**LPT1 Encodes a Membrane-bound O-Acyltransferase Involved in the Acylation of Lysophospholipids in the Yeast Saccharomyces cerevisiae**

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The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1 and S2.

The abbreviations used are: PA, phosphatidic acid; PAF, platelet-activating factor; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPS, lysophosphatidylserine; LPG, lysophosphatidylglycerol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; CL, cardiolipin; G-3-P, glycerol 3-phosphate; DHAP, dihydroxyacetone phosphate; LPAAT, lysophosphatidic acid acyltransferase; MBOA, membrane-bound O-acyltransferase; LC/MS, liquid chromatography/mass spectrometry; HA, hemagglutinin; ORF, open reading frame; GFP, green fluorescent protein; ER, endoplasmic reticulum; aa, amino acid(s); CHAPS, 3-[N-(cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; LPAAT, lysophosphatidic acid acyltransferase.

Phospholipids are major components of cellular membranes that participate in a range of cellular processes. Phosphatidic acid (PA) is a key molecule in the phospholipid biosynthetic pathway. In *Saccharomyces cerevisiae*, SLC1 has been identified as the gene encoding lysophosphatidic acid acyltransferase, which catalyzes PA synthesis. However, despite the importance of PA, disruption of SLC1 does not affect cell viability (Nagiec, M. M., Wells, G. B., Lester, R. L., and Dickson, R. C. (1993) *J. Biol. Chem.* 268, 22156–22163). We originally aimed to identify the acetyl-CoA:lysophosphatidic acid platelet-activating factor acetyltransferase (lysoPAF AT) gene in yeast. Screening of a complete set of yeast deletion clones (4741 homozygous diploid clones) revealed a single mutant strain, YOR175c, with a defect in lysoPAF AT activity. YOR175c has been predicted to be a member of the membrane-bound O-acyltransferase superfamily, and we designated the gene *LPT1*. An Lpt1-green fluorescent protein fusion protein localized at the endoplasmic reticulum. Other than lysoPAF AT activity, Lpt1 catalyzed acyltransferase activity with a wide variety of lysophospholipids as acceptors, including lysophosphatidic acid, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysophosphatidylinositol, and lysophosphatidylserine. A liquid chromatography-mass spectrometry analysis indicated that lysoPAF AT accepts various lysophosphatidic acid (LPA) analogs including 1-acyl-LPA and other lysophosphatidic acid analogs that have been previously identified. LPAAT revealed a single mutant strain, YOR175c, with a defect in the biosynthesis of lysophospholipids. PA is synthesized by two major de novo biosynthetic pathways that utilize either glycerol 3-phosphate (G-3-P) or dihydroxyacetone phosphate (DHAP) as precursors (1, 2). G-3-P is acylated by G-3-P acyltransferase at the sn-1 position to form lysophosphatidic acid (LPA). DHAP is acylated at the sn-1 position by DHAP acyltransferase to produce 1-acyl-DHAP, which is reduced by 1-acyl-DHAP reductase to form LPA. LPA produced by these two different pathways is further acylated by LPA acyltransferase in the sn-2 position to yield PA.

The cell membrane is a semipermeable lipid bilayer found in all living cells that physically separates the cytoplasm of the cell from the extracellular environment. Glycerophospholipids and sphingophospholipids are the major components of most cell membranes. Phosphatidic acid (PA)2 is a key intermediate in the biosynthesis of glycerophospholipids. PA is synthesized by two major de novo biosynthetic pathways that utilize either glycerol 3-phosphate (G-3-P) or dihydroxyacetone phosphate (DHAP) as precursors (1, 2). G-3-P is acylated by G-3-P acyltransferase at the sn-1 position to form lysophosphatidic acid (LPA). DHAP is acylated at the sn-1 position by DHAP acyltransferase to produce 1-acyl-DHAP, which is reduced by 1-acyl-DHAP reductase to form LPA. LPA produced by these two different pathways is further acylated by LPA acyltransferase in the sn-2 position to yield PA.
phospholipids is carried out by phospholipases and lysophospholipid acyltransferases, but the specific enzyme involved in the remodeling of glycerophospholipids had not been identified. Very recently, a gene for mouse lysophosphatidylcholine (LPC) acyltransferase was cloned by two different research groups and designated LPCAT1 (20, 21). LPCAT1 is highly expressed in alveolar type II cells and is thought to be involved in the production of surfactant lipids. A related gene, LPCAT2, which encodes an enzyme having both acetyl-CoA:lysophospholipid-activating factor acyltransferase (lysoPAF AT) activity and LPC acyltransferase activity, has also been reported (22).

