The LPP1 and DPP1 Gene Products Account for Most of the Isoprenoid Phosphate Phosphatase Activities in Saccharomyces cerevisiae*

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Two genes in Saccharomyces cerevisiae, LPP1 and DPP1, with homology to a mammalian phosphatidic acid (PA) phosphatase were identified and disrupted. Neither single nor combined deletions resulted in growth or secretion phenotypes. As observed previously (Toke, D. A., Bennett, W. L., Dillon, D. A., Wu, W.-I., Chen, X., Ostrander, D. B., Oshiro, J., Cremesti, A., Voelker, D. R., Fischl, A. S., and Carman, G. M. (1998) J. Biol. Chem. 273, 3278–3284; Toke, D. A., Bennett, W. L., Oshiro, J., Wu, W.-I., Voelker, D. R., and Carman, G. M. (1998) J. Biol. Chem. 273, 14331–14338), the disruption of DPP1 and LPP1 produced profound losses of Mg2+-independent PA phosphatase activity. The coincident attenuation of hydrolytic activity against diacylglycerol pyrophosphate prompted an examination of the effects of these disruptions on hydrolysis of isoprenoid phosphates. Disruption of either LPP1 or DPP1 caused respective decreases of about 25 and 75% in Mg2+-independent hydrolysis of several isoprenoid phosphates by particulate fractions isolated from these cells. The particulate and cytosolic fractions from the double disruption (dpp1Δ dpp1Δ) showed essentially complete loss of Mg2+-independent hydrolytic activity toward dolichyl phosphate (dolichyl-P), dolichyl pyrophosphate (dolichyl-P-P), farnesyl pyrophosphate (farnesyl-P-P), and geranylgeranyl pyrophosphate (geranylgeranyl-P-P). However, a modest Mg2+-stimulated activity toward PA and dolichyl-P was retained in cytosol from lpp1Δ dpp1Δ cells. The activity of Dpp1p on isoprenyl pyrophosphates was confirmed by characterization of the hydrolysis of geranylgeranyl-P-P by the purified protein. These results indicate that LPP1 and DPP1 account for most of the hydrolytic activities toward dolichyl-P, dolichyl-P-P, farnesyl-P-P, and geranylgeranyl-P-P but also suggest that yeast contain other enzymes capable of dephosphorylating these essential isoprenoid intermediates.

Phosphorylated lipids serve diverse roles in cellular metabolism, including signal transduction, membrane biosynthesis, and energy storage. These lipid phosphates are created through direct phosphorylation of lipids by lipid kinases such as diacylglycerol kinase and dolichol kinase which produce phosphatic acid (PA) and dolichyl monophosphate (dolichyl-P), respectively. Alternatively, these molecules can be formed by degradation of precursor molecules as in the hydrolysis of phosphorylases by phospholipase D to form PA or the transfer of oligosaccharides from a dolichol carrier to produce dolichyl pyrophosphate (dolichyl-P-P) or dolichyl-P (1). The phosphorylated lipids can be metabolized by several phosphatases or used in synthetic reactions to produce a variety of phospholipids or dolichyl oligosaccharides (2–6).

PA serves as both a signaling molecule and as an important precursor of several phospholipids. In signal transduction, PA, created by the action of phospholipase D, may act directly as a second messenger or be hydrolyzed to diacylglycerol, another well-characterized signaling molecule. At least two types of phosphatase activity can metabolize PA. A type I activity is dependent on Mg2+ and inhibited by N-ethylmaleimide, whereas a type II activity is independent of Mg2+ and insensitive to the alkylating agent (3). The type I activity in Saccharomyces cerevisiae is found in both cytosolic and particulate fractions (7, 8). The activity in the particulate fraction has been purified to apparent homogeneity and corresponds to two integral membrane proteins with molecular masses of 104 and 45 kDa (9). Although these activities have been characterized extensively (9–13), the genes encoding the enzymes responsible have not been identified.

