Mammalian Mg\(^{2+}\)-independent Phosphatidate Phosphatase (PAP2) Displays Diacylglycerol Pyrophosphate Phosphatase Activity*

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Recent studies indicate that the metabolism of diacylglycerol pyrophosphate (DGPP) is involved in a novel lipid signaling pathway. DGPP phosphatases (DGPP phosphohydrolase) from Saccharomyces cerevisiae and Escherichia coli catalyze the dephosphorylation of DGPP to yield phosphatidate (PA) and then catalyze the dephosphorylation of PA to yield diacylglycerol. We demonstrated that the Mg\(^{2+}\)-independent form of PA phosphatase (PA phosphohydrolase, PAP2) purified from rat liver catalyzed the dephosphorylation of DGPP. This reaction was Mg\(^{2+}\)-independent, insensitive to inhibition by N-ethylmaleimide and bromoeno lactone, and inhibited by Mn\(^{2+}\) ions. PAP2 exhibited a high affinity for DGPP (K\(_{m}\) = 0.04±0.01 μM). The specificity constant (V\(_{max}\)/K\(_{m}\)) for DGPP was 1.3-fold higher than that of PA. DGPP inhibited the ability of PAP2 to dephosphorylate PA, and PA inhibited the dephosphorylation of DGPP. Like rat liver PAP2, the Mg\(^{2+}\)-independent PA phosphatase activity of DGPP phosphatase purified from S. cerevisiae was inhibited by lyso-PA, sphingosine 1-phosphate, and ceramide 1-phosphate. Mouse PAP2 showed homology to DGPP phosphatases from S. cerevisiae and E. coli, especially in localized regions that constitute a novel phosphatase sequence motif. Collectively, our work indicated that rat liver PAP2 is a member of a phosphatase family that includes DGPP phosphatases from S. cerevisiae and E. coli. We propose a model in which the phosphatase activities of rat liver PAP2 and the DGPP phosphatase of S. cerevisiae regulate the cellular levels of DGPP, PA, and diacylglycerol.

PA\(^{3}\) phosphatase (3-sn-phosphatidate phosphohydrolase, EC 3.1.3.4) catalyzes the dephosphorylation of PA to yield DG and P\(_{i}\). Two forms of PA phosphatase exist in mammalian cells. Data indicate that one form of PA phosphatase (PAP1) is primarily responsible for the synthesis of phospholipids and triacylglycerols (2–5), whereas the other form of PA phosphatase (PAP2) is primarily involved in lipid signaling pathways (4–7). PA, the substrate of the PA phosphatase reaction, regulates the activity of several lipid-dependent enzymes (8–12) and exhibits mitogenic effects in mammalian cells (12–16). Thus, the action of PA phosphatase is thought to attenuate the signaling functions of PA (7). In addition, Brindley and co-workers (7, 17) have shown that PAP2, purified from rat liver, has the ability to dephosphorylate LPA, SPP, and CerP. These substrates and their hydrolysis products have been shown to play a role in signaling pathways in mammalian cells (7, 17).

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 thio reactive agent NEM (4, 6, 7). PAP2 does not have a Mg\(^{2+}\)-ion requirement and is insensitive to NEM (4, 6, 7). PAP2, purified from rat liver (18–20) and porcine thymus (21, 22), shares enzymological properties that are strikingly similar to a PA phosphatase activity exhibited by a DGPP phosphatase (DGPP phosphohydrolase) that has recently been isolated from Saccharomyces cerevisiae (23) and Escherichia coli (24). DGPP phosphatase catalyzes the dephosphorylation of the novel lipid DGPP to form PA and P\(_{i}\) (23, 25). When DGPP is supplied as a substrate in vitro, the enzyme removes the β-phosphate of DGPP to generate PA and then removes the phosphate of PA to generate DG (23, 24). Although DGPP phosphatase utilizes PA as a substrate in the absence of DGPP, the enzyme has a preference for DGPP as a substrate (23, 24). This PA phosphatase activity (23) is distinctly different from that of the PAP1 enzymes that have been purified from S. cerevisiae (26, 27) 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 (23, 24). In addition, the PAP2 (18) and DGPP phosphatase (23, 24) enzymes can utilize LPA as a substrate. Given these similarities, we hypothesized that mammalian PAP2 would display DGPP phosphatase activity. Using purified PAP2 from rat liver we demonstrated that PAP2 catalyzed the DGPP phosphatase reaction. Recent data indicate that DGPP and the enzymes responsible for its metabolism are involved in a novel lipid signaling pathway (23, 28, 29). The implications of PAP2 having DGPP phosphatase activity are discussed in relation to lipid signaling pathways.

