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IDENTIFICATION OF A NOVEL LYSOPHOSPHOLIPID ACYLTRANSFERASE IN SACCHAROMYCES CEREVISIAE

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Running title: Identification of LPLAT in yeast

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The incorporation of unsaturated acyl chains into phospholipids during *de novo* synthesis is primarily mediated by the 1-acyl-sn-glycerol-3-phosphate acyltransferase reaction. In *S. cerevisiae*, Slc1 has been shown to mediate this reaction but distinct activity remains after its removal from the genome. To identify the enzyme that mediates the remaining activity, we performed synthetic genetic array analysis using a *slc1Δ* strain. One of the genes identified by the screen, *LPT1*, was found to encode for an acyltransferase that uses a variety of lysophospholipid species, including 1-acyl-sn-glycerol-3-phosphate. Deletion of *LPT1* had a minimal effect on 1-acyl-sn-glycerol-3-phosphate acyltransferase activity but over-expression increased activity 7-fold. Deletion of *LPT1* abrogated the esterification of other lysophospholipids and over-expression increased lysophosphatidylcholine acyltransferase activity 7-fold. The majority of this activity co-purified with microsomes. To test the putative role for this enzyme in selectively incorporating unsaturated acyl chains into phospholipids, *in vitro*, substrate concentration series experiments were performed with the four acyl-CoA species commonly found in yeast. While the saturated palmitoyl-CoA and stearoyl-CoA showed a lower apparent K_m, the monounsaturated palmitoleoyl-CoA and oleoyl-CoA showed a higher apparent V_{max}. Arachidonyl-CoA, although not abundant in yeast, also had a high apparent V_{max}. Pulse-labeling of *lpt1Δ* strains showed a 30% reduction in [^{3}H]oleate incorporation into phosphatidylcholine only. Therefore, Lpt1p, a member of the membrane bound o-acyltransferase gene family, seems to work in conjunction with Slc1 to mediate the incorporation of unsaturated acyl-chains into the sn-2 position of phospholipids.

It has long been known that phospholipids commonly contain an asymmetrical distribution of acyl chains (1,2). Saturated acyl chains are usually found at the sn-1 position and unsaturated acyl chains at sn-2. The reason for this asymmetry may be that the liquid crystal transition temperature (Tc) for phospholipids with two saturated acyl chains, such as distearoyl phosphatidylcholine (58° C), is much higher than that for 1-stearoyl-2-oleoyl phosphatidylcholine (3° C) (3). The incorporation of unsaturated acyl chains prevents membranes from assuming the gel phase at common physiological temperatures.

During the *de novo* synthesis of phospholipids (Fig. 1a), acyl chains are incorporated into the sn-2 position by 1-acyl-sn-glycerol-3-phosphate acyltransferase (AGPAT)[4], also known as lysophosphatidic acid acyltransferase (LPAAT). The acyl-CoA substrate specificity for the reaction in rat liver microsomes (4) and by over-expressed human AGPAT1 (5,6) and AGPAT2 (6,7) show only a mild, if any, preference for unsaturated species. Thus, while the reaction produces a necessary intermediate, phosphatidic acid, in the synthesis of phospholipids and triglycerides, the substrate specificity does not match the acyl chain composition of most phospholipids. However, the reaction clearly has physiological importance since mutations in AGPAT2 result in the dramatic lack of many, but not all, adipose depots in congenital general lipodystrophy (8). Given this necessity of AGPAT2 function, it is surprising that there are nine AGPAT paralogs in humans. AGPAT6 has been implicated in triglyceride synthesis in mammary glands (9). AGPAT8 encodes for an endoplasmic reticulum glycerol 3-phosphate acyltransferase (10) and AGPAT9 is

highly homologous to lysophosphatidylcholine acyltransferase 1 (LPCAT1) (11). Whether the members of this gene family are responsible for all AGPAT activity in mammals cannot be practically determined. The only AGPAT gene family member in the *S. cerevisiae* is *SLC1*. *SLC1* deletion mutants contain no AGPAT activity in lipid particles and about 50% activity in microsomes (12). Clearly, a unique AGPAT exists in the yeast genome.

AGPAT's product, phosphatidic acid, can be used to synthesize phospholipids by two pathways (Fig. 1a) (reviewed in (13)). Additionally, after *de novo* synthesis, phospholipids can undergo remodeling (Fig. 1b). This involves deacylation at the sn-2 position by phospholipase A_2 and reacylation by acyl-CoA-dependent lysophospholipid acyltransferase (Lands' Cycle) (14). There are many possible physiological functions of this remodeling which may be organism and cell type specific. In addition to incorporating unsaturated acyl chains for membrane fluidity, specific incorporation of the polyunsaturated arachidonyl into the sn-2 position may prime phospholipase A_2 inducible signal cascades that involve eicosanoid synthesis (15). Alternatively, preferential incorporation of saturated acyl chains occurs during surfactant production in lungs (16). The remodeling enzymes may also re-esterify exogenous lysophospholipids generated by extracellular lipases. For instance, yeast can absorb and esterify lysophosphatidylethanolamine (lysoPE) (17). In mammals, this process may prevent the inflammation that lysophospholipids can induce (18). To date, the only cloned lysophospholipid acyltransferases are the mouse LPCAT1 and lyso-platelet activating factor acyltransferase (lysoPAFAT) / LPCAT2 (19-21). These are primarily expressed in lungs, have a mild preference for saturated acyl-CoA, are specific for lysophosphatidylcholine (lysoPC) and have conserved motifs in common with the AGPAT gene family. In yeast, acyl-CoA-independent LPCAT (i.e. transacylase) activity is mediated by Taffazin (22) and acyl-CoA dependent LPCAT activity has been reported (2,23) but the enzyme had not been identified.

In this study, the application of synthetic genetic array (SGA) analysis lead to the identification of the enzyme, Lpt1p. SGA is an automated approach to create double gene deletion mutants by crossing a gene deletion strain of interest and the ~5,000 viable yeast gene deletion strains. Inviability of the double deletion mutants (i.e. synthetic lethality) defines a relationship whereby mutation in two different genes, neither by itself lethal, causes cell death when combined in a haploid genome (24). Such genetic interactions are of particular interest because they can identify genes whose products perform complementary biological functions.