The characterization of type II PA-phosphatase activities is more advanced. The purification of a type II activity from the particulate fraction of porcine thymus led to the isolation of a corresponding cDNA from mouse kidney (14). Yeast also contains type II activities associated with a particulate fraction (15). In S. cerevisiae, two genes have been identified that have similar hydrophy profiles and are homologous to the mammalian enzyme. The conservation of amino acid sequence is particularly strong in three regions thought to form the active sites of the enzymes (4, 16–18). One of the type II activities in yeast also hydrolyzed diacylglycerol pyrophosphate (diacylglycerol-P-P) and was purified to homogeneity on the basis of this activity (19). This enzyme proved to be one of the identified

1 The abbreviations used are: PA, phosphatidic acid; LPA, lysophosphatidic acid; Man-P-Dol, mannosylphosphoryldolichol; dolichyl-P, dolichyl phosphate; dolichyl-P-P, dolichyl pyrophosphate; MES, 4-morpholineethanesulfonic acid; ORF, open reading frame.
yeast homologs (YDR284C) and has been designated diacylglycerol-pyrophosphate phosphatase, Dpp1p. Although identified as a pyrophosphatase, Dpp1p can further metabolize the PA formed to diacylglycerol, albeit with lower catalytic efficiency (19). Dpp1p, along with other enzymes that hydrolyze diacylglycerol-P-P, act on a variety of lipid monophosphates including lysophosphatidic acid (LPA), ceramide phosphate, sphingosine 1-phosphate, and phosphatidylglycerol phosphate (19–21).

The second homolog from yeast (lipid phosphate phosphatase, LPP1, YDR503c), also has broad specificity as demonstrated by hydrolysis of PA, LPA, and diacylglycerol-P-P (15). A type II PA phosphatase from rat liver was similar in its ability to hydrolyze many different phosphorylated lipid substrates including those listed for Dpp1p (21–23). Further evidence indicated that this mammalian activity could hydrolyze dolichyl-P (23). The existence of a relatively non-selective lipid phosphatase was also suggested by observations that Mg\textsuperscript{2+}-independent dolichyl-P phosphatase activities from mammalian sources were inhibited by PA and LPA (24, 25). Furthermore, a dolichyl-P phosphatase that was purified to apparent homogeneity from the particulate fraction of porcine brain used both dolichyl-P and PA with similar catalytic efficiency (26).

This evidence and the knowledge that the yeast enzymes use multiple substrates suggested that DPP1 and/or LPP1 may catalyze hydrolysis of phosphorylated and pyrophosphorylated isopenoids. Here, evidence for such activities and their virtual absence after disruption of LPP1 and DPP1 is presented and discussed.

EXPERIMENTAL PROCEDURES

General Reagents and Methods—All chemicals were reagent grade. Radiochemicals, unless noted, and EN'\(\text{HANCE}'\)were from NEN Life Science Products. Triton X-100, isopentyl alcohol, isopentyl pyrophosphate, farnesol, farnesyl pyrophosphate, geraniol, geranyl pyrophosphate, geranylerganyrl pyrophosphate, and bovine serum albumin were purchased from Sigma. Other reagents were obtained from the sources indicated: Silica Gel 60 thin layer chromatography plates (EM Science), Silica Gel-loaded SG81 chromatography paper (Whatman, Inc.),\[\text{HI}farnesyl pyrophosphate (60 mCi/mmole), and\[\text{HI}geranylerganyl pyrophosphate (American Radiolabeled Chemicals, Inc.),\text{Tran}^{32}\text{P}-\text{label (ICN Biochemicals)}, Zymolase 100-T (Seikagaku Kogyo Co.), Glusulase (DuPont), and oligonucleotide primers (Genosys). Molecular biology reagents were from New England Biolabs unless otherwise noted. Protein concentration was determined by described methods (27, 28) using bovine serum albumin as the standard.

