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Chapter IVA

Phosphatidate phosphatases and diacylglycerol pyrophosphate phosphatases in *Saccharomyces cerevisiae* and *Escherichia coli*

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Abstract

Phosphatidate phosphatase plays a major role in the synthesis of phospholipids and triacylglycerols in the yeast *Saccharomyces cerevisiae*. Membrane- and cytosolic-associated forms of the enzyme have been isolated and characterized. These enzymes are Mg^{2+}-dependent and *N*-ethylmaleimide-sensitive. The expression of a membrane-associated form of phosphatidate phosphatase is regulated by growth phase and inositol supplementation, whereas enzyme activity is regulated by lipids, nucleotides, and by phosphorylation. Phosphatidate phosphatase is coordinately regulated with other phospholipid biosynthetic enzymes including phosphatidylserine synthase. Diacylglycerol pyrophosphate phosphatase is a novel enzyme of phospholipid metabolism which is present in *S. cerevisiae*, *Escherichia coli*, and mammalian cells. This enzyme possesses a phosphatidate phosphatase activity which is Mg^{2+}-independent and *N*-ethylmaleimide-insensitive and is distinct from the Mg^{2+}-dependent and *N*-ethylmaleimide-sensitive form of phosphatidate phosphatase. Genes encoding for diacylglycerol pyrophosphate phosphatase have been isolated from *S. cerevisiae* and *E. coli*. The deduced protein sequences of these genes show homology to the sequence of the mouse PAP2 (Mg^{2+}-independent and *N*-ethylmaleimide-insensitive phosphatidate phosphatase) protein, especially in a novel phosphatase sequence motif. Rat liver PAP2 displays diacylglycerol pyrophosphate phosphatase activity. © 1997 Elsevier Science B.V.

Keywords: Phosphatidate; Diacylglycerol; Diacylglycerol pyrophosphate; Phosphatases; Yeast; Bacteria

1. Discovery, occurrence, and localization of PA phosphatase in *Saccharomyces cerevisiae*

Phosphatidate (PA) phosphatase (3-*sn*-phosphatidate phosphohydrolase, EC 3.1.3.4) catalyzes the dephosphorylation of PA yielding diacylglycerol (DG) and P_i [1]. This reaction was first characterized by Kennedy and co-workers [1] using chicken liver extracts. Hosaka and Yamashita [2] were the first workers to identify PA phosphatase activity in the yeast *Saccharomyces cerevisiae*. PA phosphatase activity is

Abbreviations: PA, phosphatidate; DG, diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TG, triacylglycerol; CDP-DG, CDP-diacylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; CL, cardiolipin; PG, phosphatidylglycerol; PGP, phosphatidylglycerophosphate; DGPP, diacylglycerol pyrophosphate; NEM, *N*-ethylmaleimide; protein kinase A, cAMP-dependent protein kinase; PAP1, Mg^{2+}-dependent and NEM-sensitive PA phosphatase; PAP2, Mg^{2+}-independent and NEM-insensitive PA phosphatase

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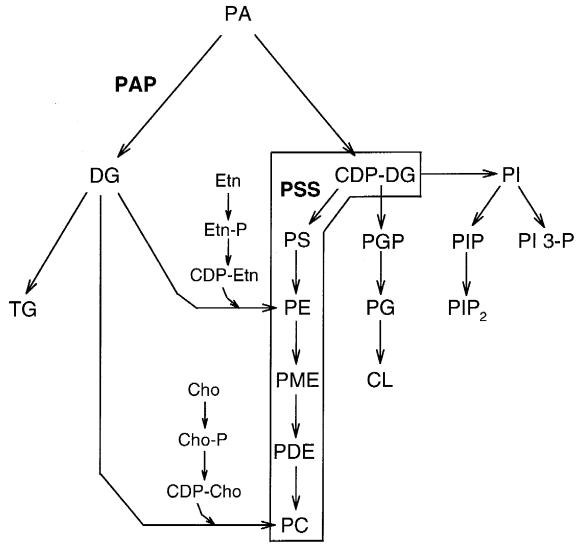