EXPERIMENTAL PROCEDURES

Materials—All chemicals were reagent grade. Radiochemicals and EN\(^{3}\)HANCE were from DuPont NEN. Scintillation counting supplies were from National Diagnostics. Nucleotides, NEM, Triton X-100, and bovine serum albumin were purchased from Sigma. Phospholipids were purchased from Avanti Polar Lipids and Sigma. Protein assay kits were purchased from Bio-Rad (Coomassie Blue) and Pierce (BCA). silica gel 60 thin-layer chromatography plates were from EM Science. Protein
A-Sepharose and Sephacryl S-200 were from Pharmacia Biotech Inc. E. coli DG kinase was obtained from Lipidex Inc. or Calbiochem. Bromo- 2-nanol lactone [(E)-6-(bromomethylene) tetrahydro-3-(1-naphthalenyl)-2H-pyran-2-one] was obtained from Dr. Edward A. Dennis (University of California, San Diego, CA).

Preparation of Enzymes—PAP2 (anionic form) was purified from rat liver plasma membranes as described by Waggner et al. (18). DGPP phosphatase (23) and the 104-kDa Mg$^{2+}$-dependent PA phosphatase (26, 27) were purified from the microsomal fraction of S. cerevisiae as described previously. PA kinase was purified from plasma membranes of Catharanthus roseus cells as described by Wissing and Behrbohm (30).

Preparation of Substrates—DGPP standard and $^{32}$P-labeled (α$^{32}$P and β$^{32}$P) DGPP were synthesized enzymatically using purified C. roseus PA kinase as described by Wu et al. (23). [$^{32}$P]IPA and [$^{32}$P]CerP were synthesized enzymatically from DG and long-chain ceramide, respectively, using E. coli DG kinase (33) as described previously (17, 26). Unlabeled CerP was also synthesized enzymatically via the DG kinase reaction (17). SPP was prepared from CerP by acid hydrolysis (34).

Preparation of Triton X-100/Lipid-mixed Micelles—Lipids in chloroform were transferred to a test tube, and solvent was removed in vacuo for 1 h. Triton X-100/lipid-mixed micelles were prepared by adding various amounts of a 5% (w/v) solution of Triton X-100 to the dried lipids. After the addition of Triton X-100, the mixture was vortexed. The surface concentration of lipids in mixed micelles was varied by the addition of Triton X-100. The total lipid concentration in Triton X-100/lipid-mixed micelles did not exceed 20 mol % to ensure that the structure of the mixed micelles was similar to the structure of pure Triton X-100 (35, 36). The uniformity of Triton X-100/DGPP-mixed micelles was determined by Sephacryl S-300 gel filtration chromatography (37). The mole percent of a lipid in a mixed micelle was calculated using the formula: mol %lipid = ([lipid (bulk)]/[lipid (bulk) + [Triton X-100]) × 100.

Enzyme Assays—DGPP phosphatase activity was measured by following the release of water-soluble $^{32}$P, from chloroform-soluble [γ$^{32}$P]DGPP (5,000–10,000 cpm/nmol) or by following the formation of [$^{32}$P]IPA from [α$^{32}$P]DGPP (2,000–5,000 cpm/nmol) as described by Wu et al. (23). The reaction mixture contained 50 mM Tris-maleate buffer (pH 6.5), enzyme protein, and the indicated concentrations of Triton X-100 and DGPP in a total volume of 50 μl. Kinetic experiments were performed with 50 mM citrate buffer (pH 5.5). The chloroform-soluble phospholipid product of the reaction, PA, was analyzed by thin-layer chromatography on potassium oxalate-treated plates using the solvent system chloroform/acetone/methanol/glacial acetic acid/water (50:15:13:12:4) (23). The positions of the labeled phospholipids on the chromatograms were determined by autoradiography. The amount of labeled phospholipids was determined by scintillation counting.

