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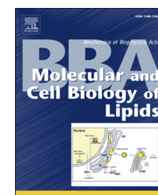

Defects in triacylglycerol lipolysis affect synthesis of triacylglycerols and steryl esters in the yeast

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ABSTRACT

Tgl3p, Tgl4p and Tgl5p are the major triacylglycerol lipases of the yeast *Saccharomyces cerevisiae* catalyzing degradation of triacylglycerols stored in lipid droplets. Previous results from our laboratory (Athenstaedt and Daum, 2005, *J. Biol. Chem.* 280, 37301–37309) demonstrated that a yeast strain lacking all three triacylglycerol lipases accumulates not only triacylglycerols at high amount, but also steryl esters. Here we show a metabolic link between synthesis and mobilization of non-polar lipids. In particular, we demonstrate that a block in triacylglycerol degradation in a *tgl3*Δ*tgl4*Δ*tgl5*Δ triple mutant lacking all major triacylglycerol lipases causes marked changes in non-polar lipid synthesis. Under these conditions formation of triacylglycerols is reduced, whereas steryl ester synthesis is enhanced as shown by quantification of non-polar lipids, *in vivo* labeling of lipids using [^{14}C]oleic acid and [^{14}C]acetic acid as precursors, and enzyme analyses *in vitro*. In summary, this study demonstrates that triacylglycerol metabolism and steryl ester metabolism are linked processes. The importance of balanced storage and degradation of these components for lipid homeostasis in the yeast is highlighted.
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1. Introduction

The major non-polar lipids triacylglycerols (TGs) and steryl esters (SEs) are depot forms of metabolic energy. In the yeast *Saccharomyces cerevisiae*, however, fatty acids and sterols stored in TG and SE, respectively, are mainly used as building blocks for membrane biosynthesis [1,2]. Furthermore, formation of non-polar lipids "neutralizes" a possible toxic effect of free fatty acids and sterols. TG and SE form the hydrophobic core of a unique organelle called lipid droplet (LD), lipid particle, lipid body, oil body or oleosome [1–3]. In *S. cerevisiae* the core of LD is formed from TG and SE at similar amounts [4]. This non-polar lipid core is enwrapped by a phospholipid monolayer with a small amount and number of proteins embedded [5,6]. During the last decade the protein equipment of LD has been well studied, and LD proteins were characterized in some detail [6,7]. Most of the LD proteins are involved in lipid metabolism, although other cellular functions were also attributed to LD such as storage and degradation of protein aggregates or proteins which are incorrectly folded [8].

Non-polar lipid synthesizing enzymes have been studied in detail not only in *S. cerevisiae* but also in other yeasts [9–12]. The four acyltransferases Dga1p, Lro1p, Are1p and Are2p contribute to TG and SE synthesis in *S. cerevisiae* [13]. Dga1p is the major TG synthase under standard growth condition in the stationary growth phase [9,14]. This acyl-CoA:diacylglycerol acyltransferase requires an activated fatty acid (acyl-CoA) for the esterification of diacylglycerols and shows a dual localization to the endoplasmic reticulum (ER) and LD. The highest specific Dga1p acyltransferase activity *in vitro* was measured in LD [15]. The second TG synthase is encoded by *LRO1* (lecithin:cholesterol acyltransferase related open reading frame) [16,17]. This phospholipid:diacylglycerol acyltransferase is located to the ER and catalyzes an acyl-CoA independent diacylglycerol acyltransferase reaction. Lro1p requires a phospholipid, preferentially phosphatidylethanolamine or phosphatidylcholine, as acyl-donor for TG synthesis. In contrast to Dga1p, Lro1p seems to be the major TG synthase in the logarithmic growth phase of yeast cells [14].

The two acyl-CoA:sterol acyltransferases Are1p and Are2p which are localized to the ER [18,19] are members of the membrane-bound *O*-acyltransferase family [20,21]. Whereas Are2p prefers ergosterol as a substrate, Are1p uses ergosterol and ergosterol precursors, mainly lanosterol as substrates. *In vitro* Are2p was verified as the major SE

Abbreviations: BSA, bovine serum albumin; E, ergosterol; ER, endoplasmic reticulum; LD, lipid droplet; PL, phospholipids; SE, steryl ester; TG, triacylglycerol; TLC, thin layer chromatography; TM, triple mutant

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synthase accounting for 65–75% of total acyl-CoA:sterol acyltransferase activity [22]. Additionally, the two SE synthases show minor acyl-CoA: diacylglycerol acyltransferase activity and thus may contribute to TG synthesis, although with minor efficiency [23].