Yeast Media and Methods—S. cerevisiae was propagated in yeast extract/peptone/dextrose (YPD) or yeast nitrogen broth (YNB) supplemented with amino acids as required. Mating, sporulation, and other standard techniques used established methods (29). The strains used are presented in Table I. Yeast total genomic DNA was isolated after disruption of cells with glass beads (30). Immunoprecipitation of carboxypeptidase Y was done as described (31).

Isolation of LPP1 (ORF YDR503C) and DPP1 (ORF YDR284C)—Two yeast homologs of a mouse Mg\textsuperscript{2+}-independent PA phosphatase (14), corresponding to yeast open reading frames YDR503C and YDR284C, were found by searching the Stanford University Saccharomyces Genome Data base (2). During the course of this study, these genes became known as LPP1 and DPP1, respectively (15, 32).

LPP1 and DPP1 were amplified with PCR and the genomic DNA from yeast strain YDR284C using Vent\textsuperscript{®} DNA Polymerase (New England Biolabs), supplied buffers, and 5% Me SO. Oligonucleotide primers for LPP1 (YDR503C) (5'GTTAGGATCCGTCCTATCCGTAATGTCATGCTC3') contained BamHI and EcoRI sites for insertion into the Bluescript SK\textsuperscript{+} vector (pBSK\textsuperscript{+}), and the primers used to amplify DPP1 (YDR284C) (5'ATTGAGTTCGGAATACATTCTTATTGGCT3') contained SacI and PstI sites for insertion into pBSK\textsuperscript{+}. Amplified products were verified by sequencing.

\[\text{Stanford University Saccharomyces Genome Data base address: http://genome-www.stanford.edu/Saccharomyces/}\]
LPP1 and DPP1 Account for Isoprenoid Phosphate Phosphatase

Two Genes from Yeast Encoding Putative Homologs of a Mouse Mg$^{2+}$-independent PA Phosphatase Gene—Two putative yeast proteins (YDR503c and YDR284c) with 49% similarity at the amino acid level to the published sequence for a mouse Mg$^{2+}$-independent PA phosphatase (14) were identified in the S. cerevisiae genome. During the course of this study, these genes were termed LPP1 (lipid phosphate phosphatase) and DPP1 (diacylglycerol-pyrophosphate phosphatase), respectively (15, 32). The amino acid sequences of LPP1 and DPP1 are 51% similar and 26% identical to each other. The proteins encoded by these three genes share a common hydropathy plot with less than 3% of parental activity (Fig. 1A). Other strains are described in this report. The terms wild-type, lpp1Δ, and lpp1Δ dpp1Δ refer to SEY6211 strains unless explicitly stated.

**RESULTS**

Two Genes from Yeast Encoding Putative Homologs of a Mouse Mg$^{2+}$-independent PA Phosphatase Gene—Two putative yeast proteins (YDR503c and YDR284c) with 49% similarity at the amino acid level to the published sequence for a mouse Mg$^{2+}$-independent PA phosphatase (14) were identified in the S. cerevisiae genome. During the course of this study, these genes were termed LPP1 (lipid phosphate phosphatase) and DPP1 (diacylglycerol-pyrophosphate phosphatase), respectively (15, 32). The amino acid sequences of LPP1 and DPP1 are 51% similar and 26% identical to each other. The proteins encoded by these three genes share a common hydropathy plot with less than 3% of parental activity (Fig. 1A). Other strains are described in this report. The terms wild-type, lpp1Δ, and lpp1Δ dpp1Δ refer to SEY6211 strains unless explicitly stated.

**FIG. 1.** PA phosphatase activity in the wild-type (WT) and disrupted strains of S. cerevisiae in the presence and absence of Mg$^{2+}$. A, particulate fractions were prepared from the indicated strains of S. cerevisiae and assayed for PA phosphatase activity in the absence (open bars) or presence (filled bars) of Mg$^{2+}$ as described under “Experimental Procedures.” B, the amount of PA phosphatase activity in particulate fractions due solely to stimulation by the presence of Mg$^{2+}$ (difference between the shaded and open bars in A). C, PA phosphatase activity in cytosolic fractions of WT and lpp1Δ dpp1Δ cells measured in either the absence or presence of Mg$^{2+}$ as indicated.