In this study, we searched for lysoPAF AT genes in yeast by measuring the enzyme activity of each strain in a complete set of yeast deletion clones. By this brute force approach, we identified a novel lysoPAF AT gene that did not show any homology with LPCAT2, and which we designated LPT1 (lysoPAF AT). LPT1 gene products possess lysophospholipid acyltransferase activity with a wide variety of substrate acceptors, including LPC, LPE, LPA, LPI, LPS, and LPG. We also characterized in detail the enzymatic activity and cellular role of Lpt1.

**EXPERIMENTAL PROCEDURES**

**Strains and Media**—The complete set of Yeast Deletion Clones (Homozygous Diploid) and the parental strain BY4743 were purchased from Invitrogen. Other yeast strains used in this study are shown in Table 1. Strains were grown on either YPD medium (1% yeast extract, 2% Bacto-peptone, and 2% glucose), synthetic dextrose (0.67% yeast nitrogen base without amino acids (Difco), 2% glucose, and appropriate supplements) or synthetic complete (SC) medium (SD medium, with dropout amino acids (Difco), 2% glucose, and appropriate supplements)

**Plasmid Construction**—YEplac112 (24) was digested with AatII, blunted, and BAP treated, and then the BsaB1-BsaA1 fragment containing the rop region from pBR322 was ligated to an AatII, blunted, and BAP treated, and then the BsaB1-BsaA1 fragment containing the rop region from pBR322 was ligated to the SmaI site of plasmid pUC19 to form LPT1-pUC19. The open reading frame (ORF) of LPT1 was amplified by PCR using primers 5’-GGTGCAGCTCATGTAC-AATCCGTGGAGCCT-3’ and 5’-TTTAGCTCTACTAGCGGCGCCCTCTTCTTCTTTTTGAAATAGGC-3’ to introduce a Sall site before the first ATG codon and NotI and SacI sites just before and after the stop codon, respectively. The resultant fragment was ligated into pMW119 (Nippongene, Japan), and a NotI DNA fragment containing the triple hemagglutinin (HA) epitope coding sequence or a NotI-Sacl DNA fragment containing the green fluorescent protein (GFP) coding sequence was inserted to yield LPT1-ORF(HA)/pMW119 or LPT1-ORF(GFP)/pMW119. A 2.7-kb Sall-Sacl fragment of LPT1/pUC19 was inserted into pMW119 (HindIII–), in which the HindIII site is disrupted. Then the plasmid was cleaved with HindIII and SacI, and the 3’-region of the LPT1 gene was replaced with the corresponding region of LPT1-ORF(HA)/pMW119 or LPT1-ORF(GFP)/pMW119 to create LPT1-HA/pMW119 (HindIII–) or LPT1-GFP/pMW119 (HindIII–). Finally, the SacI-Sacl fragment containing LPT1-HA and LPT1-GFP was inserted into YEplp (rop) to form LPT1-HA/YEp(rop) and LPT1-GFP/YEp(rop), respectively. His-32, a potential active site residue, was changed to asparagine by using the QuikChange site-Directed mutagenesis kit (Stratagene). Site-directed mutagenesis was performed by using LPT1-HA/YEp(rop) as a template with the following primers: 5’-CTTCGCGCATTTTTGGAGTGACACGCGCAGC-3’ and 5’-AGTACCAGGTCTGTTACCATCCAAAATGCGGAA-GTTAGG-3’ (altered nucleotides are underlined).

The ORF of the PLB1 gene was amplified by PCR using primers (5’-AGTCCGACATGAATGGTGAATGGTGG-3’ and 5’-GGCTGGCCGAATTAGCAAGACGCGCT-3’) to introduce a Sall site before the first ATG codon and a NotI site just before the stop codon, and the ORF was ligated into the Sall site of plasmid pUC19 to form PLB1/pUC19. A Sall-NotI fragment containing the PLB1 ORF was inserted into YEGAp (25) to form PLB1/YEGAp, in which the PLB1 gene was expressed under the control of the glyceraldehyde-3-phosphate dehydrogenase promoter.

**Strain Construction**—The LPT1 allele was deleted in strains W303-1A and 1278b by using a PCR-derived lpxK-kanMX-loxP cassette containing the kanamycin resistance gene (26) with the following primers: 5’-CGGCAAGACAAACCGTGTT-GTGATTTAATTCGCTGATCGCCTACGGACTGAA-AATCCGTGGAGCCT-3’ and 5’-GACAACAGAAGACTGTGACCT

