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Metabolic link between phosphatidylethanolamine and triacylglycerol metabolism in the yeast *Saccharomyces cerevisiae*

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ABSTRACT

In the yeast *Saccharomyces cerevisiae* triacylglycerols (TAG) are synthesized by the acyl-CoA dependent acyltransferases Dga1p, Are1p, Are2p and the acyl-CoA independent phospholipid:diacylglycerol acyltransferase (PDAT) Lro1p which uses phosphatidylethanolamine (PE) as a preferred acyl donor. In the present study we investigated a possible link between TAG and PE metabolism by analyzing the contribution of the four different PE biosynthetic pathways to TAG formation, namely *de novo* PE synthesis *via* Psd1p and Psd2p, the CDP-ethanolamine (CDP-Etn) pathway and lyso-PE acylation by Ale1p. In cells grown on the non-fermentable carbon source lactate supplemented with 5 mM ethanolamine (Etn) the CDP-Etn pathway contributed most to the cellular TAG level, whereas mutations in the other pathways displayed only minor effects. In *cki1Δdpl1Δeki1Δ* mutants bearing defects in the CDP-Etn pathway both the cellular and the microsomal levels of PE were markedly decreased, whereas in other mutants of PE biosynthetic routes depletion of this aminoglycerophospholipid was less pronounced in microsomes. This observation is important because Lro1p similar to the enzymes of the CDP-Etn pathway is a component of the ER. We conclude from these results that in *cki1Δdpl1Δeki1Δ* insufficient supply of PE to the PDAT Lro1p was a major reason for the strongly reduced TAG level. Moreover, we found that Lro1p activity was markedly decreased in *cki1Δdpl1Δeki1Δ*, although transcription of *LRO1* was not affected. Our findings imply that (i) TAG and PE syntheses in the yeast are tightly linked; and (ii) TAG formation by the PDAT Lro1p strongly depends on PE synthesis through the CDP-Etn pathway. Moreover, it is very likely that local availability of PE in microsomes is crucial for TAG synthesis through the Lro1p reaction.

1. Introduction

Storage lipids in all types of eukaryotic cells accumulate mostly as triacylglycerols (TAG) and steryl esters (SE) which are the major components of a specialized globular compartment of the cell named lipid particle (LP), lipid droplet, lipid body or oil body [1]. LP consist of a randomly packed TAG core surrounded by several more or less ordered SE shells and a phospholipid surface monolayer with a small set of specific proteins embedded [1–3]. Mechanism(s) of LP biogenesis are still a matter of dispute [4], but the most convincing hypothesis for this process is budding of LP from the endoplasmic reticulum (ER) [2,5–7]. According to this model, TAG and SE formed in the ER accumulate in certain microdomains of the ER membrane. Upon further synthesis of neutral lipids, LP precursors are formed, which at a critical size bud off the ER and are released into the cytosol [2,4].

In *Saccharomyces cerevisiae* two proteins localized to the ER, Are1p and Are2p, catalyze the synthesis of SE with slightly different substrate specificity. Are2p is the major SE synthase of the yeast and prefers ergosterol as a substrate, whereas Are1p uses ergosterol and its precursors at nearly equal efficiency with a slight preference for lanosterol. Mutants lacking *ARE1* and *ARE2* are completely devoid of SE and accumulate free sterols [5,8]. In addition, Are1p and Are2p can also catalyze TAG synthesis although with minor efficiency compared to the two major yeast TAG synthesizing enzymes, Dga1p and Lro1p [8–12]. In *Saccharomyces cerevisiae* two primary mechanisms of TAG formation were identified, namely an acyl-CoA dependent reaction catalyzed by the diacylglycerol acyltransferase (DGAT) Dga1p, and an acyl-CoA independent pathway involving the phospholipid: diacylglycerol acyltransferase (PDAT) Lro1p [9–14]. Dga1p is dually localized to the ER and LP and appears to be more efficient than