EXPERIMENTAL PROCEDURES

Materials - Synthetic complete media and yeast nitrogen base were obtained from Q-biogene (MP Biomedicals). Myristoyl lysophosphatidylethanolamine and oleoyl lysophosphatidylserine were from Avanti Polar Lipids (Alabaster, AL). [1-^{14}C] palmitoyl – lysophosphatidylcholine (lysoPC) (55mCi/mmol) was from Perkin-Elmer Life Sciences. Nourseothricin was obtained from Werner BioAgents. All other chemicals were obtained from either Sigma or Fisher.

Yeast strains - Molecular biology and yeast genetic procedures were performed according to conventional protocols (25). Deletion mutant strains for *SLC1* and *LPT1* were generated by homologous recombination in a W303-1B haploid (*MATa ade2–1, can1–1, trp1–1, ura3–1, his3–11, 15, leu2–3, 112*) (26) by transformation with PCR derived products containing 50 bp of gene-specific sequence flanking the *Kluyveromyces lactis URA3* sequence (27,28). For *LPT1*: LPT1F, 5' ATGTACAATCCTGTGGACGCTGTTTTAACA AAGATAATTACCAACTATGGACATGGCAA TTCCCGGGGATCGL and LPT1R, 5' CTACTCTTCCTTTTTTGAAATAGGCTTTGGT GAGTAACCACTAAAACTCATCAACATGGT GGTCAGCTGGAATT. For *SLC1*: SLC1F, 5' ATGAGTGTGATAGGTAGGTTCTTGTATTAC TTGAGGTCCGTGTTGGTCGTACATGGCAAT TCCCGGGGATCG and SLC1R, 5' TTAATGCATCTTTTTTACAGATGAACCTTC GTTATGGGTATTGACATCGTTCAACATGGT GGTCAGCTGGAATT. Successful deletion was confirmed by PCR using gene specific primers. Yeast were grown in YP media (1% yeast extract, 2% peptone) with 2% glucose (YPD) or in synthetic complete (SC) media (29).

Synthetic lethality screen - SGA was performed as described previously (24). A *MAT*α

slc1Δ::NatMX4 can1Δ::STE2pr-Sp_his5 lyp1Δ strain was crossed to an array containing the set of ~5, 000 viable deletion mutant strains.

Yeast Expression Plasmid Construction - A PCR product including the *LPT1* open reading frame, 90 bp of 5' flanking sequence and 46 bp of 3' flanking sequence was generated using the primers: 175-1, CAAAATACAGGCACAGGTCAAGC; 175-2, GACAACAAGACTGTGACTTCCAC, and W303-1B genomic DNA. The 2.0-kb PCR product was cloned into pCR2.1 TOPO (Invitrogen) and subsequently subcloned (NotI / SacI) into pRS423GP to create pRS423GP-*LPT1*.

Pulse-labeling - Pulse labeling of yeast was performed for 30 minutes with log phase cultures cultured in YPD with [^{3}H]oleate as described previously (30). Phospholipids were resolved by thin layer chromatography in chloroform: methanol: acetic acid: water (50:25:8:4) and neutral lipids were resolved as previously (30). Each lane was cut according to lipid standards and counted by liquid scintillation. Assays were performed on a minimum of two independent strains of each genotype on two different days. Statistical analysis was performed using *t* tests.

Preparation of cell lysates, mitochondria and microsomes - Yeast cells were grown in YP containing either 2% glucose or 3% glycerol at 30° C into early stationary phase ($O.D._{660} = 1.0$). SC-His media containing 2% galactose was used for yeast harboring a plasmid. The cells were harvested, washed and cell lysates prepared as described previously (30). Mitochondria were prepared using a previously published protocol (31). Briefly, cells were grown in YP media containing 3% glycerol, harvested, lysed and crude mitochondria were precipitated by centrifugation at 12,000 x g. Resuspension of the pellet, followed by sucrose step gradients allowed purified mitochondria to be collected. Microsomes were purified from the 12,000 x g supernatant by precipitation by centrifugation at 100,000 x g for 1 hour (30). Lowry assays were used to measure protein concentration.

AGPAT and LPLAT assays, spectrophotometric method - 1-acyl-sn-glycerol-3-phosphate acyltransferase (AGPAT) activity was measured by the reaction of thiol groups of the released coenzyme A (CoA) with DTNB resulting in absorbance at 412nm (32). The reaction mixture contained 100 mM Tris-HCl, pH 7.4, 1.5 mM DTNB, 50 μM lysophosphatidic acid (lysoPA), 50 μM acyl CoA, cell lysate (210 - 840 μg) in a total volume of 1 ml. The reaction was monitored in real time for 3 minutes in a Spectronic Genesys 2 spectrophotometer at room temperature. A molar absorbance of 13,600 M^{-1} cm^{-1} was used to calculate relative specific activity. Lysophospholipid acyltransferase (LPLAT) activity was measured by replacing lysoPA with lysoPC, lysoPE, lysoPI, or lysoPS

LPCAT assay, radioactive substrate - Lysophosphatidylcholine acyltransferase (LPCAT) activity was measured by the incorporation of [1-^{14}C] palmitoyl lysoPC into PC. The reaction contained 100 mM Tris-HCl, pH 7.4, 50 μM [1-^{14}C] palmitoyl lysoPC (50,000 dpm / nmol), 1 - 110 μM of the respective acyl-CoA, and 3μg of cell lysate protein in a final volume of 100 μl. Fixed time assays were performed for 5 minutes at 28 °C. The reactions were stopped by adding chloroform: methanol (2:1) and lipids were extracted and resolved as described elsewhere (20). EZ-Fit software was used for non-linear regression, curve fit analysis to calculate K_m and V_{max}. To calculate V_{max} / K_m, V_{max} was first changed to nM / min / mg to remove volume from the units of the ratio.