### Table 1

Yeast strains used in this study

| Strain     | Relevant genotype                                      | Source or reference |
|------------|--------------------------------------------------------|---------------------|
| BY4743     | MATα/his3delta1/α/his3delta1 leu2delta0/α/leu2delta0 lys2delta0/α/lys2               | Invitrogen          |
| YOR175c    | MATα/YOR175c-kanMX/YOR175c-kanMX                       | Invitrogen          |
| W303-1A    | MATα ade2-1/iα3-11,15 leu2-3,112 trp1-1 ural3-1        | (61)                |
| HTY210     | MATα lpt1-kanMX                                        | This study          |
| HTY228     | MATα slc1-kanMX                                        | This study          |
| HTY229b    | MATα lpt1-kanMX/LPT1 slc1-kanMX/SLC1                   | This study          |
| HTY231b    | MATα taz1-kanMX                                        | This study          |
| HTY232b    | MATα lpt1-kanMX/LPT1 taz1-kanMX/TAZ1                   | This study          |
| HTY233     | MATα lpt1-kanMX taz1-kanMX                             | This study          |
| MLY40      | MATα ura3-52                                          | This study          |
| HTY212     | MATα lpt1-kanMX                                        | This study          |

* Strain is congenic with S288C.
* Strain is congenic with W303-1A.
* Strain is congenic with 1278b.
CACACGCGATCTGCGTTTTTGCCGAGCATAGGGCCACTAGTGGATCTG-3′.

The deletion of the SLC1 allele in strain W303-1A was made by transforming the cells with the loxP-kanMX-loxP cassette derived by PCR with the following primers: 5′-TTCAATAGA- GAAGTTTATGTTTCTCCCGATGAACTCGAGCAGGCTGAGCTGTGCCAC-3′ and 5′-CAGTTTGTGGG-TCTATATAACTACTCTAAAAATGTGGTGGCC- ATAGGCACACTGGATCTG-3′. Haploid strains with single gene mutations were crossed, sporulated, and dissected to try to obtain double mutant strains.

**Enzyme Preparation for Screening**—Each strain of the complete set of Yeast Deletion Clones were grown in 5 ml of YPD medium containing 200 μg/ml G418 at 30 °C overnight. Cells were collected by centrifugation and suspended in 2 volumes of 50 mM Tris-HCl buffer (pH 7.5) containing 5 mM 2-mercaptoethanol and 2 mM phenylmethylsulfonyl fluoride. The cells were disrupted with glass beads by vortexing at 4 °C, and then subjected to an enzyme assay as described below, except that the reaction was performed at 30 °C.

**Preparation of Microsomal Fractions**—Yeast cells were suspended in 2 volumes of 50 mM Tris-HCl buffer (pH 7.5) containing 5 mM 2-mercaptoethanol and 2 mM phenylmethylsulfonyl fluoride, and disrupted with glass beads by vortexing at 4 °C. The cell debris was removed by centrifugation at 18,800 × g for 15 min at 4 °C to obtain the crude enzyme fraction. The microsomal fraction was pelleted by centrifugation of the crude enzyme fraction at 100,000 × g for 60 min at 4 °C, and the pellet was resuspended in a small amount of the same buffer. Alternatively, cells were suspended in 5 volumes of spheroplasting buffer (20 mM Tris-HCl (pH 7.5), 1.2 mM sorbitol, 50 mM potassium acetate, and 1 mM 2-mercaptoethanol); Zymolyase 100T (Seikagaku, Japan) was added to a concentration of 1 mg/ml, and the cells were incubated at 30 °C for 10–30 min. The spheroplasts were washed with spheroplasting buffer and lysed in ice-cold lysis buffer (20 mM Tris-HCl (pH 7.5), 100 mM sorbitol, 50 mM potassium acetate, 1 mM 2-mercaptoethanol, and 2 mM phenylmethylsulfonyl fluoride) using a Teflon homogenizer. The cell lysate was further subjected to centrifugation and ultracentrifugation to obtain the microsome fraction as described above.

We performed centrifugation at 18,800 × g before ultracentrifugation to avoid the degradation of Lpt1-HA. When we examined the enzyme properties of samples obtained at lower centrifugation speeds before ultracentrifugation, the reaction was not linear, and quick enzyme degradation was observed during the assay even in the presence of protease inhibitors. We found that the microsome fraction prepared after performing centrifugation at 18,800 × g was more stable and performed better in the enzyme assay even if the procedure resulted in the removal of a large amount of nuclei and nucleus-attached ER.

**Enzyme and Protein Assay**—LysOPAF AT activity was measured by a modification of the procedure of Gomez-Cambronero et al. (27). The reaction mixture (0.4 ml) contained 50 mM Tris-HCl buffer (pH 7.5), 50 μM lysophosphatidylcholine (C16:0, Cayman Chemical Co.), 3 μM of [14C]acetyl-CoA (185 GBq/mmol, Moravek Biochemicals, Inc.), 60 μM unlabeled acetyl-CoA, 2 mM phenylmethylsulfonyl fluoride, 5 mM 2-mercaptoethanol, and the enzyme solution. The reaction was performed at 0–15 °C for 20 min, after which total lipid was extracted by the method of Bligh and Dyer (28) and subjected to TLC. Samples were applied to DC-Alufolien Kieselgel 60 TLC plates (Merck) and developed with chloroform/methanol/water (65:25:4, v/v). The TLC plate was exposed to an imaging plate, and synthesized [14C]PAF was visualized and quantified with a BAS-2500 Bio-imaging analyzer (Fujifilm). The authentic [3H]PAF (6.0 Ci/mmol, PerkinElmer Life Sciences) was detected by fluorography using EN3HANCE Spray (PerkinElmer Life Sciences).