Mg$^{2+}$-independent PA phosphatase and Mg$^{2+}$-dependent PA phosphatase activities were measured by following the release of water-soluble $^{32}$P, from chloroform-soluble [γ$^{32}$P]IPA (10,000 cpm/nmol) (18, 38). The reaction mixture contained 50 mM Tris-maleate buffer (pH 6.5), enzyme protein, and the indicated concentrations of Triton X-100 and PA in a total volume of 50 μl. Kinetic experiments were performed with 50 mM citrate buffer (pH 5.5). The reaction mixture for Mg$^{2+}$-dependent PA phosphatase contained 50 mM Tris-maleate buffer (pH 7.0), 2 mM MgCl$_2$, 0.1 mM PA, 1 mM Triton X-100, 10 mM 2-mercaptoethanol, and enzyme protein in a total volume of 0.1 ml. CerP phosphatase activity was measured by following the release of water-soluble $^{32}$P, from chloroform-soluble [γ$^{32}$P]CerP (10,000 cpm/nmol) (17). The reaction mixture contained 100 mM Tris-maleate buffer (pH 6.5), 1 mM EDTA, enzyme protein, and the indicated concentrations of Triton X-100 and CerP in a total volume of 25 μl. All enzyme assays were conducted for 15 min at 30 °C in triplicate. The average S.D. of the assays was ± 5%. The enzyme reactions were linear with time and protein concentration. A unit of enzymatic activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol of product/min. Specific activity was defined as units/mg of protein. Protein concentration was determined using the BCA assay or by the method of Bradford (39) using bovine serum albumin as the standard.

Analysis of Kinetic Data—Kinetic data were analyzed according to the Michaelis-Menten and Hill equations using the EZ-FIT Enzyme Kinetic Model Fitting Program (40). EZ-FIT uses the Nelder-Mead Simplex and Marquardt/Nash nonlinear regression algorithms sequentially and tests for the best fit of the data among different kinetic models.

FIG. 1. Time dependence of the DGPP phosphatase reaction catalyzed by rat liver PAP2. DGPP phosphatase activity was measured with 0.1 mM [α$^{32}$P]DGPP and 5 mM Triton X-100 using 40 ng of rat liver PAP2. After incubation for the indicated time intervals, the chloroform-soluble [α$^{32}$P]DGPP was separated from [α$^{32}$P]DGPP by thin-layer chromatography (A) and then analyzed by scintillation counting (B). The water-soluble [α$^{32}$P]DGPP was hydrolyzed by [β$^{32}$P]DGPP and analyzed by scintillation counting (B). The positions of standard DGPP and PA after thin-layer chromatography are indicated in A.

Rat Liver PAP2 Displays DGPP Phosphatase Activity—Rat liver PAP2 was examined for its ability to catalyze the removal of the β phosphate from DGPP using [α$^{32}$P]DGPP as the substrate. The chloroform-soluble product of the reaction was analyzed by thin-layer chromatography followed by autoradiography. The enzyme catalyzed a time-dependent conversion of DGPP to PA (Fig. 1A). Quantification of the PA spots on the thin-layer chromatogram by scintillation counting showed that the DGPP phosphatase reaction was linear (Fig. 1B). A DGPP phosphatase reaction was also conducted over the same time period using [β$^{32}$P]DGPP as the substrate. In this case, enzyme activity was followed by measuring the water-soluble product of the reaction by scintillation counting. The amount of β-labeled P$_2$ produced in the reaction was also linear with time and paralleled the amount of PA produced in the reaction using the α-labeled substrate (data not shown). Because rat liver PAP2 catalyzes the dephosphorylation of PA (18), we examined the water-soluble fraction of the DGPP phosphatase reaction for the production of PA$_2$. Over the time period of the reaction, the P$_2$ phosphatase catalyzed essentially the stoichiometric conversion of DGPP to PA and P$_2$. When the DGPP phosphatase reaction was followed for longer time intervals (e.g., 1 h), the enzyme then removed the phosphate of PA to produce DG (data not shown).

Although the rat liver PAP2 preparation used in these studies was highly purified, it was not a homogeneous protein sample (18). We obtained a small sample of homogeneous enzyme from this preparation by immunoprecipitation with anti-PAP2 antibodies (18). This immunoprecipitated enzyme catalyzed the DGPP phosphatase reaction. Because the amount of the immunoprecipitated enzyme was limiting, we examined the properties of the DGPP phosphatase reaction using the nonhomogeneous but highly purified PAP2 preparation (18).

