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The yeast acylglycerol acyltransferase LCA1 is a key component of Lands cycle for phosphatidylcholine turnover☆

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Abstract Cellular phospholipids undergo deacylation and reacylation through a process known as Lands cycle. In this report, we provide evidence demonstrating that yeast *YOR175c*, herein designated as *LCA1*, encodes a key component of the Lands cycle, the acyl-CoA: lysophosphatidylcholine acyltransferase (LPCAT). Deletion of *LCA1* resulted in a drastic reduction in LPCAT activity, while over expression led to a several fold increase in enzyme activity. We further show that disruption of *LCA1* caused an enhanced production of glycerophosphorylcholine, a product of phosphatidylcholine (PC) deacylation and that the lysophosphatidic acid acyltransferase SLC1 was not involved in this process. Identification of LCA1 provides an essential molecular tool for further study of Lands cycle in PC turnover.

Keywords: Lands cycle; Acyl-CoA: lysophosphatidylcholine acyltransferase; PC turnover; *Saccharomyces cerevisiae*

1. Introduction

Phosphatidylcholine (PC) serves not only as a major component of cellular membranes, but also as a fatty acyl donor for neutral lipid biosynthesis in eukaryotic organisms and a source for the production of lipid messengers. There are two metabolic routes through which PC is generated [1]: (i) the Kennedy cytidine 5′diphosphocholine (CDP-choline) pathway, where diacylglycerol (DAG) is a precursor and directly condensed with CDP-choline; and (ii) a pathway where CDP–DAG is a direct precursor, involving phosphatidylserine formation and decarboxylation and phosphatidylethanolamine methylation. In the presence of choline, the Kennedy pathway is believed to be the main route of PC generation in yeast. As a major phospholipid of eukaryotic cell membrane systems, the level of PC is maintained by a balance of synthesis and degradation. An important aspect of PC metabolism ubiquitous in eukaryotic systems is a process first discovered by Lands [2] that concerns the active turnover of the acyl groups in PC. The eponymous ''Lands Cycle'' proposes a deacylation–reacylation process that starts with the deacylation of PC to produce lysophosphatidylcholine (LPC), followed by a reaction that reacylates LPC to PC. The significance of this pathway in the homeostasis of glycerolipids has received a considerable attention since the deacylation–reacylation process concerns the remodeling of phospholipid molecules. In animal cells, it has been shown that this pathway is important for the incorporation of long chain fatty acids (e.g. arachidonic acid) into PC, which is released from PC during inflammatory responses [3].

The enzymatic components responsible for the deacylation of PC in yeast have recently been identified [4,5]. The reacylation of LPC is mediated by an acyl-CoA: lysophosphatidylcholine acyltransferase (LPCAT, E.C. 2.3.1.23). Although the yeast microsomal LPCAT activity was demonstrated three decades ago [6,7], the identity of the enzyme remains elusive. A yeast tafazzin homolog was previously reported as capable of acylating LPC [8], but this enzyme appeared to be acyl-CoA independent [9,10]. More recently, a LPCAT involved in pulmonary surfactant PC production was reported [11,12] in mammals, but it was not involved in general membrane lipid synthesis [11]. Here we provide evidence demonstrating that YOR175c, herein designated as LCA1, possesses LPCAT activity and plays a pivotal role in PC turnover as a component of a lipid remodeling cycle first proposed by Lands [2].

2. Materials and methods

2.1. Strains and reagents

Yeast strains: BY4741 (WT, *MATa his*Δ*1 leu*Δ*0 met*Δ*0 ura*Δ*0*), Y02431 (*lca1*Δ, *MATa his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ *0 YOR175c::KanMX4*) and Y03749 (*slc1*Δ, *MATa his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0 YDL052c::KanMX4*) were purchased from European *Saccharoymces Cerevisiae* archive for functional analysis (EUROSCARF). Various lysophospholipids and acyl-CoAs were obtained from Avanti Polar Lipids (Alabaster, AL). [^{14}C] oleoyl-CoA, [^{14}C] palmitoyl-CoA, [^{14}C] palmitoyl-LPC were purchased from American Radiolabeled Chemicals Inc. Yeast extract, Yeast Nitrogen Base, Bacto-peptone, and Bacto-agar were purchased from Difco™, D-glucose,

☆ While this manuscript was in preparation, four papers [25–28] describing the same gene (*YOR175c*) were published electronically.