Mg$^{2+}$-independent and Mg$^{2+}$-dependent PA Phosphatase in the Wild-type and Disrupted Strains—Biochemical analysis of cellular extracts revealed substantial changes in lipid phosphatase activities in the disrupted strains. Particulate fractions prepared from lpp1Δ and dpp1Δ strains exhibited only 77 and 24%, respectively, of the Mg$^{2+}$-independent PA phosphatase activity present in the wild-type strain. The disruption of both genes (lpp1Δ dpp1Δ strain) resulted in a particulate fraction with less than 3% of parental activity (Fig. 1A, open bars). The Mg$^{2+}$-dependent PA phosphatase activities associated with the cytosol were unchanged in disrupted strains (Fig. 1C, solid bars; data not shown). However, the apparent Mg$^{2+}$-dependent PA phosphatase activity in particulate fractions increased significantly in preparations from dpp1Δ and lpp1Δ dpp1Δ cells.
FIG. 2. Dephosphorylation of PA, dolichyl-P, and dolichyl-P-P by particulate fractions from wild-type and disrupted strains of S. cerevisiae. A, particulate fractions of the wild-type (WT), lpp1Δ, dpp1Δ, and lpp1Δ dpp1Δ strains of S. cerevisiae were prepared and assayed for phosphatase activity using PA (open bars), dolichyl-P (striped bars), and dolichyl-P-P (solid bars) as substrates. B, the specific activities from the disrupted strains are expressed as a percentage of wild-type activities.

The Disruption of LPP1 and DPP1 Caused Decreases in the Dephosphorylation of Substrates Other Than PA—Particulate fractions from wild-type yeast dephosphorylated both dolichyl-P and dolichyl-P-P in the absence of Mg2+ (Fig. 2A). The rates of hydrolysis of dolichyl-P (1.2 nmol/min/mg) and dolichyl-P-P (0.5 nmol/min/mg) were substantial albeit less than for PA (3.1 nmol/min/mg). Rates of dephosphorylation of dolichyl-P catalyzed by the particulate fractions prepared from lpp1Δ, dpp1Δ, and lpp1Δ dpp1Δ strains were 80, 16, and 0.5%, respectively, of activity in the wild-type strain (Fig. 2). The same samples yielded relative rates for the dephosphorylation of dolichyl-P-P of 91, 25, and 1% (Fig. 2). These changes, observed in the absence of Mg2+, closely paralleled the altered rates for Mg2+-independent PA phosphatase activity. Similar losses in activity were observed in multiple preparations and homogenates of the cells. In contrast to PA (Fig. 1B), a small Mg2+-dependent hydrolysis of dolichyl-P and dolichyl-P-P in particulate preparations from lpp1Δ dpp1Δ cells was unchanged from wild type (data not shown).

Since dolichyl-P-P phosphatase activity was diminished in the particulate fraction of the yeast strains where the genes LPP1 and DPP1 were disrupted, two other biologically relevant isoprenoid pyrophosphates, farnesyl-P-P and geranylgeranyln-P-P, were examined. When compared with the wild-type strain, the decreases in rates of hydrolysis of farnesyl-P-P and geranylgeranyln-P-P paralleled the decreases in the hydrolysis of dolichyl-P-P by the particulate fractions from lpp1Δ, dpp1Δ, and lpp1Δ dpp1Δ strains (Fig. 3).