Properties of the DGPP Phosphatase Activity of Rat Liver PAP2—The characteristic properties of rat liver PAP2 are the absence of any divalent cation requirement and its insensitivity to inhibition by the thiorreactive compound NEM (7, 18). The DGPP phosphatase activity of the rat liver PAP2 was not dependent on any divalent cation under standard assay conditions. Moreover, the addition of 1 mM EDTA plus 1 mM EGTA to the assay system did not affect activity. DGPP phosphatase activity was insensitive to inhibition by NEM at concentrations up to 10 mM. As a control, the PA phosphatase activity of PAP2 was shown not to require a divalent cation and was insensitive to inhibition by NEM.
to treatment with NEM. The rat liver PAP1 enzyme is totally inhibited by 2 mM NEM (6).

Balsinde and Dennis (5) have recently discovered a new property that can be used to characterize the differences between the mammalian Mg2+-independent and Mg2+-dependent PA phosphatase activities. Using P388D1 macrophages, these workers have shown that the Mg2+-dependent enzyme is potently inhibited by bromoeno lactone (IC50 = 8 μM), whereas the Mg2+-independent enzyme is insensitive to this reagent (5). We examined if the DGPP phosphatase and PA phosphatase activities of rat liver PAP2 were sensitive to bromoeno lactone. Samples of the purified enzyme were preincubated with increasing concentrations of bromoeno lactone for 10 min and then assayed for each activity. Concentrations of bromoeno lactone up to 100 μM had no effect on the DGPP phosphatase and PA phosphatase activities of PAP2. Bromoeno lactone had no effect on S. cerevisiae DGPP phosphatase activity. As a positive control, we examined the effect of bromoeno lactone on the 104-kDa Mg2+-dependent PA phosphatase purified from S. cerevisiae. As described previously (5), this Mg2+-dependent enzyme was inhibited by bromoeno lactone.

A characteristic property of the DGPP phosphatases isolated from S. cerevisiae (23) and from E. coli (24) is the inhibition of their activities by Mn2+ ions. We examined the effect of Mn2+ ions on the DGPP phosphatase and PA phosphatase activities of rat liver PAP2. The addition of Mn2+ ions to the assay system for DGPP phosphatase resulted in a dose-dependent inhibition of activity (Fig. 2A). The inhibition of DGPP phosphatase activity by Mn2+ ions followed positive cooperative kinetics (n = 3.3). An IC50 value of 0.27 mM was calculated based on the analysis of the data according to the Hill equation. On the other hand, the PA phosphatase activity of PAP2 was relatively insensitive to inhibition by Mn2+ ions up to a concentration of 2.5 mM (Fig. 2B).

Jamal et al. (6) have previously shown that the pH optimum for the Mg2+-independent PA phosphatase activity associated with plasma membranes of rat liver cells was 6.5. However, the pH dependence of the purified enzyme activity has not been examined (18). In this study we examined the effect of pH on the DGPP phosphatase and PA phosphatase activities of purified rat liver PAP2. Maximum DGPP phosphatase activity was observed between pH 5.0 and 6.0, and maximum PA phosphatase activity was observed between pH 5.0 and 6.5. The Mg2+-independence and insensitivity to NEM and bromoeno lactone of the DGPP phosphatase and PA phosphatase activities were not affected when measured at pH 5.5.

Kinetics of the DGPP Phosphatase Activity of Rat Liver PAP2—The kinetics of DGPP phosphatase and PA phosphatase activities of rat liver PAP2 were examined using Triton X-100/phospholipid-mixed micelles. The kinetic analyses of these activities using mixed micelle substrates required that these micelles were homogeneous in size. Previous studies have shown that Triton X-100 forms uniform mixed micelles with PA (37). It was important for us to demonstrate that Triton X-100 formed uniform mixed micelles with DGPP. Gel filtration analysis of a mixture of Triton X-100 plus 5 mol % DGPP showed that Triton X-100 formed uniform mixed micelles with DGPP. The Triton X-100/phospholipid-mixed micelle system permitted the kinetic analyses of DGPP phosphatase and PA phosphatase activities using surface dilution kinetics (41). Surface dilution kinetics is a model system that mimics the physiological nature of the membrane where two-dimensional surface interactions occur (41). Accordingly, the concentrations of DGPP and PA in the mixed micelles were expressed as a surface concentration (in mol %) as opposed to a molar concentration (41). In addition, these activities were independent of the molar concentrations of DGPP and PA at the Triton X-100/phospholipid-mixed micelle concentrations used in this study (17, 23). DGPP phosphatase activity displayed by PAP2 exhibited saturation kinetics with respect to the surface concentration of DGPP (Fig. 3A). The Vmax was 1.24 μmol/min/mg, and the Ks value for DGPP was 0.04 mol % as described previously (17), the PA phosphatase activity of PAP2 displayed saturation kinetics with respect to the surface concentration of PA (Fig. 3B). The Vmax was 0.93 μmol/min/mg, and the Ks was 0.04 mol %.