To investigate the substrate specificities of this enzyme, various lysophosphatidylcholines and acyl-CoAs (Sigma or Avanti Polar Lipids, Alabaster AL) were used in place of either lysoPAF or acetyl-CoA. The protein concentration was determined by the method of Bradford (29).

**Western Blot Analysis**—Microsome fraction (2 μg of total protein) was electrophoresed on a 10% SDS-PAGE and then electroblotted onto polyvinylidene difluoride membranes. The polyvinylidene difluoride membranes were blocked with TBS-T (Tris-buffered saline, pH 7.6, 0.1% Tween 20) containing 5% skim milk for 1 h at room temperature and then incubated with 0.1 μg/ml of 12CA5 monoclonal antibody or GFP antibody. The protein concentration was determined by the Bradford method (29).

**Platelet Aggregation Assay**—Total lipids were extracted by the method of Bligh and Dyer and applied to an alumina column. The column was washed with chloroform, and the PAF fraction was eluted with chloroform/methanol (1:1). The eluted fraction was further loaded onto a paper fiber-made TLC sheet (Chromato sheet, Wako) and developed with chloroform/methanol/water (65:25:4). The area containing PAF was cut off, and the PAF fraction was eluted with chloroform/methanol (1:1), dried under N2 gas, and dissolved in 100 μl of bovine serum albumin/saline. Washed rabbit platelets were prepared by the method of Pincard et al. (30). Tyrode’s solution (160 μl, pH 7.2, containing 1.3 mM Ca2+) was mixed with 40 μl of washed rabbit platelets and preincubated at 37 °C for 1 min. Then 10 μl of sample lipid was added. Aggregation was assayed by monitoring the change in light transmittance with a Hematrac PAT-4A (Nikko, Japan).

**Confocal Microscopy**—The ΔLpt1 mutant strain transformed with LPT1-GFP/YEp(rop) was grown in SC(Trp-) liquid medium to early log phase, and the cells were analyzed using a confocal microscope (FV1000, Olympus) equipped with a 100 × oil-immersion objective.

**Liquid Chromatography/Mass Spectrometric Analysis**—LC/MS analysis was performed in the positive mode on an LCMS-2010 mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ion source. High performance liquid chromatography separation was carried out on a normal-phase column (Develosil 60, 2.0 × 150 mm, Nomura Chemicals, Seto, Japan). The column was eluted with solvent A (ace-
tonitrile-methanol (2:1) containing 0.1% ammonium formate (pH 6.4)) and solvent B (methanol-H2O (2:1) containing 0.1% ammonium formate (pH 6.4)) at a flow rate of 0.2 ml/min. The gradient separation was carried out using the following conditions: isocratic elution with 100% solvent A for 5 min followed by a linear gradient of 100% to 70% solvent A over 40 min. Spectra were obtained over a mass range from m/z 400–1200 with a scan time of 1 s.

RESULTS

Identification of lysoPAF AT in the Yeast S. cerevisiae—We previously reported that Saccharomyces cerevisiae produce platelet-activating factor (PAF) (31). To identify the gene for lysoPAF AT, we screened a complete set of homozygous diploid yeast deletion clones as described under “Experimental Procedures.” Using [14C]acetyl-CoA and lysoPAF, we detected lysoPAF AT activity in both crude cell-free extracts and microsome fractions (100,000 g pellets) of yeast. In the 4741 strains screened, we found one clone that did not produce [14C]PAF. This clone lacked ORF YOR175c, an as-yet-unidentified gene encoding a putative membrane-bound O-acyltransferase. We designated this gene LPT1. We deleted the corresponding gene in several yeast strains with different genetic backgrounds. All strains tested possessed lysoPAF AT activity, and when the LPT1 gene was deleted, the activity completely disappeared (Fig. 1A). In contrast, the enzyme activity increased when the LPT1 gene was introduced with a multicopy vector (Fig. 1A). We also prepared microsome fractions from the Δlpt1 strain transformed with the LPT1 overexpression vector or a control vector and used these fractions for PAF synthesis with lysoPAF and acetyl-CoA as the substrates. The lipids were extracted from the enzyme reaction products and analyzed by LC/MS. Synthesized PAF was identified by comparing their retention time in the column and m/z 524.4 with those of standard C16:PAF. The reaction with microsomes obtained from the Δlpt1 strain transformed with the LPT1 overexpression vector produced a time-dependent increase of C16:PAF production; this increase was not observed in the Δlpt1 strain harboring the control vector (data not shown), suggesting that LPT1 is required for PAF synthesis. The lipid fractions were further purified, and the PAF fractions were subjected to a platelet aggregation assay. The PAF fraction from the LPT1-overexpressing strain showed strong platelet aggregation activity, which was hardly observed when the PAF fraction from the Δlpt1 strain with the control vector was used (Fig. 1B) or when lysoPAF was omitted from the reaction mixture. The platelet aggregation activity was also strongly inhibited in the presence of 10−6 M WEB2086, a PAF antagonist (Fig. 1B). From these results we identified LPT1 as a lysoPAF AT gene in the yeast S. cerevisiae.