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Abbreviations: PC, Phosphatidylcholine; CDP-choline, cytidine 5′-diphosphocholine; DAG, diacylglycerol; LPCAT, acyl-CoA: lysophosphatidylcholine acyltransferase; LPAAT, acyl-CoA: lysophosphatidic acid acyltransferase; EUROSCARF, European *Saccharoymces cerevisiae* archive for functional analysis; DTT, dithiothreitol; PAF, platelet-activating factor; TCA, trichloroacetic acid; MBOAT, membrane-bound *O*-acyltransferase; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; LPI, lysophosphatidylinositol; GroPC, glycerophosphorylcholine

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D-galactose and D-raffinose were from Sigma. SC minimal medium and plates was prepared according to Invitrogen's recipe described for the pYES2.1 TOPO TA Cloning Kit.

2.2. Gene expression vector construction

For TOPO TA-cloning and yeast complementation, *Saccharomyces cerevisiae YOR175c* ORF was PCR-amplified with primers FP: 5′GGTGATTCTAGACTGCTGCTGATCGCTT3′ and RP: 5′GCATCTGTCGTTTTTGGAGCTCTACTCTT3′, and cloned into pYES2.1 vector (Invitrogen). Correctly-oriented plasmids were identified by DNA sequencing and subsequently introduced into yeast strain Y02431.

2.3. Microsomal preparation

Yeast strains were first grown in 15 ml of SC-leu-his-ura medium containing 2% glucose. Protein expression induction was carried out as described in Invitrogen manufacturer manual for yeast expression vector pYES2.1. After 24 h of growth in SC + 2% galactose + 1% raffinose induction conditions, the cells were washed, first with distilled water and then with wall-breaking buffer [50 mM Tris–HCl, 1 mM EDTA, 0.6 M sorbitol, pH 7.4, 1 mM dithiothreitol (DTT)]. After centrifugation at 4000 rpm (Eppendorf Centrifuge 5145C), the cells were resuspended in 1 ml wall-breaking buffer with 10 μl yeast protease cocktail (Sigma), and shaken vigorously in the presence of acid-washed glass beads (diameter 0.5 mm). The resultant homogenate was centrifuged at 12000 rpm for 10 min at 4 °C. The decanted supernatant was further centrifuged at $100000 \times g$ for 90–120 min at 4 °C. The supernatant was discarded, and the pellet was suspended in homogenization buffer containing 20% glycerol and frozen at −80 °C until use. Protein concentration was measured using Bio-Rad Protein Assay Kit for final enzyme activity calculation.

2.4. In vitro assay of LPCAT activity

LPCAT substrate specificity was determined by measuring incorporation of [^{14}C] LPC or [^{14}C] palmitoyl-CoA into PC. All assays were performed at least twice. For lysophospholipid substrate specificity assessment, 400 μl HEPES buffer contained 3 μg microsomal protein, 50 μM of lysophospholipid substrates and 112.5 μM [^{14}C] palmitoyl-CoA (5.5 nCi/nmol). For acyl-CoA substrate selectivity analysis, 400 μl HEPES reaction buffer (pH 7.4, 0.1 M) contained 3 μg microsomal protein, 50 μM acyl-CoA and 112.5 μM [^{14}C] palmitoyl-PC (1.35 nCi/nmol). Reaction was allowed for 2 min at 30 °C with 100 rpm shaking. The reaction products were extracted with chloroform/methanol (2/1, v/v) and separated with Merck silica G60 TLC plates. Spots corresponding to different phospholipid species products were scraped off and ^{14}C incorporation were scintillation counted. Different concentrations of $ZnCl_2$ were added in to reactions for Zn^{2+} inhibitory effect assay.

2.5. Lyso-PAF sensitivity

Yeast strains Y02431 over-expressing *LCA1* or harboring empty vector were first grown in 15 ml of SC-ura medium containing 2% glucose then transferred to SC-ura + 2% galactose and 1% raffinose. After 12 h *LCA1* expression induction, the culture was diluted to correspond to OD_{600} value of 0.5, 1, 2, 3, 4. Five microliters of each dilution was spotted to a YPD plate supplemented with varying concentrations of lyso-platelet-activating factor (PAF). The plates were incubated at 28 °C for 2 days.