RESULTS

SGA screen with slc1Δ identified LPT1 - In order to identify the second 1-acyl-sn-glycerol-3-phosphate acyltransferase (AGPAT) in *S. cerevisiae*, a synthetic genetic array (SGA) screen (24) was carried out using an *slc1Δ* query strain. The primary screen identified 52 non-essential genes that resulted in a synthetic lethal/sick phenotype when disrupted in combination with deletion of *SLC1* (data not shown). While the primary screen often contains a number of false positive interactions, some functionally relevant genes were identified including the fatty acid elongase gene, *ELO2*. The screen also identified two genes, *PMT5* and *YOR175C*, which were previously shown to exhibit genetic interactions with *SLC1* (33). *PMT5* encodes for a component of the dolichyl-phosphate-mannose-protein mannosyltransferase complex while a function for *YOR175c* (from here on referred to as *LPT1*[5]) was uncharacterized previously. To confirm the synthetic-lethal interaction, independent *slc1Δ* and

lpt1Δ haploid strains were generated and mated to form compound heterozygous diploids. Following meiosis, eighty haploid spores were isolated by tetrad dissection. Sixty spores were viable and all were of the wild-type, *slc1Δ* or *lpt1Δ* genotype. In line with Hardy Weinberg equilibrium, ¼ of the progeny were therefore inviable and *slc1Δlpt1Δ*, confirming the synthetic lethal interaction.

Lpt1p primary structure - The *LPT1* gene encodes for a predicted protein of 619 amino acids and 72.2 kDa. Computer algorithm analysis predicts seven transmembrane domains (33). The C-terminal four amino acids, KKEE, agree with the conserved ER retention signal KKXX (34). This is consistent with studies that have shown Lpt1p to co-purify with ribosomes (35) and a Lpt1p-green fluorescent protein chimera to localize to the endoplasmic reticulum (36). In terms of homology, Lpt1p belongs to the membrane bound o-acyltransferase (MBOAT) family of proteins (37). Within the MBOAT domain (Pfam PFO3062), Lpt1p shares about 25% identity with three characterized members of the MBOAT family in humans (Fig. 2). The presence of this domain in Lpt1p is consistent with it being an acyltransferase.

Determining if altering LPT1 expression alters AGPAT activity - Initial experiments characterizing the acyltransferase activity in yeast cell lysates were performed by measuring the release of coenzyme A from acyl-CoA substrates. Assays with and without lysophosphatidic acid (lysoPA) were performed in parallel so that lysoPA-independent CoA generation could be measured and subtracted. This "background" activity was not different between wild-type and *lpt1Δ* samples. Two acyl-CoA species, palmitoyl-CoA and oleoyl-CoA, were provided to represent saturated and monounsaturated substrates. To establish the amount of Slc1-independent AGPAT activity in yeast, *slc1Δ* cell lysates were assayed and showed a 60% reduction, with either acyl-CoA, compared to wild-type yeast (Fig. 3A). In contrast, *lpt1Δ* cell lysates showed no statistically significant changes in AGPAT activity. However, over-expression of *LPT1* resulted in a 7-fold increase in AGPAT activity (Fig. 3B). This suggests that Lpt1p can mediate the reaction but in the early stationary phase of growth, from which these cells were harvested, Slc1 is the primary AGPAT.

Lpt1p esterifies other lysophospholipids - Since yeast have been shown to mediate lysophosphatidylcholine acyltransferase activity (LPCAT) (2,23) we tested if Lpt1p is also responsible for that activity. Similar assays to the above were performed by replacing lysoPA with lysoPC. Activity was seen with wild-type yeast cell lysates supplied with palmitoyl-CoA, oleoyl-CoA, and arachidonyl-CoA representing saturated, monounsaturated, and polyunsaturated substrates (Fig. 4A). Unsaturated acyl-CoAs showed 4-7 fold higher activity than the saturated palmitoyl-CoA. No activity was observed in *lpt1Δ* cell lysates with any of the acyl-CoAs, suggesting that Lpt1p is the main, if not only, LPCAT in yeast. Similar assays using [^{14}C]lysoPC and oleoyl-CoA showed essentially no activity with *lpt1Δ* cell lysates (0.2 ± 0.2 nmol / min /mg) and robust activity in wild-type lysates (55.6 ± 4.7 nmol / min / mg). To address the possibility that Lpt1p is an accessory protein, necessary for the expression and/or activity of the true LPCAT, Lpt1p was over-expressed in wild-type yeast. LPCAT activity was increased 7-fold compared to wild-type yeast harboring an empty vector (Fig. 4B), consistent with Lpt1p being the catalyst of the observed activity. LysoPC-independent activity was modestly increased (11.5 vs. 6.9 nmol/min/mg) with *LPT1* over-expression.

Since Lpt1p may be the only LPCAT in yeast, we hypothesized that it will have broad substrate specificity regarding lysophospholipids. Replacing lysoPC with lysophosphatidyl-ethanolamine (lysoPE), lysophosphatidylinositol (lysoPI) or lysophosphatidylserine (lysoPS) showed distinct acyltransferase activity (Fig. 5A, B). Comparing the enzyme activities of each lysophospholipid with oleoyl-CoA suggests that Lpt1p has a substrate preference of lysoPC = lysoPI = lysoPS > lysoPE (Figs. 4, 5). Since Lpt1p can use multiple lysophospholipids as substrates, it should be referred to as a lysophospholipid acyltransferase (LPLAT).

Acyl-CoA substrate specificity for Lpt1p - The ability to esterify lysophospholipids suggests that Lpt1p is involved in phospholipid remodeling. This hypothesis predicts that Lpt1p will preferentially utilize unsaturated acyl-CoA substrates. To test this, we performed substrate

concentration series experiments with [^{14}C]lysoPC and the four acyl-CoA species commonly found in yeast: palmitoyl-CoA (16:0), palmitoleoyl-CoA (16:1), stearoyl-CoA (18:0), and oleoyl-CoA (18:1). Arachidonyl-CoA (20:4) was also included to represent polyunsaturated acyl-CoAs. Curve fitting of the Michaelis - Menten plots (Fig. 6) determined the apparent K_m and V_{max} (Table I). Palmitoyl-CoA and stearoyl-CoA showed relatively low K_m and low V_{max} values while the monounsaturated palmitoleoyl-CoA and oleoyl-CoA showed relatively high K_m and high V_{max} values and arachidonyl-CoA showed intermediate values. Since all the reactions have the same, although undetermined, enzyme concentration, V_{max} / K_m should be proportional to the catalytic efficiency (k_{cat} / K_m). This value tended to be higher for the unsaturated acyl-CoAs than the saturated acyl-CoAs. However, due to the very low K_m for palmitoyl-CoA, this trend was not absolute.

