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Triacylglycerol lipolysis is linked to sphingolipid and phospholipid metabolism of the yeast *Saccharomyces cerevisiae* ☆

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ABSTRACT

Previous work from our laboratory had demonstrated that deletion of *TGL3* encoding the major yeast triacylglycerol (TAG) lipase resulted in decreased mobilization of TAG, a sporulation defect and a changed pattern of fatty acids, especially increased amounts of C22:0 and C26:0 very long chain fatty acids in the TAG fraction [K. Athenstaedt and G. Daum, J. Biol. Chem. 278 (2003) 23317–23323]. To study a possible link between TAG lipolysis and membrane lipid biosynthesis, we carried out metabolic labeling experiments with wild type and deletion strains bearing defects in the three major yeast TAG lipases, Tgl3p, Tgl4p and Tgl5p. Using [^{3}H]inositol, [^{32}P]orthophosphate, [^{3}H]palmitate and [^{14}C]acetate as precursors for complex lipids we demonstrated that *tgl* mutants had a lower level of sphingolipids and glycerophospholipids than wild type. ESI-MS/MS analyses confirmed that TAG accumulation in these mutant cells resulted in reduced amounts of phospholipids and sphingolipids. *In vitro* and *in vivo* experiments revealed that TAG lipolysis markedly affected the metabolic flux of long chain fatty acids and very long chain fatty acids required for sphingolipid and glycerophospholipid synthesis. Activity and expression level of fatty acid elongases, Elo1p and Elo2p were enhanced as a consequence of reduced TAG lipolysis. Finally, the pattern of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine molecular species was altered in *tgl* deletion strain underlining the important role of TAG turnover in maintaining the pool size of these compounds and the remodeling of complex membrane lipids.

1. Introduction

Triacylglycerols (TAG) are a highly condensed form of metabolic energy because of the large number of reduced CH groups available for oxidation dependent energy generation processes, but also a reserve of essential fatty acids [1,2]. In addition, TAG provides diacylglycerols (DAG) and fatty acids for membrane lipid biosynthesis. Moreover, TAG depots may serve as a defensive measure to neutralize excess of biologically active and potentially harmful lipids such as DAG and coenzyme A esters of fatty acids [1,3].

TAG is stored in a unique organelle called lipid particle (LP), lipid droplet, fat globule, oil body or adiposome. These particles contain a core of non-polar lipids surrounded by a phospholipid monolayer [4,5]. Yeast LP have a size of 300 to 500 nm in diameter, contain TAG and steryl esters (SE) at equal amounts in the core and small amounts of phospholipids and proteins in the surface monolayer membrane. SE form several ordered shells beneath the surface phospholipid monolayer of LP, whereas TAG are more or less randomly packed in the center of the LP [6]. For many decades, TAG has been deemed only as a cellular storage molecule, but recent studies focusing on the relevance of TAG catabolism for cell regulation showed that this non-polar storage lipid is more than just an inert depot [1,7].

In the yeast, TAG hydrolysis is primarily catalyzed by three different lipases encoded by *TGL3*, *TGL4* and *TGL5* [8–10]. All three corresponding gene products are localized to LP. Among these three lipases, Tgl3p was found to have the highest TAG hydrolytic activity with broad specificity towards TAG containing acyl chains of various lengths [8]. Most interestingly, *TGL3* deletion altered the fatty acid pattern of stored TAG and led to an enhanced concentration of short chain fatty acids and a slightly increased level of C22 and C26 species. Besides these effects, *tgl3Δ* showed high sensitivity to cerulenin and exhibited a sporulation defect. On the other hand, *tgl4Δ* accounted for a 1.7-fold increased TAG level, enriched amounts of myristic acid and

Abbreviations: TAG, triacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid; VLCFA, very long chain fatty acid; LCFA, long chain fatty acid; LP, lipid particle/droplet; SE, steryl ester; $M(IP)_2C$, mannosyldiinositolphosphorylceramide; MIPCs, mannosyl-IPCs; IPCs, inositolphosphoceramides

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palmitic acid in the TAG fraction and a reduced mobilization of TAG in the presence of cerulenin. In contrast, *TGL5* deletion neither affected the total amount of TAG nor its mobilization rate. In the *tgl4Δ tgl5Δ* double mutant, however, impaired TAG hydrolysis was observed. Moreover, deletion of these two genes displayed a marked accumulation of very long chain fatty acids (VLCFAs) in stored TAG [9].