2.6. PC turnover analysis

PC turnover analysis was performed according to previously described method [13] with slight modification. Briefly, Y02431 and BY4741 yeast cells were grown overnight in chemically defined synthetic media without inositol and choline. Yeast at OD_{600} = 1.5 were used to inoculate fresh chemically defined synthetic media containing 0.15 μCi/ml [^{14}C] choline chloride (20 μM). Cells were harvested through centrifugation after 5 h labeling, washed twice in fresh non-radioactive medium, and then inoculated into in medium containing 10 mM non-radioactive choline. At different time points, 1 ml aliquots were removed and centrifuged. The supernatant was saved as the "medium" fraction. The cell pellet was suspended in 0.5 ml 5% trichloroacetic acid (TCA) and incubated on ice with frequent vortexing. Following centrifugation at 14000 rpm (5414D, Eppendorf), the TCA-containing supernatant was decanted as "intracellular water-soluble fraction", and neutralized by adding 1 M Tris–HCl (pH 8.0) to avoid acid-induced luminescence in scintillation counting. The pellet was saved as the "membrane" fraction. The labeling of each fraction was measured and presented as percentage of total counts in all the three fractions.

To confirm that the majority of choline-containing compounds in the TCA fraction were glycerophosphorylcholine (GroPC), the fractions from WT and *lca1*Δ yeast cells chased for 2 h at 37 °C were applied to Merck silica G60 gel and developed in a solvent system containing methanol/0.5% $NaCl/NH_3 \cdot H_2O$ (50/50/1, v/v/v)[14]. After separation, only one major [^{14}C]-labeled band was detected. This band was scraped off, and re-extracted with distilled water to get rid of TCA. After lyophilization, it is re-applied onto Merck silica G60 plate along with other choline-containing standards. [^{14}C] choline and [^{14}C] choline containing compound from the TCA fractions were detected with a scanner (Bioscan Inc.). LPC was stained by iodine exposure, and other choline-containing standards were visualized by molybdenum blue spray, a detection reagent specific for phosphorus in GroPC, phosphocholine and CDP-choline [15].

3. Results and discussion

3.1. Deletion of YOR175cp leads to reduced LPCAT activity

A seminal work by Schuldiner et al. reported a comprehensive genetic-interaction map on the organization of the yeast early secretory pathway [16]. Several fatty acyltransferase genes were analyzed in the study, including the previously identified sn-2 acyltransferase *SLC1*. While a negative score <−3 would imply synthetic sick/lethal interaction, the assigned score for double mutant of *slc1* and *yor175c* was −20 (http://phoibe.med.utoronto.ca/erg/php/menu.php). This was striking in light of the fact that residual acyltransferase activity in *slc1*Δ mutant was at a level sufficient for balanced cellular growth [17,18]. A simplest explanation of the exacerbated phenotype of combined deficiency in *SLC1* and *YOR175c* is that YOR175c is a sn-2 acyltransferase, either like SLC1, a lysophosphatidic acid acyltransferase (LPAAT), or alternatively another lysophospholipid acyltrasferase. YOR175c does not exhibit significant sequence similarity to other sn-2 acyltransferases reported to date, but it is a membrane-bound *O*-acyltransferase (MBOAT) family protein [19], and was shown to be localized in endoplasm reticulum [20]. Members of the MBOAT family with confirmed functions include diacyglycerol acyltransferase, sterol acyltransferase.

In a preliminary experiment, we first examined if disruption of *YOR175c* would have any impact on LPAAT and LPCAT activities using both the parental strain and a *slc1*Δ mutant as controls. Consistent with previous report [17], when lysophosphatidic acid (LPA) (18:1) was supplied as acyl acceptor, the cell lysate of *slc1*Δ mutant had a LPAAT level reduced to 63% of the parental strain, but we detected no significant LPAAT reduction in the *yor175c*Δ mutant. In marked contrast, when LPC was provided as acyl acceptor, our in vitro assay showed acyltransferase activity reduction in *yor175c*Δ to a level approximately 28% of the parental strain. The *slc1*Δ displayed no significant decrease in LPCAT activity as compared with WT strain (Fig. 1).