Fig. 1. Phospholipid biosynthetic pathways in *S. cerevisiae*. The pathways shown include the relevant steps discussed in the text. PA, phosphatidate; DG, diacylglycerol; PE, phosphatidylethanolamine; Etn, ethanolamine; PC, phosphatidylcholine; Cho, choline; CDP-DG, CDP-diacylglycerol; PS, phosphatidylserine; PME, phosphatidylmonomethylethanolamine; PDE, phosphatidyldimethylethanolamine; PGP, phosphatidylglycerophosphate; PG, phosphatidylglycerol; CL, cardiolipin; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; PI 3-P, phosphatidylinositol 3-phosphate; PAP, PA phosphatase; PSS, PS synthase. The CDP-DG-dependent pathway is indicated by the boxed area.

associated with the total membrane and cytosolic fractions of yeast cells [3]. The membrane-associated activity is about equally distributed between the mitochondrial and microsomal fractions [4]. As in higher eukaryotic cells [5], PA phosphatase in *S. cerevisiae* [6] catalyzes the committed step in the synthesis of the major membrane phospholipids phosphatidylethanolamine (PE) and phosphatidylcholine (PC) by the Kennedy (CDP-ethanolamine- and CDP-choline-based) pathway [7] (Fig. 1). The utilization of the Kennedy pathway for PC synthesis is primarily used by wild-type yeast cells grown in the presence of choline [6]. This pathway becomes more important for PC synthesis when the enzymes in the CDP-DG-dependent pathway (Fig. 1) are repressed or defective [6]. PA phosphatase also catalyzes the committed step in the synthesis of triacylglycerols (TG) [6] (Fig. 1).

2. Purification and properties of PA phosphatases in *S. cerevisiae*

A 91-kDa form of PA phosphatase has been purified to apparent homogeneity from the total membrane fraction of *S. cerevisiae* [8]. The purification procedure includes sodium cholate solubilization of total membranes followed by chromatography with DE53, Affi-Gel Blue, hydroxylapatite, Mono Q, and Superose 12. Immunoblot analysis of cell extracts using antibodies specific for the 91-kDa form of PA phosphatase revealed that the 91-kDa enzyme is a proteolysis product of a 104-kDa enzyme [9]. The immunoblot analysis also revealed the existence of a 45-kDa form of PA phosphatase [9]. The mitochondrial fraction contains the 45-kDa PA phosphatase, whereas the microsomal fraction contains the 104- and 45-kDa enzymes [9]. In vivo labeling experiments have shown that the 104-kDa PA phosphatase is not a precursor of the 45-kDa enzyme [9]. The 104- and 45-kDa forms of PA phosphatase have been purified to apparent homogeneity from the microsomal and mitochondrial fractions, respectively, by the procedure used to purify the 91-kDa enzyme [9]. Proteolysis of the 104-kDa PA phosphatase is prevented by the utilization of a protease-deficient strain [9].

A cytosolic-associated form of PA phosphatase has been partially purified from *S. cerevisiae* [3]. The purification scheme for the cytosolic enzyme includes ammonium sulfate and polyethylene glycol fractionation steps followed by chromatography with DEAE-Sepharose, Sephadex G-100, and Blue-Sepharose. The native molecular mass for the enzyme is approximately 75 kDa as determined by gel filtration chromatography [3].

The pH optima for the 104-, 45-, and 75-kDa forms of PA phosphatase are 7, 6–7, and 7–8, respectively [3,8,9]. All three PA phosphatase enzymes have a specific Mg^{2+} ion requirement for activity and each of the enzymes are specific for PA as a substrate [3,8,9]. The 104- and 45-kDa enzymes are dependent on Triton X-100 for maximum activity, are inhibited by *N*-ethylmaleimide (NEM), phenylglyoxal, and propranolol, and are unstable to temperatures above 30°C [8,9]. Although the enzymological properties of the 104- and 45-kDa PA phosphatase

enzymes are similar, the 104-kDa enzyme has a higher turnover number [8,9].

A detailed kinetic analysis of the 91-kDa (104-kDa) PA phosphatase has been performed using Triton X-100/PA-mixed micelles [10]. PA phosphatase activity is dependent on the molar and surface concentrations of PA. These results are characteristic of surface dilution kinetics [11] where the enzyme binds to the mixed micelle surface before binding to its substrate and catalysis occurs. Surface dilution kinetics is a model system that mimics the physiological surface of the membrane where two-dimensional surface interactions occur [11]. Accordingly, the K_m value is an interfacial Michaelis constant expressed as a surface concentration (e.g., in mol.%) as opposed to a molar concentration [11]. Since PA phosphatase activity is dependent on the molar and surface concentrations of PA [8–10], kinetic analyses of the enzyme are conducted under conditions where activity is dependent on the surface concentration of PA and independent of the molar concentration of PA [12].