In the yeast, long chain fatty acids (LCFAs) and VLCFAs are required for *N*-acylation reactions producing ceramides [11]. VLCFAs are constituents of sphingolipids, but also of inositolglycerophospholipids and the phosphatidylinositol moiety of GPI anchors. All these molecules are important components of lipid rafts and other detergent-insoluble lipid microdomains [12,13]. In addition, VLCFAs are involved in the targeting of proteins, e.g., the plasma membrane ATPase Pma1p to the cell periphery [14]. Finally, various studies indicated that VLCFAs strongly influence structure, function and fluidity of biological membranes.

Accumulation of VLCFAs in stored TAG of *tgl* deletion mutants led us to investigate a possible link of TAG hydrolysis with sphingolipid metabolism. Therefore, we analyzed the involvement and the physiological relevance of each individual TAG lipase in sphingolipid, but also in glycerophospholipid biosynthesis. Most interestingly, metabolic labeling studies revealed that *tgl* mutations indeed affected the biosynthesis of most of these membrane lipids. We show that fatty acid channeling but also fatty acid elongation is altered in *tgl* mutants. These results demonstrate that TAG lipolysis is not an isolated process in lipid metabolism but directly linked to the biosynthesis of complex membrane lipids in *Saccharomyces cerevisiae*.

2. Materials and methods

2.1. Strains and culture conditions

Yeast strains used throughout this study are listed in Table 1. Yeast strains were grown on YPD (1% yeast extract, 2% bacto-peptone, 2% glucose) or synthetic minimal media (SM) containing 0.67% Yeast Nitrogen Base (Difco) supplemented with the appropriate amino acids. All experiments were conducted using cultures grown on a rotary shaker at 200 rpm to an A_{600} of 0.8–1 at 30 °C unless otherwise specified.

2.2. Preparation of cytosolic and membrane fractions from S. cerevisiae

Yeast cells were grown in YPD for 24 h and harvested by low speed centrifugation. Cells were suspended in 50 mM Tris/HCl buffer, pH 7.4, 0.3 M sucrose and 1 mM 2-mercaptoethanol containing the protease inhibitors 0.1 mM phenylmethylsulfonyl fluoride and 1 μg/ml leupeptin. Cells were disrupted using glass beads, and unbroken cells and debris were removed by centrifugation at 3000×*g* for 10 min. The cell-free extract was centrifuged at 100,000×*g* for 90 min to obtain cytosolic and total membrane fractions. The membrane fraction was suspended in the lysis buffer. The protein concentration was measured by the method of Lowry et al. [15] using bovine serum albumin as a standard.

Table 1
Strains used in this study.

Strain	Genotype	Source
BY4741 wild-type	*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	Euroscarf collection, Frankfurt, Germany
tgl3Δ	BY4741,*Mat a; his3Δ1; leu2Δ0; met15Δ0;ura3Δ0; YMR313c*::*kanMX4*	Euroscarf collection, Frankfurt, Germany
tgl4Δ	BY4741,*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YKR089c*::*kanMX4*	Euroscarf collection, Frankfurt, Germany
tgl5Δ	BY4741,*Mat a; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YOR081c*::*kanMX4*	Euroscarf collection, Frankfurt, Germany
tgl3Δ tgl4Δ tgl5Δ	*his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; YMR313c*::*kanMX4; YKR089c*::*kanMX4; YOR081c*::*kanMX4*	Athenstaedt, K. and Daum, G. (2005)