Subcellular location of LPLAT activity - Previous studies have identified acyl-CoA dependent LPCAT activity in both yeast microsomes (23) and mitochondria (22). To determine the subcellular location of LPLAT activity, wild-type yeast organelles were separated by differential and density-gradient centrifugation. Using lysoPC as a representative lysophospholipid in LPLAT assays, microsomes contained 10-fold enriched activity compared to whole cell lysates while mitochondria showed no enrichment (Table II). Since microsomes comprised a much larger fraction of total cell protein, they contained 65% of total cellular LPLAT activity. Mitochondria contained 2% of total activity with the remainder either lost during extraction or in another cellular compartment. No activity was detected in *lpt1Δ* mitochondria, suggesting that there is not a second, mitochondrial specific LPCAT.

Role of Lpt1p in oleate incorporation into glycerolipids - We next addressed the physiological importance of Lpt1p mediated LPLAT activity. Wild-type and *lpt1Δ* strains were pulse-labeled for 30 minutes with [^{3}H]oleate. Incorporation of the radiolabel into PC in *lpt1Δ* strains was 30% less than in wild-type yeast (Fig. 7). Incorporation into PE, PI, and PS and neutral lipids was not affected. Since the product of AGPAT activity, PA, is required for the *de novo* synthesis of phospholipids and triglycerides, the selective effect on PC synthesis may be due to attenuated remodeling. If this is the case, then of the exogenous oleate incorporated into PC in wild-type yeast, 70% is via *de novo* synthesis and 30% is via remodeling. Why the phenotype was limited to PC is unclear. It may be that the phospholipase A_2 component of the remodeling cycle preferentially generates lysoPC.

DISCUSSION

1-acyl-sn-glycerol-3-phosphate acyltransferase (AGPAT) mediates the second step in *de novo* triglyceride and phospholipid synthesis. This pathway seems to be well conserved through evolution as evidenced by all of the pathway's enzymes in *S. cerevisiae* identified to date having a human homolog (38). These studies were initiated to identify a novel AGPAT in yeast that would allow a more complete analysis of phospholipid metabolism in this model organism and potentially identify novel human homologs. Since AGPAT activity is required for the *de novo* synthesis of phospholipids, a major component of cellular membranes, if there is only one additional AGPAT in yeast besides *SLC1*, a synthetic lethality screen should identify this gene. Systematically crossing *slc1Δ* haploids with each of the approximately 5000 viable single-gene deletion haploids identified a total of 52 synthetic-lethal interactions. One of the identified genes was *ELO2* (*FEN1*) which encodes for an acyl-CoA elongase required for ceramide synthesis (39). Interestingly, a gain of function mutation, *SLC1-1*, complements yeast that have deficient sphingolipid synthesis. Viability is conferred by the synthesis of atypical PI species that contain very long chain acyl groups, presumably with similar biophysical properties to sphingolipids (40). Perhaps the non-mutated *SLC1* produces a small amount of such PI species which are required when acyl-CoA elongation is limited.

LPT1 was also identified in the screen and chosen for further characterization due to its containing a motif found in a family of membrane bound o-acyltransferases (MBOATs) (37). In *S. cerevisiae*, the family members are the acyl-CoA sterol acyltransferases, Are1, Are2 (41), and the glycosylphosphatidylinositol anchor remodeling protein Gup1 (42) and its paralog, Gup2. In humans, the family includes ACAT1, ACAT2, and DGAT1. Lpt1p does not share sequence similarity with Slc1 or any other AGPAT. It also does not

share sequence similarity to the lysophosphatidylcholine acyltransferases, LPCAT1 (20) and LPCAT2 (21), recently identified in mouse lung nor the human lysophosphatidylglycerol acyltransferase (43). Lpt1p does, however, share about 25% overall amino acid sequence identity to three uncharacterized human genes' translation products. Whether these are functional homologs is under current investigation.

The function of Lpt1p was initially investigated by comparing *in vitro* AGPAT activity in wild-type and *lpt1Δ* cell lysates. Coenzyme A release was used to monitor AGPAT activity as has been described for measuring AGPAT, LPCAT, and DGAT activity in rat liver homogenates (32). In a validation of the assay, deletion of *SLC1* caused a 60% reduction in AGPAT activity which agreed with a previous study that found *slc1Δ* microsomes incorporate 50% less [^{14}C]glycerol 3-phosphate into phosphatidic acid than wild-type cells (12). No statistically significant decrease in activity resulted by deleting *LPT1*. However, over-expression of *LPT1* in a wild-type strain conferred a 7-fold increase in AGPAT activity. This data, along with the inviability of *scl1Δlpt1Δ*, suggests that Lpt1p is the second yeast AGPAT. The largely unchanged AGPAT activity in *lpt1Δ* may be due to *SLC1* up-regulation.

Subsequent experiments found that Lpt1p has a more pronounced role in esterifying lysophospholipids besides lysoPA. Deletion of *LPT1* completely abrogated LPCAT activity and over-expression of *LPT1* in a wild-type yeast strain increased this activity 7-fold. Cell fractionation studies detected the majority (65%) of whole-cell LPCAT activity in microsomes, consistent with Lpt1p residing in the ER (35,36). While others detected acyl-CoA dependent LPCAT activity in yeast mitochondria (22), we recovered little acyl-CoA dependent activity (2%) in wild-type mitochondria and none in *lpt1Δ* mitochondria. Supplying wild-type yeast lysates with lysoPE, lysoPI, and lysoPS also showed acyl-CoA dependent acyltransferase activity. No activity was observed in *lpt1Δ* lysates even with providing saturated and unsaturated acyl-CoA species. The substrate preference for lysophospholipids seems to be lysoPC = lysoPI = lysoPS > lysoPE. However, these differences may reflect a difference in solubility in the assay buffer and / or ability to intercalate into membranes during the assay and not a difference in enzyme affinity. The ability to esterify lysoPE agrees with the recent finding that yeast can transport exogenous lysoPE and esterify it (17). The use of lysoPI as an acyl acceptor has been rarely described in the literature: in rat brain (44) and pancreas (45) and cow brain (46). This broad use of acyl acceptors by Lpt1p prompted using the term lysophospholipid acyltransferase (LPLAT) to describe its activity. While no evidence was found for Lpt1p-independent LPLAT activity in yeast, other such enzymes may exist that are not expressed under the growth conditions used here, require assay components that were not supplied, or use other substrates such as short or very long acyl-CoAs.