Lpt1 Is a Member of the MBOAT Superfamily—YOR175c (LPT1) has been reported to be a member of the membrane bound O-acyltransferase (MBOAT) superfamily (32). The MBOAT superfamily proteins harbor several membrane-spanning regions and share sequence similarity regions. Four other MBOAT genes are present in S. cerevisiae. ARE1 and ARE2 were identified as genes encoding ergosterol acyltransferases (33), and the Gup1 and Gup2 proteins were initially reported to be involved in glycerol uptake (34). Gup1 was recently proposed to be an acyltransferase involved in the remodeling of glycosylphosphatidylinositol anchors (35); however, Lpt1 showed low similarity to Gup1 (15%/260 aa), Gup2 (14%/93 aa), Are1 (18.5%/108 aa), and Are2 (16.7%/234 aa).

A search of databases for homologues of yeast Lpt1 identified several MBOATs, including SPBC16A3.10 in the fission yeast Schizosaccharomyces pombe, OACT1 and -2 in both human and mouse (Fig. 2), and C54G7.2 and C08F8.4 in Caenorhabditis elegans. The predicted amino acid sequence of LPT1 shared 27.5% identity with OACT1 (per 458 aa) and 28.8% identity with OACT2 (per 458 aa). It was predicted that a conserved histidine residue within a long hydrophobic region might be a candidate for the active-site residue (32). The corresponding histidine residue was found at position of 382 in Lpt1 (Fig. 2). To test whether His-382 is involved in the catalytic center, we constructed an HA-tagged mutant Lpt1 expression vector in which His-382 was replaced by asparagine. Although we confirmed the expression of both native and mutant Lpt1-ΔHA in the microsomal fraction of the Δlpt1 mutant strain by Western blot analysis, we did not detect lysoPAF AT activity in the mutant enzyme fraction (see Fig. 6, B and C). From this result, we concluded that His-382 is critical for the catalytic reaction and is most likely an active-site residue.
FIGURE 2. Amino acid sequence alignment of Lpt1 homologues. Lpt1 homologues found in fission yeast (SPBC16A3.10), human (OACT2p), and mouse (mOact1p and mOact2p) are aligned with Lpt1. The asterisks indicate identical amino acids, the dots indicate conserved amino acids, and the dashes indicate gaps in the sequence.

**Substrate Selectivity of Lpt1**—We tried to elucidate the mechanism by which Lpt1 acts on lysophospholipids in fission yeast. Lpt1 prefers lysophospholipids as acceptors over soluble surfactants, including CHAPS and octylglucoside, but the enzyme activity was considerably decreased by surfactants (data not shown). We therefore used the microsomal fraction from the Lpt1-overproducing strain to characterize the enzyme reaction. Although the enzyme reaction was performed at 30 °C during the incubation, we noticed that the enzyme activity decreased rapidly at this temperature because the Lpt1 protein degraded even in the presence of various protease inhibitors (data not shown). For these reasons, the reaction was performed at 0–15 °C in buffer A as described under “Experimental Procedures.” In this condition, the reaction was stable for the first 15 min (data not shown).