We further investigated sn-2 lysophospholipid acyltransferase activity in *yor175c*Δ by using microsomal enriched fractions with different lysophospholipid acyl acceptors and palmitoyl-CoA (16:0-CoA). In keeping with the results of total cell lysate, microsomal fractions of the *yor175c*Δ strain showed a striking decrease in LPCAT activity. Lysophosphatidylethanolamine (LPE) and lysophosphatidylglycerol (LPG) acyl-

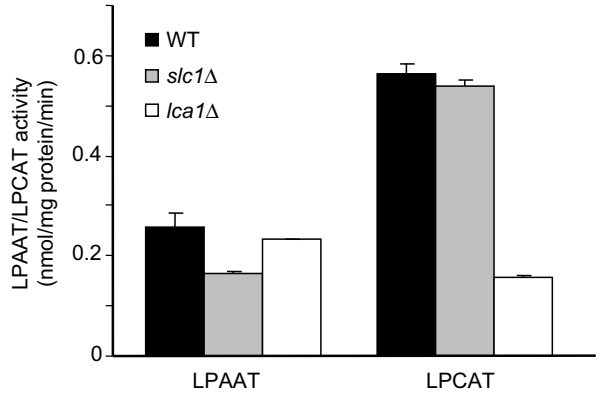

Fig. 1. Comparison of LPAAT and LPCAT activity of *slc1*Δ, *lca1*Δ, and their congenic WT yeast strain. Cell lysates equivalent to 200 μg protein were assayed for acylation of oleoyl-LPA and oleoyl-LPC with [^{14}C] oleoyl-CoA. The reaction mixture contained 45 μM 18:1-LPA or 18:1-LPC, 18 μM (10 nCi/nmol) 18:1-CoA. The reaction was to proceed for 30 min at 30 °C with 100 rpm shaking. The results were presented as a mean of three assays.

transferase were also slightly decreased, but to a much lesser degree (Fig. 2). Since LPCAT activity was singularly the most drastically affected, we thus designate *YOR175c* as *LCA1*, which stands for lysophosphatidylcholine acyltransferase.

3.2. LCA1 displays in vitro acyltransferase activity with preference for LPC

We next resorted to over-expression to assess the enzyme properties of LCA1. Microsomal preparations of *lca1*Δ mutant expressing LCA1 and *lca1*Δ harboring the empty vector (VO) were used to perform acyltransferase assays with [^{14}C] palmitoyl-CoA and various lysophospholipids substrates including LPA, LPC, LPE, LPG, LPI and LPS. As shown in Fig. 3, the highest activity was found with LPC as substrate. The activity of LPC acylation was linear at 30 °C for 20 min, and the conversion of LPC to PC is negligible in the absence of 16:0-CoA (data not shown). Over-expression of *LCA1* also caused substantial increases in the acylation of LPG and LPE. But the rates of LPG and LPE acylation were at a level

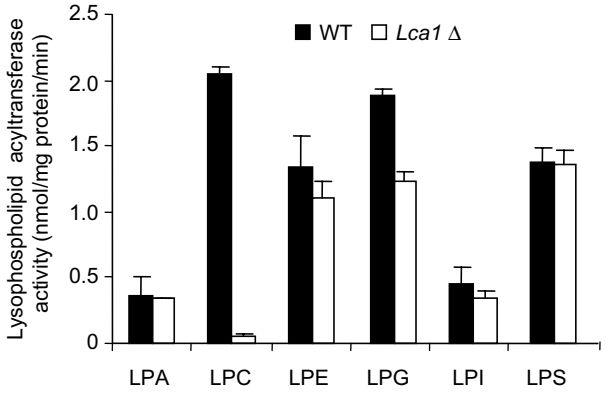

Fig. 2. Lysophospholipid acyltransferase activity in *lca1*Δ, and its congenic WT yeast strains. Microsomal preparations were assayed for acylation of palmitoyl-LPA, LPC, LPE, LPG, LPI and LPS with [^{14}C] palmitoyl-CoA. The reaction mixture contained 45 μM lysophospholipid, 27 μM (10 nCi/nmol) 16:0-CoA and 50 μg protein. The results were presented as a mean of three assays.