As for the four long chain acyl-CoA species commonly found in yeast (47), their utilization was studied in depth with substrate concentration series experiments. Since these substrates likely undergo kinetically distinct steps, such as membrane insertion, prior to interacting with Lpt1p, all kinetic parameters are considered to be apparent. With lysoPC as the acyl acceptor, the saturated acyl-CoAs showed low K_m and low V_{max} values and the monounsaturated acyl-CoAs showed relatively high K_m and high V_{max} values. For oleoyl-CoA, the K_m is lower and the V_{max} higher compared to a previous study of yeast LPCAT (K_m = 152 μM, V_{max} = 11 nmol/min/mg) (23). The values also differed from the rat liver LPCAT activity with unsaturated acyl-CoA (K_m = 1 - 10 μM; V_{max} = 8 - 13 nmol/min/mg) (48). Since V_{max} is proportional to the turnover rate of the enzyme and K_m is proportional to the enzyme's affinity for substrate, V_{max} / K_m yields a single value for comparing substrate utilization. This value tended to be higher for unsaturated acyl-CoA species than saturated species. The presence, and not number, of double bonds seems to be critical for substrate utilization since arachidonyl-CoA has four and was actively used.

Comparing the kinetic parameters to the Michaelis-Menten plots indicates some discordance. The reason may be that at high acyl-CoA concentration, activity is limited by a detergent effect. Perhaps a better way to analyze the kinetic data is to compare the activity at

physiological cellular concentrations of each acyl-CoA. Since yeast have about 0.1 nmol of each acyl-CoA in 10^9 cells (47) and the average volume of a yeast haploid is 70 μm^3, the cellular concentration of each acyl-CoA is about 2 μM. If this gross estimate is correct, then Lpt1p likely mediates similar activity with saturated and unsaturated acyl-CoA inside cells. If the physiological acyl-CoA concentrations are 10 μM or higher, then the use of unsaturated acyl-CoAs will predominate.

The physiological function of Lpt1p mediated LPLAT activity was also investigated by pulse labeling wild-type and *lpt1Δ* strains with [^{3}H]oleate. There was a selective reduction in incorporation into PC by 30%. It seems unlikely that this difference is due to decreased AGPAT activity since phosphatidic acid provides a precursor for all the phospholipid species, synthesized via diacylglycerol or CDP-diacylglycerol, and triglyceride. More likely, the difference is due to the deficient LPLAT activity in *lpt1Δ*. The lack of an effect on incorporation into PE or PI may reflect a low cellular abundance of lyso-species of these phospholipids. Whether *S. cerevisiae* has a phospholipase A_2 with preference for PC has not been determined. However, yeast actively use PC as an acyl donor for triglyceride synthesis, and thus actively produce lysoPC, via a phospholipid diacylglycerol acyltransferase reaction mediated by Lro1p (49). This may be the main source of Lpt1p substrates. In addition to constitutive remodeling, Lpt1p may also function to limit the abundance of lysophospholipids and avoid inappropriate stimulation of adenylate cyclase in yeast (50). LPLAT activity also allows exogenous lysophospholipids to be salvaged after uptake and directly utilized for cell membranes (17).

In *S. cerevisiae*, the preferential incorporation of unsaturated acyl chains into the sn-2 position of phospholipids seems to be achieved by a combination of substrate selectivity during *de novo* synthesis, primarily by Slc1p, in the Kennedy Pathway and remodeling by Lpt1p in the Lands cycle. This is supported by a deletion of both genes being lethal and the preference of both reactions for unsaturated acyl-CoA substrates. Within the family of membrane bound o-acyltransferases, *LPT1* and its orthologs may comprise a novel branch with important roles regarding phospholipid composition.

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FOOTNOTES

2. C.B is supported by grants from the Canadian Institute of Health Research, Genome Canada and Genome Ontario.
3. To whom correspondence should be addressed: Peter Oelkers, Ph.D., Drexel University, 3141 Chestnut St., Philadelphia, PA 19104 Fax: 215-895-1273; E-mail: pmo24@drexel.edu
4. The abbreviations used are: AGPAT (1-acyl-sn-glycerol-3-phosphate acyltransferase), LPAAT (lysophosphatidic acid acyltransferase), , LPCAT (lysophosphatidylcholine acyltransferase), lysoPAFAT (lyso platelet activating factor acyltransferase), SGA (synthetic genetic array), LPLAT (lysophospholipid acyltransferase), MBOAT (membrane bound o-acyltransferase), ACAT (acyl-CoA cholesterol acyltransferase), DGAT (acyl-CoA diacylglycerol acyltransferase), PA (phosphatidic acid), PC (phosphatidylcholine), PE (phosphatidylethanolamine), PI (phosphatidylinositol), PS (phosphatidylserine)
5. While this paper was under review, two papers describing the same gene have been electronically published. Riekhof et al. (51) named it *ALE1* and Benghezal et al. (52) named it *SLC4*.

FIGURE LEGENDS

Figure 1. **Pathways for phospholipid synthesis in yeast.** A. *De novo* synthesis. B. Remodeling / salvage pathways. Some substrates and products were omitted due to space constraints. PA, phosphatidic acid; DAG, diacylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine.

Figure 2. **Alignment of *LPT1* and 3 human members of the MBOAT gene family.**
The four amino acid sequences are aligned over the MBOAT motif (Pfam PFO3062). Positions where 3 out of 4 amino acids are conserved are boxed. In parentheses is the position of the first amino acid. acyl-CoA cholesterol acyltransferase (ACAT); acyl-CoA diacylglycerol acyltransferase (DGAT).

Figure 3. ***In vitro* 1-acyl-sn-glycerol-3-phosphate acyltransferase (AGPAT) assays.** A. Cell lysates from wild-type, *lpt1Δ* and *slc1Δ* haploids, grown to late logarithmic phase, were assayed for AGPAT activity by the spectrophotometric method described in "Experimental Procedures". The assay contained 50μM 1-oleoyl lysophosphatidic acid, 50μM acyl-CoA, 1.5 mM DTNB, 0.42-0.84 mg cell lysate and 100 mM Tris-HCl, pH 7.4. palmitoyl-CoA (black), oleoyl-CoA (grey). The data represent mean ± S.E. n = 3-5. Asterisk indicates statistically significant difference compared with wild-type strain ($p < 0.05$). B. Similar assays were performed with cell lysates from wild-type cells transformed with pRS423GP or pRS423GP-*LPT1*.