To examine whether Lpt1 uses other acyl-CoAs as substrates, lypoPAF AT activity was measured in the presence of various acyl-CoAs. lypoPAF AT activity was dramatically decreased in the presence of propionyl-CoA (C3:0) and butyryl-CoA (C4:0) and was not detected in the presence of palmitoyl-CoA (C16:0) or arachidonoyl-CoA (C20:4) (Fig. 1C). These results suggest that Lpt1 also catalyzes acyltransferase activity. So we first examined the substrate specificity of Lpt1 using [1-14C]linoleoyl-CoA (C18:2) as an acyl donor with a variety of lysophospholipid acceptors (Fig. 3). The microsomal fraction from the Δlpt1 mutant strain transformed with the LPT1 gene overexpression vector showed acyltransferase activity with various lysophospholipid acceptors, including LPC, LPA, LPA, LPE, LPI, and LPS (Fig. 3). Lpt1 preferred an acyl residue to an alkyl residue at the sn-1 position of lysophospholipids. Because the reaction products were hardly detectable when microsomes from the Δlpt1 mutant strain with control vector were used, LPT1 would appear to be mainly responsible for both acyl-CoA- and acyltransferase activity in the microsomal fraction in the yeast S. cerevisiae.
Next, we examined the preference of Lpt1 for various acceptor acyl groups at the sn-1 position of LPC, using [1-14C]linoleoyl-CoA as an acyl donor. Lpt1 recognized most LPCs as substrates, except 1-hexanoyl-LPC (C6:0), and preferred medium chain 1-acyl-LPCs (C16:0, C18:0, and C18:1) to 1-O-alkyl-LPCs (C1-O-16:0 and C1-O-18:0) (Fig. 4A).

We also examined the acyl-CoA selectivity of Lpt1 using [1-14C]palmitoyl-LPC as an acceptor (Fig. 4B). Lpt1 showed a preference for medium-chain fatty acyl-CoAs (C10 ~ 20), and especially for unsaturated acyl-CoAs (C16:1, C18:1 ~ C18:3, and C20:4). In contrast, it showed poor activity when short-chain (C2 to C8) saturated fatty acyl-CoAs or either saturated (C20:0) or unsaturated (C22:6) long-chain fatty acyl-CoAs were used. We could not detect 14C-labeled reaction products in the absence of acyl-CoAs, suggesting that Lpt1 catalyzes acyl-CoA-dependent acyltransferase activity.

Kinetics of Lpt1 — We examined the kinetic properties of Lpt1 by measuring acetyl- or acyltransferase activity in the microsomal fraction derived from Δlpt1 strains transformed by the LPT1 overexpression vector or control vector. We used increasing concentrations of acetyl-CoA (0–200 μM; Fig. 5A) or linoleoyl-CoA (0–5 μM; Fig. 5B) in the presence of 1 μM [1-14C]palmitoyl-LPC, or increasing concentrations (0–10 μM) of 1-palmitoyl-LPC (Fig. 5C) in the presence of 1 μM [1-14C]li-
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Cellular Localization of Lpt1-GFP—To clarify the cellular localization of Lpt1, we constructed an Lpt1-GFP fusion protein expression vector and expressed it in the ∆lpt1 strain under the control of the LPT1 promoter. Transformed cells were grown in SC(Trp-) medium, and the enzyme distribution at the exponential growth phase was examined by using confocal microscopy. Fluorescence from Lpt1-GFP was observed around the nucleus, most likely in the ER, and on the cell surface (Fig. 6A). Lpt1-GFP was functional and no degradation was observed (Fig. 6, B and C).

Lpt1 Plays a Critical Role in PA Synthesis in Concert with Slc1—Slc1 is an LPA acyltransferase present in lipid particles of S. cerevisiae (15). Because the majority of Lpt1 LPA acyltransferase activity was in the ER, we thought there might be a functional relationship between the LPT1 and SLC1 genes. We further explored the potential relationship between these genes by investigating whether null alleles of LPT1 and SLC1 genes interact genetically. To determine the phenotype of ∆lpt1 ∆slc1 double mutants, a diploid strain heterozygous for LPT1 and SLC1 deletion alleles was sporulated, and tetrads were dissected. Although strains containing single ∆lpt1 or ∆slc1 mutations grew normally, strains containing both ∆lpt1 and ∆slc1 mutations did not grow (Fig. 7, top). The synthetic lethal phenotype found in the ∆lpt1 ∆slc1 double mutant was complemented by the LPT1 expression vector (Fig. 7, bottom). These results indicate that the two different enzymes, Lpt1 and Slc1, are functionally related in PA synthesis.

A Growth Defect Caused by the Overexpression of PLB1 Is Partially Suppressed by Coexpression of LPT1—Our enzymatic characterization of Lpt1 showed that the enzyme has acyltransferase activity with a wide variety of substrate lysophospholipids. As phospholipases catalyze the reverse reaction of lysophospholipid acyltransferase, we examined the relationship between Lpt1 and phospholipases. We found that overexpression of PLB1, a gene encoding the major phospholipase in yeast (36), caused similar growth delays in both wild-type and ∆lpt1 mutant strains. However, when LPT1 was coexpressed with PLB1 in the ∆lpt1 mutant strain, the growth-delay phenotype was partially suppressed (Fig. 8). The suppression was probably only partial, because PLB1 releases fatty acids from both the sn-1 and sn-2 positions of glycerophospholipids, whereas Lpt1 catalyzes acetyl and acyl transfer reactions only at the sn-2 position of lysophospholipids. We also examined the effect of another phospholipase that localizes to the ER, NTE1 (37), on cell growth. However, we did not detect a growth-delay following NTE1 overexpression (data not shown).