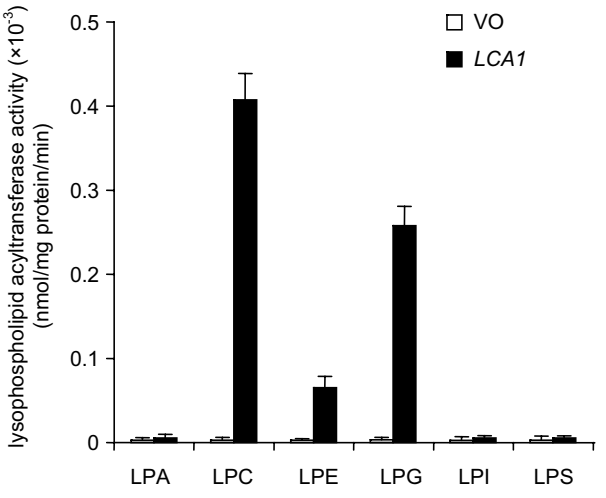

Fig. 3. Lyso-lipid substrate specificity of LCA1. The assays were preformed with 3 μg microsomal protein from *lca1*Δ harboring an empty vector (VO) and *lca1*Δ expressing LCA1. The reaction contained 112.5 μM [^{14}C] palmitoyl-CoA (5.5 nCi/nmol) and 50 μM lysophospholipid substrate (LPA, LPC, LPE, LPG, LPI and LPS). Reaction was allowed for 2 min with 100 rpm shaking. The results were presented as a mean of three assays.

approximately 60% and 20%, respectively, of the activity registered for LPC. Activities for LPA, LPS and LPI, were all less than 1% of the activity of LPCAT. Thus, LCA1 appeared capable of accepting several major lysophospholipid classes, but under our assay conditions it exhibited the highest activity with LPC. That LCA1 was capable of acylating multiple lysophospholipid molecules was not surprising because substrate

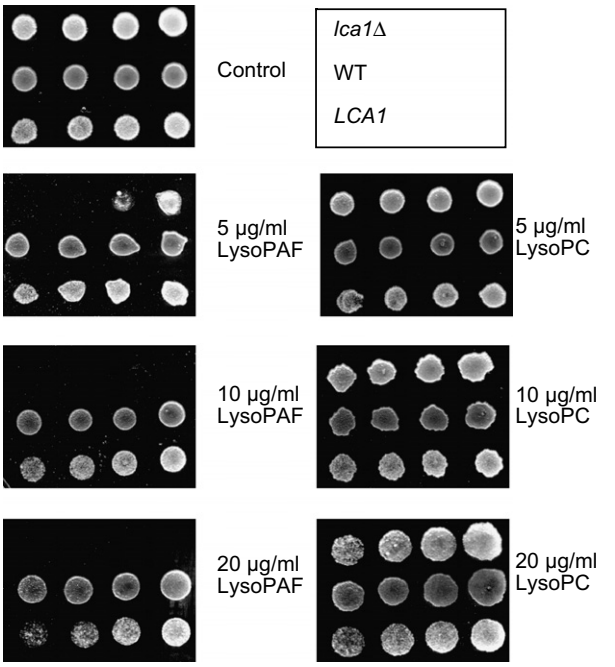

Fig. 4. Lyso-PAF and lyso-PC Sensitivity test of *lca1*Δ, WT and *lca1*Δ over expressing *LCA1*. Cells were grown first in SC-URA + 2% glucose media overnight then in protein expression induction media for 6 h. Cultures were diluted to OD_{600} value of OD_{600} 0.5, 1, 2, 4, respectively, from which 5 μl was inoculated (from left to right) onto YPD plate containing lyso-PAF or lyso-PC. The plates were incubated at 28 °C for 36 h.