Effects of PA and DGPP on the DGPP Phosphatase and PA Phosphatase Activities, Respectively, of Rat Liver PAP2—Because rat liver PAP2 utilized both DGPP and PA as substrates, we examined whether PA affected DGPP phosphatase activity and whether DGPP affected PA phosphatase activity. In these experiments, we used a surface concentration of 0.05 mol % of DGPP for the DGPP phosphatase reaction and a surface concentration of 0.05 mol % of PA for the PA phosphatase reaction. These surface concentrations were near the respective Ks values for these substrates. Thus, we could readily observe inhibitory or stimulatory effects of PA and DGPP on DGPP phosphatase and PA phosphatase activities, respectively. PA inhibited the DGPP phosphatase activity in a dose-dependent manner (Fig. 4A). An IC50 value for PA of 0.07 mol % was calculated from a replot of the log of relative DGPP phosphatase activity versus the PA concentration. DGPP inhibited the PA phosphatase activity in a dose-dependent manner (Fig. 4B). The IC50 value for DGPP was calculated to be 0.05 mol %.

Effects of LPA, SPP, and CerP on the S. cerevisiae DGPP Phosphatase—The Mg2+-independent PA phosphatase activity of rat liver PAP2 has been shown to be inhibited by LPA, SPP,
and CerP (17). These lipid phosphate compounds can also serve as substrates for PAP2 in vitro (17). Given the similarities between rat liver PAP2 and the S. cerevisiae DGPP phosphatase, we examined whether the Mg\(^{2+}\)-independent PA phosphatase activity of S. cerevisiae DGPP phosphatase was inhibited by LPA, SPP, and CerP. The surface concentration of PA (2.2 mol %) used in these experiments was the concentration of PA at its Km value for this reaction (23). All three of these compounds inhibited PA phosphatase activity in dose-dependent manners (Fig. 5). LPA was the most potent inhibitor with an IC50 value of 0.4 mol % (Fig. 5). We also examined the effect of LPA on the DGPP phosphatase activity of the S. cerevisiae enzyme. The surface concentration of DGPP (0.5 mol %) used in this experiment was the concentration of DGPP near its Km value (23). LPA inhibited the DGPP phosphatase activity of the enzyme (Fig. 6). However, the inhibitory effect of LPA (IC50 = 3.3 mol %) on the DGPP phosphatase reaction was much less potent when compared with its inhibitory effect with respect to the PA phosphatase reaction (Fig. 5). As described previously (23), PA did not inhibit the DGPP phosphatase activity of the enzyme (Fig. 6).

The inhibition of the S. cerevisiae DGPP phosphatase by LPA, SPP, and CerP suggested that these lipid phosphate compounds might serve as substrates for the enzyme. Indeed, we have recently shown that LPA is a substrate for the DGPP phosphatase (24). DGPP phosphatase catalyzed the dephosphorylation of CerP in a dose-dependent manner (Fig. 7), demonstrating that CerP was also a substrate for the enzyme. The reaction followed saturation kinetics, and the Vmax and Km values were calculated to be 110 μmol/min/mg and 4 mol %, respectively.

**DISCUSSION**

DGPP phosphatase is a recently discovered enzyme that has been identified in C. roseus, E. coli, S. cerevisiae, rat liver, pig liver, pig brain, and bovine brain (25). The discovery of DGPP phosphatase in such a wide range of organisms suggests that it plays an important role in phospholipid metabolism and cell growth. DGPP phosphatase has been purified to homogeneity from S. cerevisiae and characterized with respect to its enzymological and kinetic properties (23). DGPP phosphatase has been partially purified from E. coli and shown to be the product of the pgpB gene (24). The DGPP phosphatases isolated from S. cerevisiae (23) and E. coli (24) catalyze the dephosphorylation of the β phosphate of DGPP to yield PA and then catalyze the dephosphorylation of the PA product to yield DG. The DGPP phosphatase and PA phosphatase activities of the DGPP phosphatase enzymes from S. cerevisiae (23) and E. coli (24) are Mg\(^{2+}\)-independent and NEM-insensitive (23, 24). In addition, these DGPP phosphatase activities are potently inhibited by Mn\(^{2+}\) ions (23, 24).