The Phospholipid Contents Varied in the ∆lpt1 Mutant Strain—To test whether a lack of Lpt1 affects the phospholipid contents, we extracted total lipids from the wild-type and ∆lpt1 mutant strain and subjected them to an LC/MS analysis as described under “Experimental Procedure.” Phospholipids were identified by their retention time in the column and m/z as described previously (38). Using this method, we detected several kinds of glycerophospholipids, including PC, LPC, PE, and LPE. In the ∆lpt1 strain, although the level of PC and PE (for example, m/z 758.5 and m/z 744.5, respectively) were not affected, LPC and LPE (for example, m/z 496.4, m/z 524.4, and m/z 452.3, respectively) accumulated compared with the wild.

noleoyl-CoA. The apparent $K_m$ values of Lpt1 for acetyl-CoA, linoleoyl-CoA, and 1-palmitoyl-LPC were 23.8, 8.7, and 0.467 $\mu$M, respectively. Problems may arise when membrane fractions are used as an enzyme source, because they potentially contain other substrates for Lpt1 and/or enzymes having similar activity. Consequently, microsome from ∆lpt1 mutant harboring control vector was always used as a negative control when measuring Lpt1 enzyme activity.
type (Fig. 9 and supplemental Fig. S1), thus further supporting that Lpt1 has acyltransferase activity with a wide variety of lysophospholipids.

Although we clearly showed that Lpt1 has PAF producing ability (Fig. 1), PAF could not be detected in the lipid fraction of LPT1-overexpressing cells by LC/MS analysis. These results indicate that the amount of PAF might be too small to be detected in the yeast cell, although we cannot exclude the possibility of PAF production in yeast.

**DISCUSSION**

PAF is a phospholipid mediator that activates a G protein-coupled receptor. PAF has potent biological activities, includ-
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![Graph and Figure 10](image)

**FIGURE 9.** Phospholipid analysis of wild-type and Δlpt1 mutant strains by LC/MS. Total lipids were extracted from wild-type and Δlpt1 mutant strains and subjected to LC/MS analysis as described under “Experimental Procedures.” The intensity of the extracted ion with m/z 744.5 (PE(36:2); purple), m/z 452.3 (LPE(16:1); green), m/z 758.5 (PC(34:2); brown), m/z 524.4 (LPC(18:0); blue), and m/z 496.4 (LPC(18:0); red) are expanded 5-fold and displayed. The chromatogram for total ion is shown in black. PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine.

**FIGURE 10.** Lpt1 is involved in the phospholipid biosynthetic pathway. The phospholipid biosynthetic pathway in which Lpt1 is involved is shown. The reactions catalyzed by Lpt1 are indicated in red. The cytidine-diphosphoacylglycerol pathway is indicated in blue, and the Kennedy pathway is in green. PA is indicated with the yellow box. G 3-P, glycerol 3-phosphate; DHAP, dihydroxyacetone phosphate; LPA, lysophosphatidic acid; PA, phosphatic acid; CDP, cytidine 5′-diphosphate; DG, diacetylglycerol; TG, triacylglycerol; Etn, ethanolamine; Cho, choline; PS, phosphatidylserine; LP, lysophosphatidylserine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; LPS, phosphatidylglycerol; LPG, lysophosphatidylglycerol; CL, cardiolipin; PAF, platelet-activating factor.

Lpt1, a Novel Lysophospholipid Acyltransferase in Yeast

Lpt1, a gene encoding a novel lysophospholipid acyltransferase, was first identified in *S. cerevisiae* (53) and *S. pombe* (54). Lpt1, Taz1, and other homologs of [*S. pombe*](https://doi.org/10.1074/jbc.M410522200) were cloned. Lpt1 was shown to catalyze the acylation of G-3-P (the first step of PA biosynthesis) and the second G-3-P acyltransferase *GAT2* (54), which was initially identified as a choline transporter suppressor gene (SCT1), was also identified (55).

In this study, we identified a gene (*LPT1*) encoding an enzyme that has lysoPAF AT activity as well as lysophospholipid acyltransferase activity. To our surprise, Lpt1 did not show any homology with LPCAT2, a recently identified orthologue of LPC acyltransferase that has both lysoPAF AT and lysoPC acyltransferase activity (22), suggesting that Lpt1 is a novel lysoPAF AT.