Table 1
Inhibitory effect of Zn^{2+} on LCA1 activity

$ZnCl_2$ concentration	LPCAT activity (% control)
0 mM (control)	100 ± 7.9
20 mM	6 ± 2.0
0.1 mM	35 ± 22.4
25 μM	149.7 ± 12.0
10 μM	136.8 ± 3.9
5 μM	98 ± 5.9

Results are expressed as means ± S.D. The *lca1*Δ over-expressing *LCA1* was used to asses Zn^{2+} effect. The reactions contained 5.6 μM palmitoyl-LPC (1.35 nCi/nmol), 1.5 μg microsomal proteins, 0.1 M HEPES (pH 7.4), 11.25 μM stearyl-CoA and indicated concentration of $ZnCl_2$. The reaction was stopped after 2 min by adding 2 ml of chloroform/methanol solution (2:1).

promiscuity has been known for other acyltransferases [11,21,22].

3.3. Correlation of LCA1 activity with lyso-PAF sensitivity

Although not an endogenous acyl acceptor, ether-linked glycerolipid, lyso-PAF, can be acylated in yeast, and the reaction was attributed to a LPCAT [7]. When lyso-PAF was used as acyl acceptor, the *lca1*Δ strain had a rate of lyso-PAF acylation reduced to 31.1% of WT strain. Conversely, over-expression of *LCA1* resulted in 86.3-fold increase in lyso-PAF acyltransferase activity. It was established previously that high lyso-PAF level exerts toxic effect on yeast cells [23]. Consistent with in vitro results, LCA1 mediating lyso-PAF acylation was also evident in a plate assay (Fig. 4). In our study, both the parental strain and the *lca1*Δ were capable of tolerating LPC at a level up to 20 μg/ml, but the *lca1*Δ mutant displayed hypersensitivity to lyso-PAF at a concentration above 5 μg/ml. Moreover, its sensitivity to lyso-PAF was ameliorated by the expression of LCA1. In contrast, *slc1*Δ strain could survive and grew well on lyso-PAF plate without any apparent difference from WT cells, indicating *SLC1* disruption didn't affect lyso-PAF acylation (data not shown). This result thus further supported the notion that LCA1 is the major LPCAT in yeast.

3.4. Zn^{2+} inhibitory effect on LPCAT activity

The yeast LPCAT activity was shown to be particularly sensitive to the metal ion Zn^{2+} while insensitive to Mg^{2+} [7]. To further verify that LCA1 represents the dominant contributor to LPCAT in yeast, we examined whether the LPCAT activity of LCA1 was affected by addition of Zn^{2+} in in vitro assays. Indeed, we found that Zn^{2+} caused significant reduction of LPCAT activity of LCA1 in a range between 0.1 mM and 20 mM (Table 1). Our results also suggested that a lower (10-25 μM) concentration of Zn^{2+} enhanced LPCAT activity. The maximum increase was observed with 25 μM $ZnCl_2$. We did not detect significant effect of Mg^{2+} on LPCAT activity of LCA1, in a concentration ranging from 5 to 40 μM (data not shown).

3.5. Kinetic parameters of LCA1

The above experiments assessing enzyme properties allowed us to conclude that LCA1 represented a major LCPAT in yeast. We next explored the kinetic parameters of this enzyme. Kinetics constants based on Lineweaver-Burk double-reciprocal plot analysis showed that LCA1 had an apparent K_m for acyl-CoA at 0.89 ± 0.25 μM and a V_{max} of 524 pmol/min/μg protein.

LPC molecules are distinguished by fatty acid chain length. As shown in Fig. 5a, LCA1 exhibited a LPC substrate preference in the order of oleic (18:1)-LPC > stearic (18:0)-LPC > palmitic (16:0)-LPC. The fatty acid substrate specificity of the LCA1 was also assessed using acyl-CoA with chain lengths ranging from 14 to 22 carbons. Based on assays using 50 μM acyl-CoAs, LCA1 could use a broad range of acyl-CoAs (Fig. 5b), but it displayed particularly high activities with 16:0-CoA, 18:0-CoA and 18:1-CoA, regardless whether 18:1-LPC or 16:0-LPC was used as acyl acceptor. Interestingly, LCA1 could also efficiently mediate LPC acylation using very long chain fatty acyl-CoAs, such as 20:0-CoA and 22:6-CoA.