The 104-kDa PA phosphatase has been examined with respect to specificity for the fatty acyl composition of PA [10]. The enzyme has ~6-fold greater affinity (reflected in the dissociation constant) for Triton X-100 micelles containing dioleoyl PA and dipalmitoyl PA when compared to micelles containing dicaproyl PA. However, based on specificity constants (V_{max}/K_m), dicaproyl PA is the best substrate with 11- to 14-fold greater specificity when compared to dioleoyl PA and dipalmitoyl PA [10]. The specificity constants for dioleoyl PA and dipalmitoyl PA are similar [10].

3. Biochemical regulation of PA phosphatases in *S. cerevisiae*

3.1. Regulation of PA phosphatase activity by protein kinase A

cAMP-dependent protein kinase (protein kinase A) is the principle mediator of signals transmitted through the *RAS*/cAMP pathway in *S. cerevisiae* [13]. In *S. cerevisiae*, high protein kinase A activity is associated with rapid cell growth and enhanced metabolic activity [13]. The phosphorylation of the 104- and 45-kDa PA phosphatases by protein kinase A has been examined [14]. Purified 45-kDa PA phosphatase is phosphorylated by protein kinase A, while the purified 104-kDa PA phosphatase is not a substrate. Protein kinase A catalyzes the phosphorylation of pure 45-kDa PA phosphatase on a serine residue which results in a stimulation of PA phosphatase activity. Kinetic analysis has shown that phosphorylation does not affect substrate binding but does alter the catalytic step in the reaction. Results of studies using mutants that are defective in protein kinase A activity (i.e., *bcy1* and *cyr1*) are consistent with the results of the phosphorylation studies using pure preparations of PA phosphatase. The effects of the *RAS*/cAMP pathway on lipid synthesis using a strain that contains the inducible *GAL10-RAS2*^{val19} allele has also been examined [14]. Cells containing this allele exhibit elevated levels of protein kinase A activity when induced with galactose [15]. The expression of the *GAL10-RAS2*^{val19} allele in cells results in an increase in the synthesis of DG and TG. It is possible that the activation of PA phosphatase activity by protein kinase A phosphorylation is responsible for the increased synthesis of these lipids. These data are consistent with the model that protein kinase A regulates the activity of the 45-kDa PA phosphatase in vivo.

3.2. Regulation of PA phosphatase activity by nucleotides

The nucleotides ATP and CTP are substrates for enzymes that synthesize the energy-rich intermediates (CDP-diacylglycerol (CDP-DG), CDP-choline, and CDP-ethanolamine) of phospholipid synthesis [6]. These nucleotides are used in reactions involving the substrate and the product of the PA phosphatase reaction and the effects of these nucleotides on the regulation of PA phosphatase activity has been examined [16]. ATP and CTP are, in fact, inhibitors of the 104- and 45-kDa PA phosphatase activities. Kinetic analyses revealed that the mechanism of inhibition of the 104-kDa enzyme inhibition by ATP and CTP (with respect to PA) is complex. The dependence of this PA phosphatase activity on PA is cooperative and the nucleotides alter both the V_{max} and the K_m.

ATP does not inhibit PA phosphatase activity by binding to the enzyme nor by complexing with the PA molecule. PA phosphatase dependence on Mg^{2+} ions follows saturation kinetics, and the mechanism of nucleotide inhibition with respect to Mg^{2+} ions is competitive. Taken together, this data indicates that the mechanism of enzyme inhibition by nucleotides involves the chelation of Mg^{2+} ions. The inhibitor constant for ATP is lower than its cellular concentration in glucose-grown cells, while the inhibitor constant for ATP is higher than its cellular concentration in glucose-starved cells. Changes in the cellular concentration of ATP during glucose starvation correlate with changes in the proportional synthesis of TG and phospholipids. These results are consistent with the model that regulation of PA phosphatase activity by ATP proceeds through a mechanism that involves Mg^{2+} ion chelation [16].