Examination of the Lipid Phosphatase Activity in the Cytosol—The vast majority of Mg2+-independent lipid phosphatase activity (for PA, dolichyl-P, and dolichyl-P-P) was found in particulate fractions (>95%, data not shown). In contrast, the PA phosphatase activity in the cytosol of yeast has been reported to be Mg2+-dependent (7, 8). The addition of Mg2+ to cytosol from the wild-type strain of yeast produced a robust stimulation (8.5-fold) of PA phosphatase activity (Fig. 1C). Similar amounts of Mg2+-dependent PA phosphatase activity were observed in the cytosol from the lpp1Δ dpp1Δ strain, although Mg2+-independent activity was virtually absent (Fig. 1C). The specific activity for dephosphorylation of dolichyl-P in cytosol was much lower than for PA (Figs. 1C and 4), but the profile was similar (Fig. 4) in that a 5.5-fold stimulation was observed with the addition of magnesium. Mg2+-independent dolichyl-P phosphatase activity was lost in the double mutant, yet the specific activity for dolichyl-P in the presence of Mg2+ was equivalent to the wild-type strain (Fig. 4). In contrast, Mg2+ did not stimulate the hydrolysis of dolichyl-P-P in cytosol from either wild-type or the lpp1Δ dpp1Δ strains (Fig. 4). Both cytosol and the particulate fraction from the lpp1Δ dpp1Δ strain were essentially devoid of dolichyl-P-P phosphatase activity in either the presence or absence of Mg2+ (Figs. 2A and 4). These results indicate that the activities encoded by the genes LPP1 and DPP1 may account for the modest Mg2+-independent activities in cytosol but do not account for the Mg2+-dependent hydrolysis of PA and dolichyl-P. The lack of action on dolichyl-P-P clearly distinguishes the specificity of the enzyme responsible for the Mg2+-dependent cytosolic activity from that of Lpp1p and Dpp1p.

Comparison of Man-P-Dol Synthase Activity in Wild-type and Mutant Strains—The reduction in hydrolytic activity for dolichyl phosphates in the double mutant raises the possibility that the pools of these lipids might be altered. The enzymatic transfer of [3H]mannose from GDP-[3H]mannose to endogenous dolichyl-P was used to assess the cellular pool of the isoprenoid monophosphate accessible to Man-P-Dol synthase in particulate fractions from wild-type and disrupted strains of yeast. The initial rates and extents of this reaction catalyzed by particulate fractions derived from the various strains (data not shown). These results suggest that the disruption of LPP1 and/or DPP1 does not affect the endogenous pool of dolichyl-P.

Specificity of Purified Dpp1p for Isoprenoid Compounds—
The recognition of various isoprenyl phosphate esters by purified Dpp1p was tested by competition for hydrolysis of diacylglycerol-P-P at subsaturating concentrations. In this manner, either inhibitory or stimulatory effects on enzyme activity could be observed. Isopenetyl pyrophosphate (IC\textsubscript{50} = 1.92 mol %), geranyl pyrophosphate (IC\textsubscript{50} = 0.43 mol %), farnesyl-P-P (IC\textsubscript{50} = 0.29 mol %), and geranylgeranyl-P-P (IC\textsubscript{50} = 0.26 mol %) inhibited diacylglycerol-P-P phosphatase activity with potencies that increase with the chain length of the isoprenoid. In contrast, diacylglycerol-pyrophosphate phosphatase activity was not affected by the alcohol derivatives of these isoprenoid compounds indicating that inhibition was dependent on the presence of the pyrophosphate moieties (Fig. 5).

A kinetic analysis of hydrolysis of diacylglycerol-P-P by Dpp1p exhibited saturation kinetics in both the absence and presence of farnesyl-P-P (Fig. 6A) or geranylgeranyl-P-P (Fig. 6B). Each of the isoprenoid compounds affected the apparent \( K_m \) value for diacylglycerol-P-P phosphatase activity but had no effect on \( V_{\text{max}} \). These results are consistent with farnesyl-P-P and geranylgeranyl-P-P being competitive inhibitors of diacylglycerol-P-P phosphatase. Replots of the data in Fig. 6 were linear and were used to calculate \( K_{\text{m}} \) values for farnesyl-P-P and geranylgeranyl-P-P of 0.3 and 0.1 mol %, respectively.