3.6. LCA1 is involved in PC turnover

Since LPCAT is a key component of the classic Lands cycle, we next studied PC turnover by following an established protocol [13]. In this experiment, we included a *slc1*Δ strain in the PC turnover analysis in order to differentiate the involvement of SLC1 and LCA1. The yeast cells were cultured and labeled in chemically defined synthetic medium containing [^{14}C] choline at 28 °C. Because higher growth temperature particularly accelerates the deacylation process [13], [^{14}C] choline was subse-

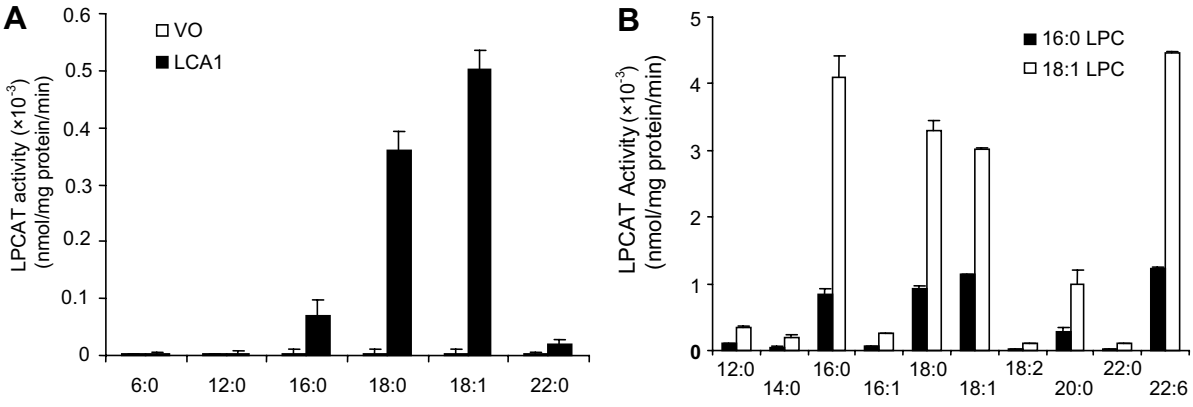

Fig. 5. Substrate preference assessment of LCA1. A. Acyl-CoA substrate preference. Assays were preformed with 3 μg microsomal protein from *lca1*Δ harboring an empty vector (VO) and *lca1*Δ expressing LCA1, with 112.5 μM [^{14}C] palmitoyl-LPC (1.35 nCi/nmol) and 50 μM acyl-CoA species. B. LPC substrate preference. Assays were preformed with 3 μg microsomal protein, 112.5 μM [^{14}C] palmitoyl-CoA (5.5 nCi/nmol), 50 μM lysophospholipid species. The values for *lca1*Δ were not shown. The results were presented as a mean of three assays.

Table 2
Phosphatidylcholine turnover in *lca1*Δ, *slc1*Δ and BY4741 (WT) strains

		28 °C			37 °C		
	Chase time (h):	0	1	2	0	1	2
*lca1*Δ	Medium	12.1 ± 1.7	16.2 ± 1.1	17.4 ± 2.7	12.1 ± 1.7	13.2 ± 3.5	13.3 ± 1.5
	Intracellular	41.1 ± 3.2	47.8 ± 2.8	53.6 ± 1.5	41.1 ± 3.2	67.9 ± 2.7	73.5 ± 2.5
	Membrane	46.8 ± 2.8	36.0 ± 2.3	28.9 ± 1.5	46.8 ± 2.8	18.9 ± 3.4	13.2 ± 2.3
*slc1*Δ	Medium	14.5 ± 1.1	16.1 ± 1.3	17.2 ± 1.6	14.5 ± 0.3	18.3 ± 2.3	19.7 ± 0.7
	Intracellular	37.4 ± 2.1	40.1 ± 2.2	42.1 ± 3.3	37.4 ± 1.5	49.1 ± 1.9	57.4 ± 4.1
	Membrane	48.1 ± 1.7	43.8 ± 2.6	40.7 ± 1.7	48.1 ± 0.9	32.6 ± 2.8	22.9 ± 1.3
WT	Medium	14.1 ± 0.8	16.1 ± 2.8	17.1 ± 3.1	14.1 ± 0.8	14.9 ± 1.5	15.3 ± 0.5
	Intracellular	36.5 ± 0.2	37.1 ± 1.6	41.6 ± 0.1	36.5 ± 0.2	55.9 ± 2.1	60.7 ± 4.3
	Membrane	49.4 ± 0.8	46.9 ± 1.2	41.3 ± 3.2	49.4 ± 0.8	29.2 ± 0.6	24.1 ± 2.7