In this study we examined the hypothesis that PAP2 purified from rat liver would display DGPP phosphatase activity. One impetus for this study was the fact that the characteristic properties of PAP2 (i.e. Mg\(^{2+}\)-independent and NEM-insensitive PA phosphatase activity) (4, 6, 18, 19, 21) are the same as those of the PA phosphatase activities described for the DGPP...
We showed in this study that PAP2 did indeed catalyze the DGPP phosphatase reaction. Like the DGPP phosphatases from *S. cerevisiae* (23) and *E. coli* (24), the DGPP phosphatase activity of PAP2 was Mg$^{2+}$-independent, NEM-insensitive, and inhibited by Mn$^{2+}$ ions. The latter effect probably reflects a specific interaction of DGPP with Mn$^{2+}$ ions that is not exhibited with PA. The rat liver and *S. cerevisiae* DGPP phosphatase activities were also insensitive to inhibition by bromoeno lactone, which has recently been used to distinguish between the Mg$^{2+}$-dependent and Mg$^{2+}$-independent forms of PA phosphatase (5). Furthermore, the *S. cerevisiae* DGPP phosphatase activities were similar in that they used other lipid phosphate compounds such as LPA and CerP as substrates.

Although our studies revealed similarities between rat liver PAP2 and the DGPP phosphatases of *S. cerevisiae* and *E. coli*, there were also differences among these enzymes. Interestingly, the affinity of PAP2 for DGPP and PA as substrates was much greater than the affinities of the DGPP phosphatases of *S. cerevisiae* and *E. coli* for these substrates (Table I). The specificity constant ($V_{\text{max}}/K_m$) of rat liver PAP2 for DGPP was slightly higher (1.3-fold) than that for PA (Table I). PA inhibited the dephosphorylation of DGPP by rat liver PAP2, and DGPP inhibited the dephosphorylation of PA by PAP2. The inhibition constants for PA and DGPP of rat liver PAP2 were 9-fold higher than those for PA (Table I) (23, 24). The ratios of PA up to 16-fold greater than the concentration of DGPP did not inhibit *S. cerevisiae* DGPP phosphatase activity.

A cDNA encoding for PAP2 has been cloned from mouse cells that encodes for a protein with a predicted minimum subunit molecular mass of 31.9 kDa (22). DGPP phosphatase activity is associated with a 34-kDa protein that we have purified to homogeneity from *S. cerevisiae* (23). We have obtained sufficient amino acid sequence information from this 34-kDa protein to identify and isolate the gene (GenBank™ accession no. U51031) encoding for this enzyme.2 We refer to this gene as *DPP1* (diacylglycerol pyrophosphate phosphatase). In addition, we have identified and isolated a second gene (GenBank™ accession no. U33057) from *S. cerevisiae* that is homologous to *DPP1* that we refer to as *DPP2.* The predicted minimum subunit molecular masses of the proteins encoded by these genes are 33.5 and 31.6 kDa, respectively. The subunit molecular mass of the protein encoded by the *E. coli pgpB* gene is 28 kDa (42). The amino acid sequences of DGPP phosphatases of *S. cerevisiae* and *E. coli* and the mouse PAP2 proteins show homology to each other. In particular there are localized regions of high homology that constitute a novel phosphatase sequence motif (44). The motif contains three domains (44). The alignment of the amino acid sequences of PAP2 and the DGPP phosphatases in these domains is shown in Table II. The size of mammalian PAP2 seems to vary in different tissues (18). These different sizes may be attributed to variations in the extent of their glycosylation because treatment of purified PAP2 from rat liver (18) and pig thymus (22) with N-glycanase decreases their apparent size from 51–53 kDa and 35 kDa, respectively, to about 30 kDa. There is no evidence of N-glycosylation of the DGPP phosphatases from *S. cerevisiae* and *E. coli*. Collectively, the work presented here indicated that mammalian PAP2 is a member of a phosphatase family that includes DGPP phosphatases from *S. cerevisiae* and *E. coli*.