3.3. Regulation of PA phosphatase activity by lipids

Phospholipids function as cofactors and activators of several membrane-associated enzymes [17]. Since PA phosphatase plays an important role in the regulation of phospholipid synthesis, the effects of phospholipids on the activities of the 104- and the 45-kDa PA phosphatases have been examined [18]. Anionic phospholipids stimulate the activities of both forms of the PA phosphatases, while zwitterionic phospholipids have a slight inhibitory effect on their activities. Cardiolipin (CL), CDP-DG, and phosphatidylinositol (PI) are the most potent anionic phospholipid activators. Enzyme activation by CL, CDP-DG, and PI follow positive cooperative kinetics. The dependence of the 104-kDa PA phosphatase on PA is cooperative in the absence and presence of phospholipid activators. CL, CDP-DG, and PI are mixed competitive activators of 104-kDa PA phosphatase activity. The major effect of the activators is to decrease the K_m for PA. Sphinganine (see below) antagonizes the activation of PA phosphatase activity by CL and PI. Sphinganine causes an increase in the cooperativity of CL activation, but has little affect on the activation constant for CL. Conversely, sphinganine has little effect on the cooperativity of PI activation, but causes an increase in the activation constant for PI. The activation constants for CL, CDP-DG, and PI are all within the range of their cellular concentrations. This is consistent with the model that the regulation of PA phosphatase activity by phospholipids is relevant in vivo [18].

The role(s) that sphingoid bases play in lipid metabolism and cell signaling in mammalian cells has been an area of intense study [19,20]. It has been suggested that sphingosine is a regulator of the PC signaling pathway since sphingosine activates phospholipase D, inhibits PA phosphatase, and inhibits protein kinase C [21]. The availability of purified preparations of the PA phosphatases has permitted the examination of the regulation of their activities by sphingosine and other sphingoid bases in a well-defined system [12]. Sphingosine, phytosphingosine, and sphinganine inhibit both the 104- and the 45-kDa PA phosphatase activities in a dose-dependent manner. The structural requirements for this are a free amino group and a long chain hydrocarbon. The dependence of the PA phosphatase on PA is cooperative in the absence and presence of sphingoid bases. Sphingosine, phytosphingosine, and sphinganine are parabolic competitive inhibitors of PA phosphatase activity. This indicates that more than one inhibitor molecule contributes to the exclusion of PA from the enzyme. The inhibitor constants for sphingosine, phytosphingosine, and sphinganine are below the K_m value for PA and are within their cellular concentrations [12]. These results are consistent with the model that the regulation of PA phosphatase activity by sphingoid bases is relevant in vivo [12].

4. Genetic regulation of PA phosphatases in *S. cerevisiae*

Mutants defective in PA phosphatase activity and the genes encoding for PA phosphatases have not been isolated from *S. cerevisiae*. Information regarding the genetic regulation of PA phosphatase activity have come from studies using antibodies [9] and mutants defective in phospholipid biosynthesis [4]. The levels of the 104- and 45-kDa forms of PA phosphatase increase as *S. cerevisiae* cells progress from the exponential to the stationary phases of growth [9]. The elevation of PA phosphatase levels in stationary-phase cells correlates with the increase in PA phosphatase activity [2,4]. This regulation correlates with the increase in TG synthesis at the expense

of phospholipid synthesis in stationary-phase cells [2,22].

The expression of PA phosphatase activity is also regulated by inositol [4,9]. The addition of inositol to the growth medium of wild-type cells results in the elevation of the levels of the 45-kDa PA phosphatase, while the levels of the 104-kDa PA phosphatase are not altered [9]. Choline, in the absence or presence of inositol, has no effect on the PA phosphatases [9]. Mutations in genes (*OPI1, INO2*) that alter the expression of inositol 1-phosphate synthase (responsible for inositol synthesis) also influence the levels of the 45-kDa PA phosphatase [4,9]. These observations are consistent with a model that predicts that the expression of the gene that encodes this PA phosphatase is regulated in response to inositol. The increase in PA phosphatase activity in response to inositol supplementation correlates with an increase in phospholipid content at the expense of TG [4].