Abbreviations: CF, cellular fraction; CL, cardiolipin; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; DMPE, dimethylphosphatidylethanolamine; ER, endoplasmic reticulum; Etn, ethanolamine; LP, lipid particle; LPL, lysophospholipid(s); MMGlu, minimal glucose media; MMLac, minimal lactate media; MAM, mitochondria associated membrane; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PDAT, phospholipid:diacylglycerol acyltransferase; RT-PCR, reverse transcription polymerase chain reaction; SE, steryl ester; TAG, triacylglycerol; TLC, thin-layer chromatography; YPD, complex glucose media; YPLac, complex lactate media

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Lro1p under standard growth conditions when cells are in the stationary phase [3,9–11]. In contrast, Lro1p which requires phospholipids as acyl donor is exclusively localized to the ER [12–15]. Lro1p preferentially uses phosphatidylethanolamine (PE) as co-substrate *in vitro* and transfers its *sn-2* acyl group to diacylglycerol (DAG), resulting in the formation of TAG and lyso-PE [13–15]. This reaction has no counterpart in mammalian cells.

The link between TAG synthesis and PE metabolism through Lro1p as described above led us to investigate metabolic interactions between biosynthesis/degradation of these two lipids in some more detail. In *Saccharomyces cerevisiae*, PE synthesis occurs by four different pathways. In brief, two of these pathways are accomplished by phosphatidylserine (PS) decarboxylases Psd1p and Psd2p which use PS as a substrate. While Psd1p is localized to mitochondria, Psd2p is a component of a Golgi/vacuolar compartment [16–21]. Yeast PE can also be synthesized through the cytidyldiphosphate ethanolamine (CDP-Etn) branch of the so-called Kennedy pathway using Etn and DAG as substrates [16,22,23]. The CDP-Etn pathway is also linked to sphingolipid metabolism through the action of the dihydrosphingosine phosphate lyase Dpl1p which sets Etn-P free [24,25]. Moreover, the lyso-PE acyltransferase Ale1p which is present in the mitochondria associated ER (MAM) catalyzes an alternative pathway to form PE [26,27]. Surprisingly, Tgl3p, the major yeast TAG lipase, can also act as a lyso-PE acyltransferase [28].

In a series of studies from our laboratory [29–33] we observed preferences in the incorporation of newly formed PE through the different pathways into different cellular compartments. These findings led us to speculate that also distinctions regarding the relative contributions of PE biosynthesis to TAG formation by Lro1p may exist between the different PE synthesizing routes. We used haploid single and multiple deletion strains bearing defects in PE biosynthesis, namely *psd1Δ*, *psd2Δ*, *psd1Δpsd2Δ*, *cki1Δdpl1Δeki1Δ* (CDP-Etn mutant) and *ale1Δ* to address this question. Yeast cells used for these experiments were grown on the non-fermentable carbon source lactate because under these conditions the level of PE in the cell becomes more critical for cell growth and viability than in glucose grown cells [31]. Here we report a clear metabolic link between TAG formation in the yeast and PE synthesis *via* the CDP-Etn branch of the Kennedy pathway, but not by other PE biosynthetic routes. In the light of these findings, the lipid metabolic network of PE and TAG metabolism in the yeast is discussed.

2. Materials and methods

2.1. Strains and culture conditions

Strains used throughout this study are listed in Table 1. Cells were cultivated aerobically in 2 l Erlenmeyer flasks to the stationary growth phase (A_{600} ~ 4) at 30 °C in minimal lactate medium consisting of 2.66% lactate (Roth), 0.67% yeast nitrogen base without amino acids (USBiological), 0.073% amino acid mix (Roth, Fluka) supplemented with 5 mM Etn (Merck) and adjusted to pH 5.5 with KOH. Main cultures were inoculated to an A_{600} of 0.1 from precultures grown aerobically for 48 h in YPD medium containing 1% yeast extract (Oxoid), 2% peptone (Oxoid) and 2% glucose (Merck) at 30 °C.