Yeast cells were labeled at starting OD_{600} = 1.5 for 5 h in chemically defined synthetic medium containing 0.15 μCi/ml. The cells were then washed twice, cultured in medium containing 10 mM non-radioactive choline at 28 °C and 37 °C. One microliter culture aliquot was removed, separated into three fractions then scintillation counted. The data were presented as mean of three analyses.

quently chased by 10 mM exogenously added choline at 28 °C and 37 °C, respectively. The ^{14}C labels in the membrane fraction, intracellular non-membrane fraction, and in the medium were monitored at different time points (Table 2). There was no significant difference with regard to the dynamics of membrane-associated labels between *slc1*Δ and WT. Each lost about 8% at 28 °C, and 25% at 37 °C of labeling, in the membrane fraction over the course of 2 h. In contrast, the *lca1*Δ strain lost 18% at 28 °C, and 33% at 37 °C, over the same period of time. The label was rising in the intracellular, non-membrane fraction, which was suggested to be of mainly GroPC [13,24], a product of PC deacylation. In order to verify the identity of the metabolite present in the intracellular fraction, we separated the TCA fraction through TLC and found that only one [^{14}C]-choline band was present. The metabolite had the same migration rate as that of GroPC (Fig. 6), thereby confirming that it was indeed GroPC. Increased GroPC level at both 28 °C and 37 °C, suggested that the metabolic impact was independent of PC deacylation, therefore strongly suggesting that *lca1*Δ was compromised in the reacylation process of the Lands cycle. That *slc1*Δ had a similar PC turnover rate to that of the WT strain indicated that, although being a major *sn*-2 acyltransferase, SLC1 did not appear to play a significant role in PC turnover.

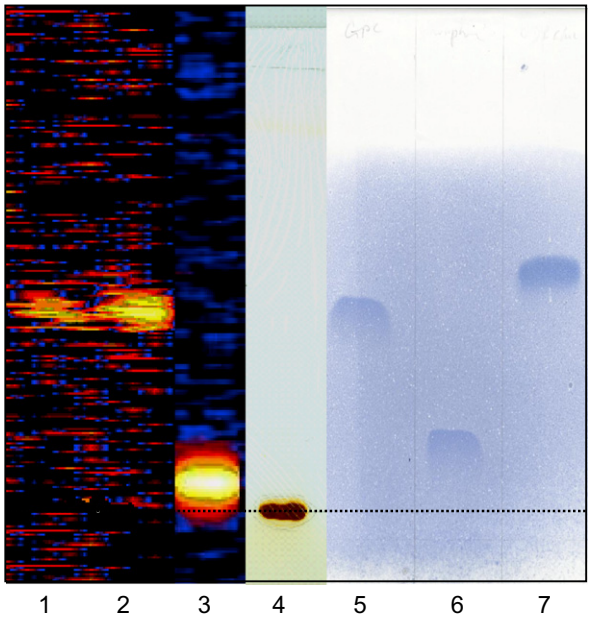

Fig. 6. TLC pattern of choline-containing compounds in the TCA fraction for PC turnover assessment. Samples were chromatographed and visualized on Merck Silica Gel G60 plate as described under Section 2. Lane 1, TCA fraction of WT yeast; lane 2, TCA fraction of *lca1*Δ yeast; lane 3, [^{14}C] choline; lane 4, palmitoleoyl-LPC; lane 5, GroPC; lane 6, phosphocholine; lane 7, CDP-choline. Dashed line indicated sample origin.

4. Conclusions

An earlier report on the interaction of *LCA1* and *SLC1* led us to investigate the *sn*-2 acyltransferase activity of LCA1. The present study provides several lines of evidence demonstrating that LCA1 is the hitherto unidentified LPCAT in yeast. Our PC turnover experiments reveal that without the participation of LCA1, the reacylation process of PC is severely crippled. These results are consistent with the role ascribed for LPCAT based on the concept of Lands cycle. We propose that LCA1 is a key enzyme involved in the PC turnover process in yeast.

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