5. Coordinate regulation of PA phosphatase and PS synthase in *S. cerevisiae*

PA phosphatase activity is regulated by biochemical and genetic mechanisms in a reciprocal manner with the regulation of the phospholipid biosynthetic enzyme phosphatidylserine (PS) synthase [6]. PS synthase catalyzes the committed step for the synthesis of PE and PC by the CDP-DG-dependent pathway in *S. cerevisiae* [6] (Fig. 1). The DG generated from PA by PA phosphatase can be used to synthesize TG and phospholipids by the Kennedy pathway, while PS synthase can use the CDP-DG derived from PA by the action of CDP-DG synthase (Fig. 1). Inositol supplementation elevates levels of the 45-kDa PA phosphatase [9] but reduces levels of the PS synthase [23,24]. Phosphorylation of the 45-kDa PA phosphatase by protein kinase A stimulates its activity [14], while phosphorylation of PS synthase inhibits its activity [25]. Both enzymes are regulated by phospholipids but in a complementary manner. PS synthase activity is activated by PA [26], while PA phosphatase activity is activated by CDP-DG [18]. Thus, the phospholipid substrate for PA phosphatase activates PS synthase, while the phospholipid substrate for PS synthase activates PA phosphatase. In addition, DG (the product of the PA phosphatase reaction) inhibits PS synthase activity [26]. Finally, CL activates PA phosphatase activity [18] but inhibits PS synthase activity [26]. These results suggest that the differential regulation of PA phosphatase and PS synthase plays a central role in controlling the pathways by which phospholipids and neutral lipids are synthesized.

6. Identification, purification, and properties of DGPP phosphatases from *S. cerevisiae*

Diacylglycerol pyrophosphate (DGPP) is a novel phospholipid that was first identified as the product of the PA kinase reaction in the plant *Catharanthus roseus* (Fig. 2) [27]. Metabolic labeling studies have shown that DGPP is rapidly metabolized to PA and then to DG by an unidentified phosphatase [28]. The fact that PA is converted to DG raised the question of whether the unidentified phosphatase was in fact a PA phosphatase. This question has been addressed using pure PA phosphatases from *S. cerevisiae*. DGPP is neither a substrate nor an inhibitor of the pure PA phosphatases [29]. However, an activity which does catalyze the dephosphorylation of DGPP to form PA has been identified in *S. cerevisiae* and has been named DGPP phosphatase [29]. This activity is localized to the microsomal fraction of the cell [29].

Two DGPP phosphatases have been purified from *S. cerevisiae* [29]. The purification scheme includes the solubilization of the enzymes from microsomal membranes with Triton X-100 followed by chromatography with DE53, Affi-Gel Blue, hydroxylapatite, and Mono Q [29]. The hydroxylapatite chromatography step results in the isolation of two distinct peaks of DGPP phosphatase activity. Fractions containing activity under each of the peaks have been further purified to homogeneity on separate Mono Q columns [29]. In each case, the Mono Q chromatogra-

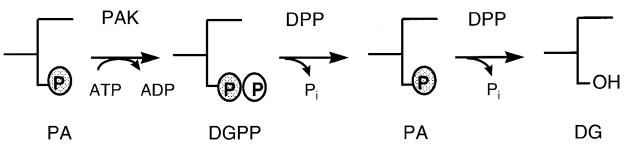

Fig. 2. Reactions catalyzed by PA kinase and DGPP phosphatase. PA, phosphatidate; DGPP, diacylglycerol pyrophosphate; DG, diacylglycerol; PAK, PA kinase; DPP, DGPP phosphatase.

phy step results in the isolation of a 34-kDa protein, which exhibits DGPP phosphatase activity [29]. The DGPP phosphatase derived from peak 1 of the hydroxylapatite column has been characterized [29]. When DGPP is supplied as a substrate, the enzyme removes the β phosphate of DGPP to generate PA and then removes the phosphate of PA to generate DG (Fig. 2). In fact, DGPP phosphatase can utilize PA as a substrate in the absence of DGPP, although the enzyme has a 10-fold higher specificity constant for DGPP. DGPP phosphatase has a pH optimum of 6.5 and is dependent on Triton X-100 for maximum activity. The enzyme is inhibited by Mn^{2+} ions, NaF, and pyrophosphate but is insensitive to thioreactive agents such as NEM. DGPP phosphatase exhibits typical saturation kinetics with respect to DGPP and the K_m value for DGPP is 3-fold greater than its cellular concentration. DGPP potently inhibits the dephosphorylation of PA by a competitive mechanism, whereas PA does not inhibit the dephosphorylation of DGPP.