**Dephosphorylation of Geranylgeranyl Pyrophosphate by Dpp1p**—The action of geranylgeranyl-P-P as a competitive inhibitor of diacylglycerol-P-P phosphatase activity and the attenuation of hydrolytic activity for this molecule by disruption of DPP1 and LPP1 indicated that Dpp1p would utilize geranylgeranyl-P-P as a substrate. Therefore, the formation of geranylgeranyl-P from geranylgeranyl-P-P was assessed using substrate labeled in the geranyl moiety of the compound. Indeed, the diacylglycerol-P-P phosphatase enzyme catalyzed a time-dependent dephosphorylation of geranylgeranyl-P-P (Fig. 7A). The production of geranylgeranyl-P was linear for 30 min. After this time the apparent production of geranylgeranyl-P was reduced, whereas production of geranylglycerol increased (data not shown). This indicated that the enzyme could also catalyze the dephosphorylation of geranylglycerol phosphate. The effect of pH on the geranylgeranyl-P-P phosphatase reaction is shown in Fig. 7B. The enzyme exhibited a pH optimum between 5.0 and 5.5.

**FIG. 4. Dephosphorylation of dolichyl-P and dolichyl-P-P by cytosolic fractions of wild-type (WT) and lpp1Δ dpp1Δ strains.** Cytosolic fractions of the indicated strains of \( S. \) cerevisiae were prepared and assayed for phosphatase activity with either dolichyl-P or dolichyl-P-P as the substrate and either the presence or absence of Mg\( ^{2+} \) as indicated.

**FIG. 5. Inhibition of diacylglycerol-pyrophosphate phosphatase activity of purified Dpp1p by other phosphorylated isoprenoids.** Diacylglycerol-pyrophosphate (DGPP) phosphatase activity was measured under standard assay conditions with 0.3 mol % diacylglycerol pyrophosphate (bulk concentration of 0.1 mM) in the absence and presence of the indicated concentrations of isopentyl alcohol (○), geraniol (◇), farnesol (□), isopentyl pyrophosphate (■), geranyl pyrophosphate (●), farnesyl pyrophosphate (▲), and geranylglycerol pyrophosphate (filled circle with white dot).

The dependence of geranylglycerol-P-P phosphatase activity on the concentration of geranylglycerol-P-P was examined using mixed micelles of Triton X-100 and geranylglycerol-P-P. The concentration of the substrate was expressed as a surface concentration (in mol %). The enzyme catalyzed a dose-dependent dephosphorylation of geranylglycerol-P-P and exhibited saturation kinetics (Fig. 7C). A double-reciprocal plot of the data was linear and was used to calculate a \( V_{\text{max}} \) of 90 units/mg and a \( K_m \) of 0.18 mol %. This compares with reported \( V_{\text{max}} \) and \( K_m \) values for diacylglycerol-P-P of 172 units/mg (at pH 6.5) and 0.55 mol % (19). However, subsequent studies have determined the pH optimum of Dpp1p for diacylglycerol-P-P to be 5.0 and the \( V_{\text{max}} \) at this pH is 260 units/mg (5). Thus, at their pH optima, the specificity constants (\( V_{\text{max}}/K_m \)) for diacylglycerol-P-P and geranylglycerol-P-P are 472 and 500, respectively, indicating that both are equally good substrates for the enzyme.

**DISCUSSION**

Two genes with homology to a Mg\( ^{2+} \)-independent PA phosphatase from mouse were identified in the \( S. \) cerevisiae genome. The disruption of these genes produced dramatic reductions in the amount of Mg\( ^{2+} \)-independent PA phosphatase activity in lysates and particulate fractions from the mutant strains. Consistent with previous studies (15, 32), these data indicate that the two genes, DPP1 and LPP1, account for most of the Mg\( ^{2+} \)-independent PA phosphatase activity in yeast. The predominant association of these activities with particulate fractions is consistent with the predicted structure of the two proteins.

