast. PA kinase activity is extremely low, and this has hampered efforts to purify the enzyme. Furthermore, the gene encoding the PA kinase has not been identified. Work is currently in progress to isolate the PA kinase gene from plants, which should facilitate identification of the gene from yeast. The availability of a mutant defective in the PA kinase enzyme would provide a useful tool for elucidating the function of DGPP in yeast. Another issue that needs to be addressed is whether DGPP and/or PA plays a role as a signaling molecule to control glycerophospholipid synthesis.

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