The PA phosphatase activity of the DGPP phosphatase enzyme is distinct from the 104- and 45-kDa PA phosphatases [8,9]. The PA phosphatase activity of the DGPP phosphatase enzyme does not have a Mg^{2+} ion requirement and is insensitive to NEM [29]. As indicated above, the 104- and 45-kDa PA phosphatases have a Mg^{2+} ion requirement and are sensitive to inhibition by NEM [8,9]. In addition, the 104-kDa PA phosphatase activity is stimulated by DGPP [29].

7. PA phosphatase and DGPP phosphatase in *Escherichia coli*

A PA phosphatase activity has been identified from the membrane fraction of *Escherichia coli* [30] and mutants defective in this activity have been isolated that are defined by the *pgpB* gene [31–33]. The PA phosphatase activity in *pgpB* mutants has been measured using the assay conditions described for the Mg^{2+}-dependent PA phosphatase [31–33]. However, these assay conditions would not reveal the existence of a Mg^{2+}-independent PA phosphatase activity such as that displayed by the yeast DGPP phosphatase [29]. Dillon et al. [34] have tested the hypothesis that *E. coli* possesses a Mg^{2+}-independent PA phosphatase activity of a DGPP phosphatase enzyme. They have provided genetic and biochemical evidence that supports the conclusion that the product of *pgpB* gene of *E. coli* exhibits DGPP phosphatase activity [34]. DGPP phosphatase activity is absent in *pgpB* mutant cells and is expressed at high levels in cells carrying the wild-type *pgpB* gene on a runaway replication plasmid. The *pgpB* mutant has been primarily characterized by a defect in PA phosphatase activity and also exhibits defects in lyso-PA (LPA) phosphatase and phosphatidylglycerophosphate (PGP) phosphatase activities. The defective PA phosphatase in the *pgpB* mutant has been shown to be a Mg^{2+}-independent PA phosphatase activity of the DGPP phosphatase enzyme. The DGPP phosphatase activity in membranes from cells overproducing the *pgpB* gene product has been characterized [34]. This DGPP phosphatase catalyzes the dephosphorylation of the β phosphate of DGPP to form PA followed by the dephosphorylation of PA to form DG. The specificity constant (V_{max}/K_m) for DGPP is 9.3-fold greater than that for PA. The pH optimum for the DGPP phosphatase reaction is 6.5. Activity is independent of a divalent cation requirement, is potently inhibited by Mn^{2+} ions, and is insensitive to inhibition by NEM. In addition, Dillon et al. [34] have shown that the yeast DGPP phosphatase is similar to the *E. coli* DGPP phosphatase in its ability to utilize LPA and PGP as substrates in vitro.

8. Isolation and characterization of *S. cerevisiae* genes encoding for DGPP phosphatase and the similarity of DGPP phosphatase to mammalian PAP2

Carman and co-workers [35] have purified sufficient DGPP phosphatase (peak 2 [29]) to obtain amino acid sequence information to peptide fragments of the enzyme. The sequences that have been obtained are MNRVSFIKTPFNIGAKWRLE, QPVEGLPLDTLFTAK, and FPPIDDPLPFKPLMD. A gene in *S. cerevisiae* (GenBank: U51031), whose predicted amino acid sequence corresponds exactly to the amino acid sequences derived from these peptide fragments has been identified. This gene has been referred to as *DPP1* (for *d*iacylglycerol *p*yrophosphate *p*hosphatase). The *DPP1* gene is located on

chromosome IV. The predicted protein product is 289 amino acids in length, has a molecular mass of 33.5 kDa, and is predicted to be an integral membrane protein with six transmembrane helices. During purification, the DGPP phosphatase enzyme is tightly associated with microsomal membranes and the purified enzyme migrates as a 34-kDa protein after SDS-PAGE analysis [29]. The identification of the *DPP1* gene has also been facilitated by studies showing that the *E. coli pgpB* gene encodes for a DGPP phosphatase activity [34] and that these enzymes share homology in a novel phosphatase sequence motif [36]. This sequence motif consists of three domains [36]. The alignment of the amino acid sequences of the *S. cerevisiae* and *E. coli* DGPP phosphatases in these domains is shown in Table 2.