DGPP is a novel phospholipid that was first identified as the product of the PA kinase reaction in the plant *C. roseus* (28). DGPP has since been found in a variety of plants (29, 30) and in *S. cerevisiae* (23). The amounts of DGPP in plants and in wild-type *S. cerevisiae* are barely detectable (23, 29). For example, DGPP accounts for only 0.18 mol % of the major phospholipids in *S. cerevisiae* (23). The low amount of DGPP is reminiscent of other lipid signaling molecules such as the in-

### Table I

| Enzyme                        | $V_{\text{max}}$ | $K_m$ | $V_{\text{max}}/K_m^{a}$ | $IC_{50}$ | $V_{\text{max}}$ | $K_m$ | $V_{\text{max}}/K_m^{a}$ | $IC_{50}$ |
|------------------------------|------------------|-------|--------------------------|----------|------------------|-------|--------------------------|----------|
| **PA**                       |                  |       |                          |          |                  |       |                          |          |
| (units/mg)                   |                  |       |                          |          | (mol%)           |       |                          |          |
| Rat liver PAP2               | 1.24             | 0.04  | 31                       | 0.05b    | 70               | 2.2   | 32                       | NDf      |
| DGPP phosphatase$^d$ (S. cerevisiae) | 172             | 0.55  | 313                      | 0.35b    | 23               | 0.04  | 32                       | NDf      |
| DGPP phosphatase$^d$ (E. coli) | 2.16             | 2.3   | 0.94                     | NDf      | 0.31             | 3.1   | 0.1                      | NDf      |

*a* Because the enzymes from rat liver and *E. coli* have not been purified to homogeneity, the specificity constants reported in the table cannot be compared.

$b$ Inhibitor constant with respect to PA as the substrate.

$c$ Inhibitor constant with respect to DGPP as the substrate.

$d$ Data taken from Ref. 23.

*e* ND, not inhibitory.

$f$ Data taken from Ref. 24.

### Table II

| Protein                          | Domain 1$^b$        | Domain 2 | Domain 3 |
|----------------------------------|---------------------|----------|----------|
| PAP2 (mouse)                     | 119-KYTIGSLRP-39-YSGLH44-SRVSDDYHHSW-283 |          |          |
| DGPP phosphatase 1 (S. cerevisiae) | 117-KNNIGKLRP-39-PSGH-46-SRTQYKHFV-289 |          |          |
| DGPP phosphatase 2 (S. cerevisiae) | 135-KLIIGNLRP-38-PSGH-46-SRTQDYHHT-275 |          |          |
| DGPP phosphatase (E. coli)        | 96-KDKVQEPRP-54-PSGH-36-SRLLGHHWPRD-254 |          |          |

*a* Data taken from Ref. 44.

$b$ The numbers preceding domain 1 indicate the length in amino acids of the N terminus of the protein. The numbers after domain 3 indicate the total amino acids in each protein.

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ositol-containing phospholipids (45–49). Recent studies have shown that DGPP accumulates in plant tissues upon G protein activation through the stimulation of PA kinase activity (29), and metabolic labeling studies have shown that DGPP is metabolized to PA and then to DG (25). At the present time the function of DGPP in phospholipid metabolism and cell signaling is unknown. It has been suggested that DGPP might attenuate the signaling functions of PA, that DGPP is the precursor of the PA that serves as a signaling molecule, or that DGPP itself might function as a signaling molecule (23, 29). DGPP and the DGPP phosphatases from S. cerevisiae and E. coli are fascinating enzymes in that the product of one reaction becomes the substrate for another reaction (Fig. 8). Based on available information, we propose models in which feedback inhibition of the activities displayed by mammalian PAP2 (Fig. 8A) and S. cerevisiae DGPP phosphatase (Fig. 8B) could regulate the cellular levels of DGPP, PA, and DG. For example, our data showed that for rat liver PAP2, PA potently inhibited the ability of the enzyme to dephosphorylate DGPP and that DGPP potently inhibited the ability of the enzyme to dephosphorylate PA. Although DGPP inhibits the ability of the yeast DGPP phosphatase to dephosphorylate PA (23), its ability to dephosphorylate DGPP is not inhibited by PA.

There is a vast literature that indicates that PAP2 acts on a number of lipid phosphate compounds, which play a role in lipid signal transduction in mammalian cells (4, 7, 50). The findings presented here demonstrated that PAP2 also has a DGPP phosphatase activity. Given that this activity could regulate the levels of DGPP, PA, and DG in cells, it is clear that the role of DGPP phosphatase activity in lipid signaling as well as phospholipid metabolism should be addressed. Studies are currently in progress in our laboratories to examine the physiological roles of DGPP and DGPP phosphatase in eukaryotic cells.

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