Enzymes identified as PA hydrolases have been shown to dephosphorylate several other lipid phosphates (4, 14, 19–21, 32, 43, 44). Purified Dpp1p was further shown to first remove the \( \beta \)- and subsequently the \( \alpha \)-phosphate from diacylglycerol-P-P and the resulting PA (19). The catalytic efficiency (\( V_{\text{max}}/K_m \)) for the hydrolysis of diacylglycerol-P-P was 10-fold greater than that for PA. In this study, the potential spectrum of action of Lpp1p and Dpp1p was further extended to the dephosphorylation of isoprenoid mono- and diphosphates, and purified Dpp1p was shown to dephosphorylate geranylglycerol-P-P with specificity constants indistinguishable from those obtained with diacylglycerol-P-P. The results represent the first demon-
FIG. 6. Kinetic analysis of the inhibitory action of farnesyl and geranylgeranyl pyrophosphate on the hydrolysis of diacylglycerol pyrophosphate by Dpp1p. A, the phosphatase activity of Dpp1p was measured as a function of the surface concentration (mol %) of diacylglycerol pyrophosphate (substrate, bulk concentration 0.1 mM) at set surface concentrations of farnesyl pyrophosphate of 0 (●), 0.15 (■), 0.45 (▲), and 0.75 (○) mol %. The data are plotted as 1/diacylglycerol pyrophosphate phosphatase (units/mg) versus the reciprocal of the diacylglycerol pyrophosphate surface concentration. The inset is a replot of the slopes versus the concentration of farnesyl pyrophosphate. B, phosphatase activity of Dpp1p was measured as a function of the surface concentration (in mol %) of diacylglycerol pyrophosphate (bulk concentration 0.1 mM) at set surface concentrations of geranylgeranyl pyrophosphate of 0 (●), 0.15 (■), 0.45 (▲), and 0.75 (○) mol %. The data are plotted as 1/diacylglycerol pyrophosphate phosphatase (units/mg) versus the reciprocal of the diacylglycerol pyrophosphate surface concentration. The inset is a replot of the slopes versus the concentration of geranylgeranyl pyrophosphate. The lines drawn in each of the panels were determined by least squares analysis. Abbreviations used are: DGPP, diacylglycerol pyrophosphate; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate.

FIG. 7. Hydrolysis of geranylgeranyl pyrophosphate (GGPP) by isolated Dpp1p; effect of time, pH, and concentration of substrate. A, hydrolysis of 2 nmol of [3H]geranylgeranyl pyrophosphate was assessed at the indicated time intervals. The production of geranylgeranyl phosphate was determined by paper chromatography with SG 81 paper as described under “Experimental Procedures.” B, the geranylgeranyl-pyrophosphate phosphatase activity of Dpp1p was measured at the indicated pH values with 50 mM citrate/maleate/Tris buffer. C, the geranylgeranyl-pyrophosphate phosphatase activity of the Dpp1p was measured as a function of the surface concentration (in mol %) of geranylgeranyl pyrophosphate. The molar concentration of geranylgeranyl pyrophosphate was held constant at 0.1 mM, whereas the Triton X-100 concentration was varied. The inset is a replot of 1/V (units/mg) versus the reciprocal surface concentration of geranylgeranyl pyrophosphate. The line was determined by least squares analysis.

...that dolichyl-P, dolichyl-P-P, farnesyl-P-P, and geranylgeranyl-P-P are dephosphorylated by activities in yeast and that most of this activity is due to expression of Dpp1p and Lpp1p.