When *DPP1* is run through GenBank, a mouse cDNA product is identified (GenBank: D84376) that Kanoh et al. [37] reported encodes a Mg^{2+}-independent PA phosphatase (PAP2). This enzyme also contains the novel phosphatase sequence motif (Table 1). As discussed elsewhere in this issue (Kanoh), two forms of PA phosphatase exist in mammalian cells. One form of PA phosphatase (PAP1) is primarily responsible for the synthesis of phospholipids and triacylglycerols [5,38–40], while the other form of PA phosphatase (PAP2) is believed to be involved in lipid signaling pathways [39–42]. The two forms of PA phosphatase have distinguishing enzymological properties that are used to differentiate them. PAP1 has a Mg^{2+} ion requirement and is inhibited by the thioreactive agent NEM [39,41,42]. PAP2 does not have a Mg^{2+} ion requirement and is insensitive to NEM [39,41,42]. PAP2, purified from rat liver [43–45] and porcine thymus [37,46], share enzymological properties that are strikingly similar to the PA phosphatase activity exhibited by the DGPP phosphatases isolated from *S. cerevisiae* [29] and *E. coli* [34]. As indicated above, this PA phosphatase activity [29] is distinctly different from that of the 104- and 45-kDa PA phosphatases that have been purified from *S. cerevisiae* [8,9], but does resemble that of the mammalian PAP2 enzymes. Like PAP2, the PA phosphatase activity catalyzed by DGPP phosphatase is Mg^{2+}-independent and NEM-insensitive [29,34]. In addition, the PAP2 [43] and DGPP phosphatase [29,34] enzymes can utilize LPA as a substrate. Dillon et al. [47] have shown that purified rat liver PAP2 catalyzes the dephosphorylation of DGPP. This reaction is Mg^{2+}-independent, insensitive to inhibition by NEM, and inhibited by Mn^{2+} ions. PAP2 exhibits a very high affinity for DGPP. The specificity constant (V_{max}/K_m) for DGPP is 1.3-fold higher than that of PA. DGPP inhibits the ability of PAP2 to dephosphorylate PA and PA inhibits the dephosphorylation of DGPP. Collectively, these results indicate that the DGPP phosphatases from *S. cerevisiae* and *E. coli* are members of a phosphatase family that includes mammalian PAP2. Interestingly, the PA phosphatase activity that was originally described in chicken liver by Kennedy and co-workers in 1957 is a Mg^{2+}-independent enzyme [1].

Table 1
Protein alignments of the phosphatase sequence motif of DGPP phosphatases from *S. cerevisiae* and *E. coli* and mouse PAP2 [a]

Protein	Domain 1[b]	Domain 2	Domain 3
DGPP phosphatase 1 (*S. cerevisiae*)	117-**KNWIGRLRP**-39-**PSGH**-46-**SRTQDYRHHFVD**-289		
DGPP phosphatase 2 (*S. cerevisiae*)	135-**KLIIGNLRP**-41-**PSGH**-38-**SRVIDHRHHWYD**-275		
DGPP phosphatase (*E. coli*)	96-**KDKVQEPRP**-54-**PSGH**-36-**SRLLLGMHWPRD**-254		
PAP2 (mouse)	119-**KYTIGSLRP**-39-**YSGH**-44-**SRVSDYKHHWSD**-283		

[a] Data taken from Ref. [36].
[b] The numbers preceding domain 1 indicate the length in amino acids of the N-terminus of the protein. The numbers between domains indicate the amino acids between each domain. The numbers following domain 3 indicate the total amino acids in each protein.

9. Unresolved issues and future developments

A summary of the properties of the PA phosphatases and DGPP phosphatases is presented in Table 2. Although a great deal is known about the biochemical regulation of the 104- and 45-kDa forms of PA phosphatase from *S. cerevisiae*, little is known about the genetic regulation of these enzymes. The isolation of the structural genes encoding for PA phosphatase enzymes are required to more fully understand their regulation and contribution to the control of lipid synthesis in yeast.