Figure 4. ***In vitro* lysophosphatidylcholine acyltransferase (LCPAT) assays.** A. Cell lysates from wild-type, *lpt1Δ* and *slc1Δ* haploids, grown to late logarithmic phase, were assayed for LPCAT activity by the spectrophotometric method described in "Experimental Procedures" and in fig. 2 legend except lysoPA was replaced with lysoPC. palmitoyl-CoA (black), oleoyl-CoA (grey) and arachidonyl-CoA (striped). The data represent mean ± S.E. n = 3-4. Asterisks indicate statistically significant difference compared with wild-type strain ($p < 0.01$). B. Similar assays were performed with cell lysates from wild-type cells transformed with pRS423GP or pRS423GP-*LPT1*.

Figure 5. **Lysophospholipid substrate specificity of Lpt1p.** A. Lysophosphatidylethanolamine (lysoPE), B. lysophosphatidylinositol (lysoPI), or C. lysophosphatidylserine (lysoPS) were used as acyl acceptors and LPLAT activity was determined by the spectrophotometric method using cell lysates prepared from wild-type yeast. palmitoyl-CoA (black), oleoyl-CoA (gray). The data represents mean ± S.E. n = 3. Asterisk indicates statistically significant difference ($p<0.01$) between wild-type and *lpt1Δ*.

Figure 6. **Acyl-CoA substrate specificity of Lpt1p.** Cell lysates from *lpt1Δ* yeast transformed with pRS423GP / *LPT1* were assayed for LPCAT activity by the radioactive substrate method described in "Experimental Procedures". The reaction contained 50μM [1-^{14}C] palmitoyl lysophosphatidylcholine, 1 - 110 μM of acyl-CoA, 3μg of cell lysate, and 100 mM Tris-HCl, pH 7.4 and incubated for 5 minutes at 28°C. A. palmitoyl-CoA B. palmitoleoyl-CoA C. stearoyl-CoA D. oleoyl-CoA E. arachidonyl-CoA. The data represent mean ± S.E. n = 3 - 5.

Figure 7. **Incorporation of [^{3}H]oleate into phospholipids and neutral lipids.** Wild-type and *lpt1Δ* yeast were grown in YPD to logarithmic phase and pulse-labeled with [^{3}H]oleate for 30 minutes. A. Incorporation into the major phospholipids (PC, black; PE, white; PS/PI, striped) B. neutral lipids (diglyceride, dark gray; triglyceride, light gray; ergosterol ester, hatched) was measured as described in "Experimental Procedures". The data in counts per minute represent mean ± S.E. n = 4. Asterisk indicates statistically significant difference between wild-type and *lpt1Δ* ($p<0.05$)

TABLES

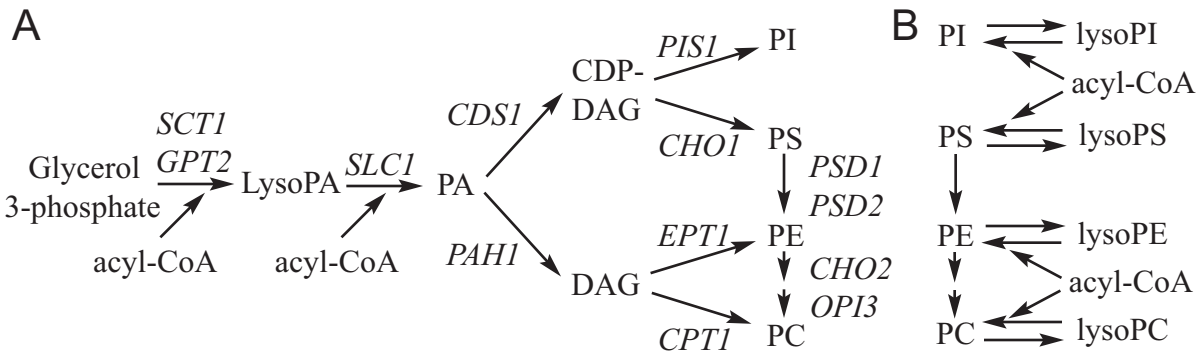
Table I. **Kinetic parameters of Lpt1p.** Kinetic data from fig. 6 were used to calculate apparent K_m and V_{max}.

Acyl-CoA	K_m (μM)	V_{max} (nmol / min / mg)	V_{max} / K_m (min^{-1} mg^{-1})
palmitoyl-CoA (16:0)	1.8	7.8	43
palmitoleoyl-CoA (16:1)	21	142	69
stearoyl-CoA (18:0)	5.9	12	20
oleoyl-CoA (18:1)	49	125	25
arachidonyl-CoA (20:4)	11	58	52

Table II. **Subcellular localization of acyl-CoA dependent LPCAT activity.** Wild-type yeast were grown in YP with 3% glycerol to $O.D._{660} = 1$. Cell lysates, mitochondria and microsomes were isolated as described under "Experimental Procedures". LPCAT activity was measured using the spectrophotometric method as described in fig. 4. The data represent mean ± S.E. n = 3.