For synthesis of radioactively labeled [^{14}C]phospholipids, a *dga1Δlro1Δ* double deletion strain was grown on minimal glucose media containing 2% glucose (Merck), 0.67% yeast nitrogen base without amino acids (USBiological) and 0.073% amino acid mix (Roth, Fluka). Details of the labeling procedure will be described below.

2.2. Strain construction

Single-step deletion of chromosomal genes was carried out using the PCR-mediated technique described by Longtine et al. [34]. The marker module His3MX6 including the *Schizosaccharomyces pombe his5*$^{+}$ gene on the vector pFA6a was used to replace *YNL169c* encoding Psd1p and *YOR245c* encoding Dga1p in the single deletion strains *psd2Δ::KanMX4* and *lro1Δ::KanMX4*, respectively [34–36]. Deletion cassettes containing homologous regions to start and stop regions of either *PSD1* or *DGA1* and the entire *HIS5*$^{+}$ gene were constructed with primers listed in Table 2. A 1.4 kbp PCR fragment was generated with *ExTaq* DNA polymerase (Takara, Otsu, Japan) by using 150 ng of plasmid as a template in a standard PCR mixture containing PCR buffer (20 mM Mg^{2+}), 0.2 mM deoxynucleoside triphosphates, each, and a 1 μM solution of primers in a total volume of 100 μl. After a denaturation step of 2 min at 94 °C, fragments were amplified for 10 cycles of 15 s at 94 °C, 60 s at 55 °C, and 100 s at 72 °C; and for 25 cycles of 15 s at 94 °C, 90 s at 68 °C, and 60 s at 72 °C, followed by a final elongation step for 10 min at 72 °C.

Overnight cultures (A_{600} ~ 0.8) of single mutants *psd2Δ::KanMX4* and *lro1Δ::KanMX4* were used for transformation with the high-efficiency lithium acetate transformation method [37]. Transformants were grown on plates lacking histidine for 3 days at 30 °C. Plates used for cultivation of *psd1Δpsd2Δ* transformants contained in addition 5 mM Etn to permit growth. Large colonies were transferred to fresh plates for further selection. Clones yielding colonies were considered as positive transformants and further checked for correct integration of the respective deletion cassette. Verification of the correct replacement of *PSD1* and *DGA1* by the His3MX6 module was done by colony PCR. In brief, oligonucleotides were designed to bind outside the target locus and within the marker module [36,38]. Correct integration of the marker resulted in the appearance of the respective PCR fragment.

2.3. Isolation and characterization of subcellular fractions

Total cell-free homogenate (3000 × *g* supernatant) and 100,000 × *g* microsomes were prepared from cells grown to the stationary growth phase as described previously [21,39]. Proteins from isolated fractions were precipitated with trichloroacetic acid at a final concentration of 10%, the obtained protein pellet was solubilized in 0.1% SDS, 0.1 M NaOH, and proteins were quantified by the method of Lowry et al. [40] with bovine serum albumin as a standard. SDS-polyacrylamide gel electrophoresis was carried out by the method of Laemmli [41], and Western blot analysis by the method of Haid and Suissa [42].

Table 1
Yeast strains used in this study.

Strain	Genotype	Source/reference
Wild type	BY4741 *MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Euroscarf
psd1Δ	BY4741 *MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 psd1Δ::His3MX6*	This study
psd2Δ	BY4741 *MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 psd2Δ::KanMX4*	Euroscarf
ale1Δ	BY4741 *MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ale1Δ::KanMX4*	Euroscarf
lro1Δ	BY4741 *MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lro1Δ::KanMX4*	Euroscarf
dga1Δ	BY4741 *MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 dga1Δ::KanMX4*	Euroscarf
cki1Δdpl1Δeki1Δ	BY4741 *MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 cki1Δ::His3MX6 dpl1Δ::LEU2 eki1Δ::KanMX4*	This study
psd1Δpsd2Δ	BY4741 *MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 psd1Δ::His3MX6 psd2Δ::KanMX4*	This study
dga1Δlro1Δ	BY4741 *MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 dga1Δ::His3MX6 lro1Δ::KanMX4*	This study