Table 2
Properties of PA Phosphatases and DGPP Phosphatases from *S. cerevisiae* and *E. coli*

Enzyme	Subcellular localization	Purified	Cloned	Activity	Substrate	Activators	Inhibitors	Other Properties	Ref.
104-kDa (91-kDa) PA phosphatase (*S. cerevisiae*)	microsomes	purified to apparent homogeneity	no	Mg^{2+} ions, pH optimum 7, Triton X-100	PA	CL, CDPDG, PI, DGPP, Triton X-100	PC, PE, nucleotides, sphingoid bases, thioreactive reagents (NEM), phenylglyoxal, propanolol	induced in stationary phase	[4], [8], [9], [10,12,16,18,29]
45-kDa PA phosphatase (*S. cerevisiae*)	microsomes mitochondria	purified to apparent homogeneity	no	Mg^{2+} ions, pH optimum 6-7, Triton X-100	PA	CL, CDPDG, PI, Triton X-100	PC, PE, nucleotides, sphingoid bases, thioreactive reagents (NEM), phenylglyoxal, propanolol,	activation by protein kinase A phosphorylation, induced in stationary phase and by inositol supplementation	[4] [9] [12,14,16,18]
75-kDa PA phosphatase (*S. cerevisiae*)	cytosol	partially purified	no	Mg^{2+} ions, pH optimum 7–8	PA			induced in stationary phase	[2–4]
34-kDa DGPP phosphatase (*S. cerevisiae*)	microsomes	purified to apparent homogeneity	*DPP1*	pH optimum 6.5, Triton X-100	DGPP, PA, LPA, CerP, PGP	Triton X-100	Mn^{2+} ions, PPi, ADP, NaF		[29,34,47]
28-kDa DGPP phosphatase (*E. coli*)	cytoplasmic membranes	partially purified	*pgpB*	pH optimum 6.5, Triton X-100	DGPP, PA, LPA, PGP	Triton X-100	Mn^{2+} ions		[31–34]

The identification of DGPP phosphatase in *S. cerevisiae* is an exciting discovery. This observation, and the initial analyses of the enzyme, indicate that DGPP phosphatase plays a previously unidentified role in lipid metabolism. Recent studies indicate that the metabolism of DGPP is involved in a novel lipid signaling pathway. DGPP has been found in a variety of plants [48,49] and in *S. cerevisiae* [29]. The amounts of DGPP in plants and in wild-type *S. cerevisiae* are barely detectable [29,49]. For example, DGPP accounts for only 0.18 mol.% of the major phospholipids in *S. cerevisiae* [29]. The low abundance of DGPP is reminiscent of other lipid signaling molecules such as the inositol-containing phospholipids [50–54]. Munnik et al. [49] have shown that DGPP accumulates in plant tissues upon G protein activation through the stimulation of PA kinase activity. It has been suggested that the function of DGPP is to attenuate the signaling functions of PA [55,56], that DGPP is the precursor of the PA which serves as a signaling molecule, or that DGPP itself functions as a signaling molecule [29,49]. DGPP phosphatase may function to regulate the cellular levels of DGPP, PA, as well as DG.

As indicated above, the deduced protein encoded by the yeast *DPP1* gene shows homology to the deduced protein encoded by the mouse *PAP2* cDNA. These protein products also show homology to the deduced protein products of the *WUN* gene of *Drosophila* [57] and the *Dri 42* gene of rat intestine [58]. Wunen, product of the *WUN* gene, repels migrating germ cells during embryonic development. The repulsive function of Wunen plays a role in guiding germ cells toward the mesoderm during development [57]. Based on the predicted protein sequence homologies of the *WUN* gene and of the PAP2 cDNA, Howard and co-workers [57] have speculated that Wunen mediates its function through lipid signaling pathways involving PA phosphatase activity. The expression of the Dri 42 protein is up-regulated during epithelial cell differentiation [58]. The Wunen and Dri 42 proteins have not been examined for DGPP phosphatase or Mg^{2+}-independent PA phosphatase activities. However, these papers are noteworthy since they provide potential evidence for physiological roles of DGPP phosphatase and/or Mg^{2+}-independent PA phosphatase.

The literature indicates that PAP2 acts on a number of lipid phosphate compounds which play a role in lipid signaling in mammalian cells [39,42,55]. The fact that PAP2 shows homology to the DGPP phosphatases and displays DGPP phosphatase activity suggests that this activity could play an important physiological role in eukaryotic cells. The availability of the yeast *DPP1* gene encoding for DGPP phosphatase and the genes encoding for mammalian PAP2 enzymes will allow a combination of genetic, molecular, and biochemical approaches to gain an understanding of the role(s) DGPP phosphatase and PAP2 play in lipid metabolism and lipid signaling.

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