Subcellular fraction	LPCAT activity (nmol / min / mg)	Fold enrichment	Percent recovery
Cell lysate	10.2 ± 0.5		
Mitochondria	8.1 ± 1.9	0.8 ± 0.1	1.9 ± 0.9
Microsomes	99.2 ± 2.7	9.7 ± 0.2	65.1 ± 14.5

Figure 1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Glycerol
3-phosphate
SCT1
GPT2
acyl-CoA
LysoPA
SLC1
acyl-CoA
PA
CDS1
PAH1
CDP-
DAG
DAG
PIS1
CHO1
EPT1
CPT1
PI
PS
PE
PC
PSD1
PSD2
CHO2
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B
PI
lysoPI
acyl-CoA
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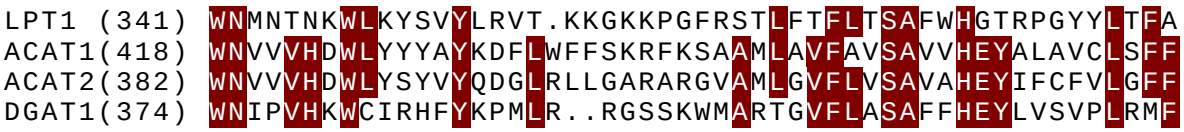
lysoPS
PE
lysoPE
acyl-CoA
PC
lysoPC

Figure 2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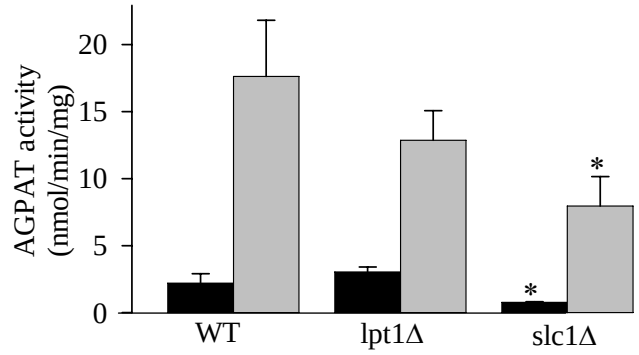
ACAT2(382) WNVVVHDWLYSYVYQDGLRLLGARARGVAMLGVFLVSAVAHEYIFCFVLGFF
DGAT1(374) WNIPVHKWCIRHFYKPMLR..RGSSKWMARTGVFLASAFFHEYLVSVPLRMF

Figure 3

A


AGPAT activity
(nmol/min/mg)
20
15
10
5
0
*
*
WT
lpt1Δ
slc1Δ

B

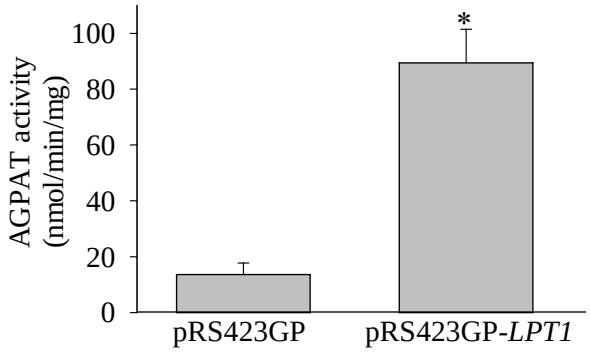

AGPAT activity
(nmol/min/mg)
100
80
60
40
20
0
*
pRS423GP
pRS423GP-*LPT1*

Figure 4

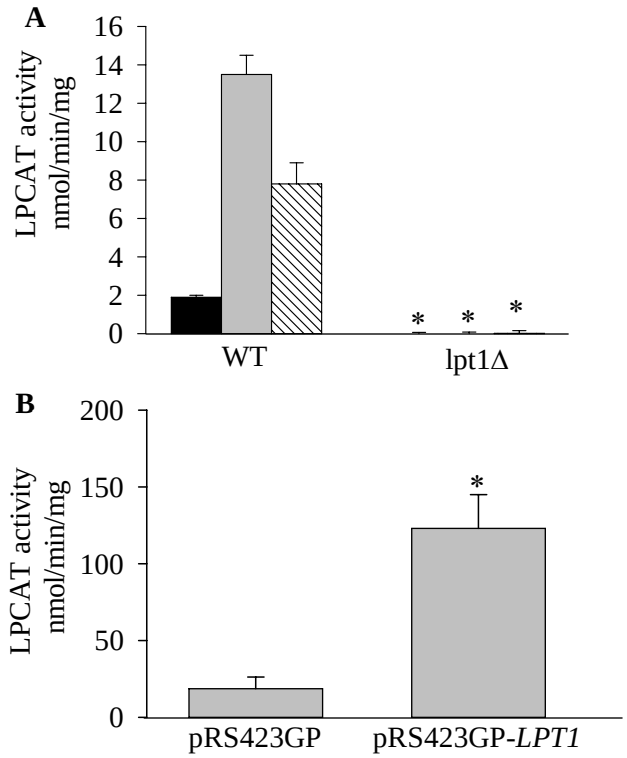

A
LPCAT activity
nmol/min/mg
16
14
12
10
8
6
4
2
0
*
*
*
WT
lpt1Δ
B
LPCAT activity
nmol/min/mg
200
150
100
50
0
*
pRS423GP
pRS423GP-LPT1

Figure 5

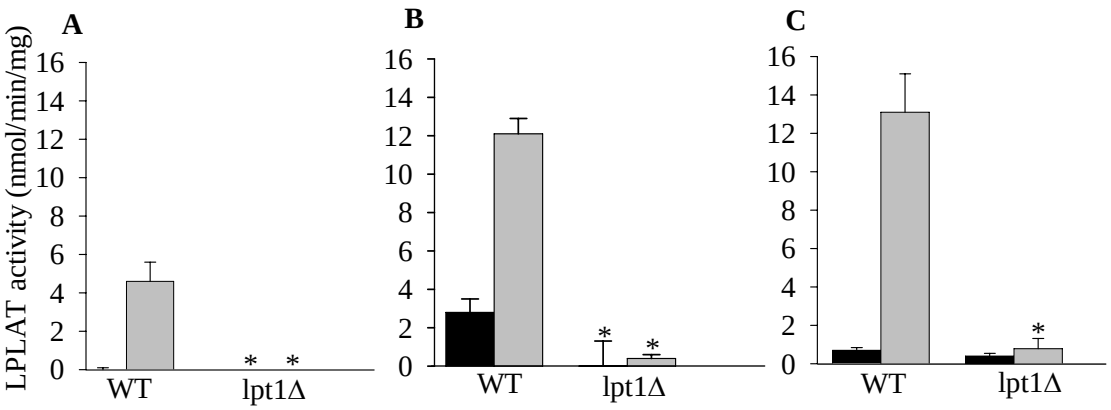

A
B
C
LPLAT activity (nmol/min/mg)
16
14
12
10
8
6
4
2
0
*
*
*
*
*
WT
lpt1Δ
WT
lpt1Δ
WT
lpt1Δ