*LPT1* encodes a protein that is classified as an MBOAT. The MBOAT superfamily, which is conserved from yeast to human (32), consists of a large and diverse group of membrane associated acyltransferases. In yeast, 5 MBOATs have been found, *ARE1, ARE2, GUP1, GUP2*, and *YOR175C (LPT1)* (32, 35). In this study, we found that Lpt1, the fifth MBOAT in yeast *S. cerevisiae*, catalyzes the transfer of acyl residues to a wide variety of substrate acceptors, including LPA, LPC, LPI, LPS, LPC, and LPE (Fig. 10).

Several lysophospholipid acyltransferase genes have been cloned (3–14, 43–52). Lysophosphatidic acid acyltransferase (LPAAT) catalyzes the acylation of LPA to produce PA. LPAAT was first identified in *Escherichia coli* by isolating a mutant lacking LPAAT activity, and the corresponding gene (*pIsC*) (53) and homologs of *pIsC* were cloned. *SLC1*, originally obtained as a suppressor of a genetic defect in sphingolipid biosynthesis, was identified as the LPAAT gene in *S. cerevisiae* (15). Slc1 catalyzes the acylation of LPA (the second step of PA biosynthesis) in the lipid particle. Gat1 was shown to catalyze the acylation of G-3-P (the first step of PA synthesis), and the second G-3-P acyltransferase *GAT2* (54), which was initially identified as a choline transporter suppressor gene (SCT1), was also identified (55).

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Inactivation of both GAT1 and GAT2/SCT1 resulted in a synthetic lethal phenotype, suggesting that these two genes cooperatively play a central role in the first step of the PA synthesis pathway. However, despite the importance of PA, disruption of SLC1 does not cause any defect in cell growth, indicating that PA biosynthesis may proceed through an alternative pathway (15). Indeed, the Δlpt1 Δslcl double mutant showed a synthetic lethal phenotype like the Δgat1 Δgat2/Δsct1 double mutant. Thus, we conclude that the concerted action of Lpt1 and Slcl is indispensable for PA synthesis in vivo and for cell growth (Fig. 10).

We could hardly detect acyltransferase activity with various lysophospholipid acceptors using [1-14C]linoleoyl-CoA in the Δlpt1 mutant (Fig. 3), however, this does not exclude the possibility that different acyltransferases with overlapping functions could be found in the different assay conditions or different growth conditions. Indeed, 1-acyl-LPA acyltransferase activity was detected in the Δlpt1 mutant when [1-14C]arachidonoyl-CoA was used (supplemental Fig. S2). The activity found in the Δlpt1 mutant might be catalyzed by Sct1, another LPA acyltransferase. We also examined the effect of the SLC1 deletion on lysophospholipid acyltransferase activity. The Δslcl mutant showed slightly reduced acyltransferase activity compared with the wild-type strain and dramatically reduced 1-acyl LPA acyltransferase activity (supplemental Fig. S2). This result indicates that Slc1 mainly catalyzes LPA acyltransferase activity and that it may also be involved in various lysophospholipid acyltransferase reactions.

Tafazzin is a protein with some similarities to LPAAT. Mutation of the tafazzin gene is associated with diseases, including Barth syndrome (43). In yeast, an orthologue of tafazzin gene has been identified as TAZ1, whereas the role of Taz1 in yeast is controversial. It has been reported that Taz1 has LPC acyltransferase activity in the mitochondrial fraction (44), and others have hypothesized that Taz1 acts as a monolysyl-CL acyltransferase (56). Although we examined lysophospholipid acyltransferase activity using the microsome fraction from the Δtaz1 mutant, we could not detect any difference in enzyme activity between the wild-type and the Δtaz1 mutant (supplemental Fig. S2).

Glycerophospholipid structure exhibits a high degree of heterogeneity. Saturated fatty acyl groups are predominated in the sn-1 position, whereas unsaturated fatty acids are commonly found at the sn-2 position. It is thought that unsaturated fatty acid incorporation at the sn-2 position does not occur through a novo synthetic pathway but through fatty acid remodeling (57). Our finding that Lpt1 catalyzes lysophospholipid acyltransferase activity with a strong preference for unsaturated medium-chain acyl-CoAs as acyl donors (Fig. 4B) may indicate the possible involvement of Lpt1 in glycerophospholipid heterogeneity in yeast.

Addendum—After submission of our manuscript, three independent reports identifying LPT1 (YOR175c) appeared. Dr. Voelker’s group identified YOR175c as the major lysophosphatidylethanolamine acyltransferase-encoding gene and named it ALE1 (58). Dr. Conzelmann’s group identified the same gene as a second 1-acyl-sn-glycerol-3-phosphate acyltransferase and named it SLC4, which has partially redundant function with SLC1, a previously identified LPA acyltransferase (59). Dr. Oelkers’s group also identified the same gene as the lysophospholipid acyltransferase-encoding gene and named it LPT1 (60).

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