2.3. Metabolic labeling of phospholipids, neutral lipids and sphingolipids

To analyze the steady-state phospholipid composition, cells were grown at 30 °C for eight generations in the presence of 50 μCi/ml [^{32}P]orthophosphate (3,000 Ci/mmol) or 2 μCi/ml [^{14}C]palmitate (50 mCi/mmol), respectively, and the uptake of the label was determined as described previously [16]. Labeling for longer time periods did not alter the distribution of the label among the various lipid species (data not shown) indicating that a steady-state labeling had been reached. For pulse-chase labeling of phospholipids and sphingolipids cells were cultivated at the A_{600} of 0.8–1. Then, cells were pulsed with 50 μCi/ml [^{32}P]orthophosphate or 2 μCi/ml [^{14}C]palmitate for 20 min followed by a 60 min chase in the presence of 2 mM cold phosphate or palmitate [17], respectively. For lipid analysis, 5×10^7 cells per time point were harvested by centrifugation at 1000×g for 5 min, washed once with sterile water and once with PBS (phosphate-buffered saline). Then, chloroform/methanol/2% phosphoric acid (1:2:2, per vol.) was added for lipid extraction [18]. Lipids were subjected to one-dimensional thin-layer chromatography (TLC) using Silica gel 60F_{254} plates (Merck) with chloroform/methanol/acetone/acetic acid/water (50:10:20:15:5, per vol.) as the solvent system. Lipids were identified by their relative migration compared to standards. Radioactively labeled lipids were analyzed by autoradiography and quantified by liquid scintillation counting (Packard, Tri-Carb 2900TR) using LSC Safety (Baker, Deventer, The Netherlands) as scintillation cocktail.

For the analysis of complex sphingolipids, [^{3}H]myo-inositol labeling was performed as described previously [19]. In brief, cells were pulse labeled with 15 μCi [^{3}H]myo-inositol (18.5 Ci/mmol) for 20 min followed by a 60 min chase initiated by adding unlabeled inositol [20]. Per time point 2.5×10^7 cells were harvested by centrifugation and washed with 4 mM sodium azide. Lipids were extracted as described by Hanson and Lester [21]. Pooled extracts were dried under a stream of nitrogen, alkali treated and desalted with water saturated butanol as described previously [22–24]. Samples were analyzed by TLC using chloroform/methanol/0.25% KCl (55:45:10, per vol.) as solvent system.

For pulse labeling of ceramides, yeast cultures were incubated with 5 μCi/ml [^{3}H]dihydrosphingosine (60 Ci/mmol) for 20 min [25]. For ceramide analysis, lipids were extracted using ethanol/diethyl ether/pyridine/4.2 N ammonium hydroxide (15:5:1:0.018; per vol.), dried under a stream of nitrogen, subjected to mild-base treatment and then desalted by butanol/water partitioning. The extracted ceramides were separated by TLC using chloroform/methanol/4.2 N ammonium hydroxide (9:7:2; per vol.) as solvent system [25].

2.4. Electrospray ionization mass spectrometry (ESI-MS/MS)

Yeast cells were grown in synthetic complete medium until A_{600} reached 1.0–2.0, harvested by centrifugation and washed with sterile water. Equal amounts of protein of the whole cell lysates from different strains were taken for lipid extraction. Then, lipids were extracted as described by Ejsing et al. [26]. The chloroform soluble fraction was dried, alkali treated and desalted with water saturated butanol as described previously [27]. Samples were suspended in HPLC grade methanol along with internal standards of IPC, MIPC and M(IP)$_2$C isolated from *scs7*Δ strains and commercially available standards for phospholipids. These samples were subjected to ESI-MS/MS (Bruker Esquire 3000 Plus electrospray ion trap instrument; Bremen, Germany) as described previously [26,28,29]. Lipid samples (10 μl) were directly applied to the ESI source through a polytetrafluoroethylene line at a rate of 4 μl/min. ESI-MS/MS settings were as follows. The turboelectrospray ionization source was maintained at