Figure 6

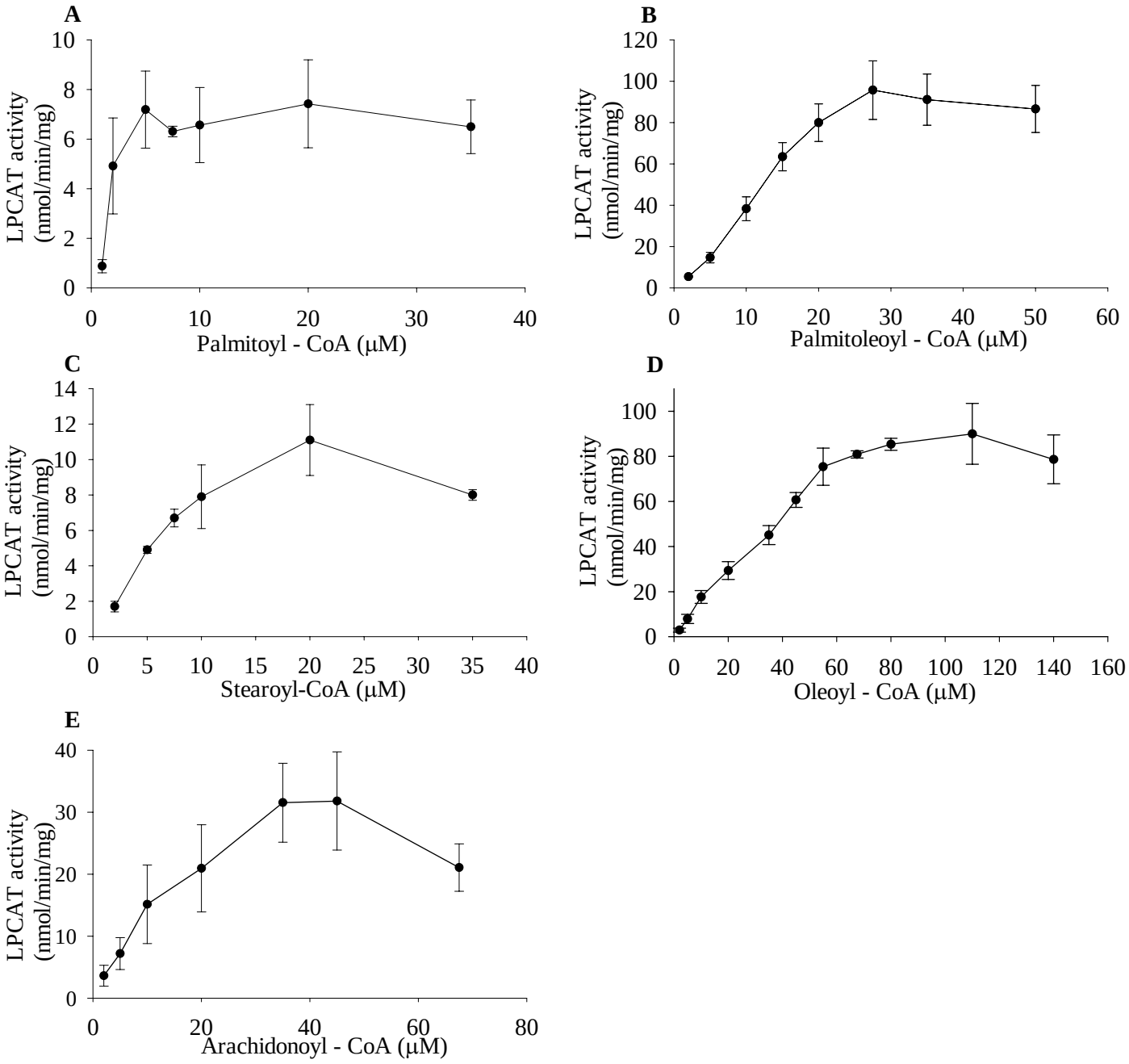
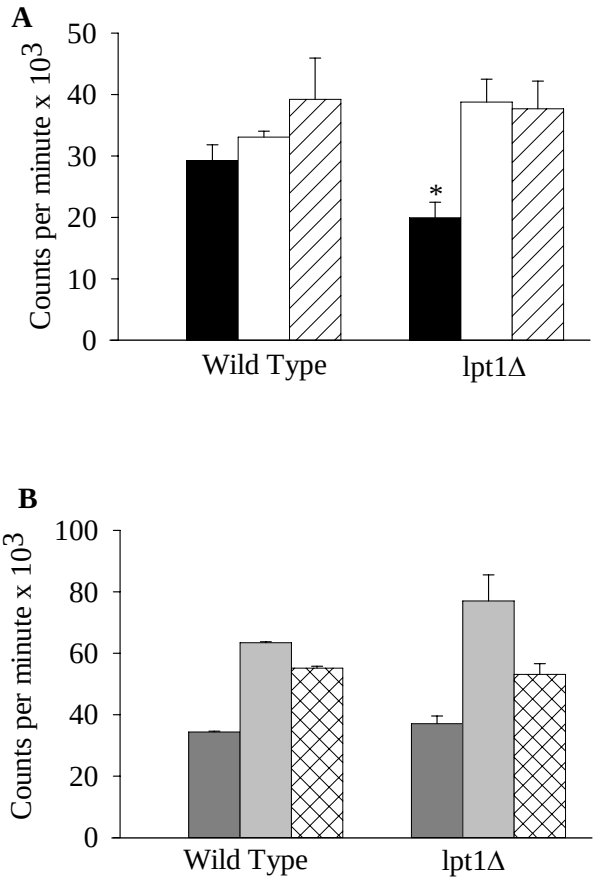

A
LPCAT activity (nmol/min/mg)
Palmitoyl - CoA (μM)
B
LPCAT activity (nmol/min/mg)
Palmitoleoyl - CoA (μM)
C
LPCAT activity (nmol/min/mg)
Stearoyl-CoA (μM)
D
LPCAT activity (nmol/min/mg)
Oleoyl - CoA (μM)
E
LPCAT activity (nmol/min/mg)
Arachidonoyl - CoA (μM)

Figure 7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Counts per minute x 10^3
50
40
30
20
10
0
*
Wild Type
lpt1Δ
B
Counts per minute x 10^3
100
80
60
40
20
0
Wild Type
lpt1Δ