Several functions could be attributed to these isoprenoid phosphatases. Conversion of polyisoprenyl pyrophosphates to monophosphates could be required for the re-cycling of the carrier lipid for another round of lipid intermediate synthesis when dolichyl-P is released during primary N-glycosylation of proteins. A polyisoprenyl-pyrophosphate phosphatase would also be necessary for the dephosphorylation of the long chain polyisoprenyl pyrophosphate intermediate formed in the de novo pathway for dolichyl-P biosynthesis prior to the reduction of the α-isoprene unit (45, 46). The metabolic and functional significance of the dephosphorylation of dolichyl-P is not completely understood. Nevertheless, dolichyl-P phosphatase activity has been characterized from several sources (25, 37, 47, 48). Such a reaction could be important for the topological redistribution of dolichols. Man-P-Dol and glucosylphosphoryldolichol are formed on the cytosolic face of the endoplasmic reticulum and then diffuse transversely to the luminal monolayer where they function as glycosyl donors (46). Dephosphorylation of the dolichyl-P molecules formed during the lumenal mannosylation and glucosylation reactions might be important to allow free dolichol to diffuse back to the cytosolic face of the endoplasmic reticulum. Dolichyl-P could then be re-synthesized by dolichol kinase and utilized again as a glycosyl carrier lipid.

Considering the important roles for dolichyl-P-P and dolichyl-P phosphatases, and the large attenuation of hydrolytic activity for dolichyl-P and, especially, dolichyl-P-P, it is surprising that defects in glycosylation or changes in the accessible dolichyl-P pool were not observed in the lpp1Δ dpp1Δ strain. A novel Mg2⁺-dependent dolichyl-P phosphatase activity was noted in the cytosolic fraction of yeast. It is possible that this activity could compensate for the loss of Mg2⁺-independent activity in the disrupted strains. However, no Mg2⁺-dependent dolichyl-P-Pase activity was noted in the cytosol. The results raise the possibility that there is another enzyme(s) in yeast capable of degrading dolichyl-P-P that may not be active under...
conditions used for the in vitro assays in this study.

The reductions in the rates of dephosphorylation of farnesyl-P-P and geranylgeranyl-P-P in the disrupted strains are also intriguing. Previous data have shown that levels of diacetylglycerol-P-P increase in yeast membranes when DPP1 is disrupted (32). It remains to be determined if comparable increases in the isoprenyl substrates (including dolichyl-P-P) are characteristics of the lpp1Δ dpp1Δ strain. The significance of dephosphorylation of the farnesyl and geranylgeranyl compounds to cellular physiology is not known. Two activities that were described in microsomal preparations from rat liver appeared to be quite specific for either farnesyl-P-P or geranylgeranyl-P-P (49). Such specificity was not apparent with purified Dpp1p. Both microsomal activities were reported to have $K_m$ values in the low micromolar range and exhibited pH optima of 5.5 to 6.0 (49), properties similar to those described for Dpp1p.

It is possible that Dpp1p and possibly Lpp1p could act to prevent a toxic accumulation of isoprenoid pyrophosphates. Such an accumulation could occur if hydroxymethylglutaryl-CoA reductase were not down-regulated concurrently with squalene synthase activity, a later step in ergosterol synthesis. A synthesis such as this is created artificially in the erg9 mutant (squalene synthase). This could cause an accumulation of farnesyl-P-P that is toxic to the cell or upset the synthesis of dolichol and ubiquinone or the isoprenylation of protein. Over-expression of the ERG20 gene (farnesyl-P-P synthase) in an erg9 mutant background led to a dramatic increase in the level of dolichol (50). It is possible that the farnesyl-P-P pool is shunted in this direction, but dephosphorylation of farnesyl-P-P may also be important.

Interestingly, a “salvage pathway” for farnesol and geranylgeraniol has been described recently in mammalian cells that can allow the free isoprenoids to be re-utilized for protein isoprenylation (51) presumably after conversion to the isoprenyl phosphates. Although similar kinase activities are not described in yeast, their presence in mammalian cells may indicate that there are pools of farnesol and geranylgeraniol that must be phosphorylated to be used by the cell. The free isoprenoids could be formed in yeast by the combined action of the phosphatases described here or perhaps by the “turnover” of isoprenylated proteins. The metabolic balance in dephosphorylation and rephosphorylation of farnesyl-P-P and geranylgeranyl-P-P could play a role in the regulation of their cellular levels.

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