Tgl3p, Tgl4p and Tgl5p are the major TG lipases of the yeast *S. cerevisiae* and responsible for most of the TG degradation from LD [24–26]. Tgl3p was shown to be the most potent TG lipase with a broad specificity to various TG species with different acyl chains [25]. Deletion of *TGL3* resulted in a markedly increased TG level compared to wild type and an altered fatty acid composition of TG. Myristic acid and palmitic acid were slightly increased in a *tgl3*Δ strain, whereas stearic acid and oleic acid were decreased. Furthermore, the long chain fatty acid hexacosanoic acid was enriched in TG from this mutant. A single deletion of *TGL4* resulted in a moderate increase of total TG compared to wild type whereas a single deletion of *TGL5* did not change the overall TG content at all. Interestingly, Tgl3p, Tgl4p and Tgl5p do not only act as TG lipases but also exhibit lysophospholipid acyltransferase activities as shown by recent studies from our laboratory [27–30].

Little is known about regulation of non-polar lipid metabolism. It was speculated that synthesis and mobilization of these components are balanced because a *tgl3*Δ*tgl4*Δ*tgl5*Δ triple mutant (TM) lacking the major TG lipases did not only accumulate TG at high amount (~2.4-fold increased over wild type), but also SE (~1.4-fold increased over wild type) [25]. These findings led us to investigate the metabolic link between synthesis and degradation of non-polar lipids in the yeast in more detail. In the present study, we demonstrate that TG metabolism and SE metabolism are indeed linked processes. These results were obtained by non-polar lipid analysis, *in vivo* labeling of lipids using [^{14}C]oleic acid and [^{14}C]acetic acids as lipid precursors, and enzyme analyses *in vitro*. The importance of these findings for balanced formation and degradation of non-polar lipids and the overall lipid homeostasis in the yeast will be discussed.

2. Material and methods

2.1. Strains and culture conditions

Strains used throughout this study are listed in Table 1. Cells were cultivated aerobically to either the logarithmic or the stationary growth phase in YPD media containing 1% yeast extract, 2% glucose and 2% peptone at 30 °C. Yeast strains bearing plasmids were cultivated in synthetic minimal medium containing 0.67% yeast nitrogen base (U.S. Biochemical Corp.), 2% glucose, and the respective amino acid supplements. Gal1 promoter-controlled genes were induced by growing cells in synthetic minimal medium containing 2% galactose as a carbon source. Media supplemented with oleic acid (YPO) contained 0.3% yeast extract, 0.5% peptone, 0.5% KH_2PO_4, and 0.1% oleic acid. For solubilizing oleic acid, 0.2% Tween 80 was added to the medium. Growth was monitored by measuring absorbance at 600 nm (A_{600}).

Table 1
Yeast strains used in this study.

Strain	Genotype	Source
Wild type (WT)	BY4741 *Mat a*; *his3*Δ*1*; *leu2*Δ*0*; *met15*Δ*0*; *ura3*Δ*0*	Euroscarf
*tgl3*Δ*tgl4*Δ*tgl5*Δ (TM)	BY4741; *tgl3*Δ::*kanMX4*; *tgl4*Δ::*kanMX4*; *tgl5*Δ::*kanMX4*	[25]
*tgl3*Δ*tgl4*Δ*tgl5*Δ*dga1*Δ	See TM; *dga1*Δ::*URA3KL*	This study
*tgl3*Δ*tgl4*Δ*tgl5*Δ*lro1*Δ	See TM; *lro1*Δ::*URA3KL*	This study
Dga1p-HA	See WT; *DGA1-HA*::*URA3KL*	This study
*tgl3*Δ*tgl4*Δ*tgl5*Δ Dga1p-HA	See TM; *DGA1-HA*::*URA3KL*	This study
Lro1p-Myc	See WT; *LRO1-13Myc*::*HIS3MX6*	This study
*tgl3*Δ*tgl4*Δ*tgl5*Δ Lro1p-Myc	See TM; *LRO1-13Myc*::*HIS3MX6*	This study
Are1p-Myc	See WT; *ARE1-13Myc*::*HIS3MX6*	This study
*tgl3*Δ*tgl4*Δ*tgl5*Δ Are1p-Myc	See TM; *ARE1-13Myc*::*HIS3MX6*	This study
Are2p-Myc	See WT; *ARE2-13Myc*::*HIS3MX6*	This study
*tgl3*Δ*tgl4*Δ*tgl5*Δ Are2p-Myc	See TM; *ARE2-13Myc*::*HIS3MX6*	This study

2.2. Genetic techniques

Single step chromosomal tagging and deletion of genes were performed by homologous recombination as described by Longtine et al. [31]. Inserts for the construction of Myc- or HA-tagged genes were obtained by PCR from plasmid pFA6a-13Myc-*HIS3MX6* or plasmid pFA6a-*URA3KL* from *Kluyveromyces lactis*. Primers used for amplification of the respective DNA-fragments are listed in Table 2. For transformation of yeast strains, 300–600 ng DNA was used according to the high-efficiency lithium acetate transformation protocol [32]. Transformants were incubated on minimal medium lacking the respective amino acid for 3 days at 30 °C. Positive transformants were verified for correct integration of the fusion cassette by colony-PCR of whole yeast cell extracts. For the construction of the pYES2-*DGA1* and pYES2-*ARE2* plasmids, the open reading frames of *DGA1* and *ARE2*, respectively, were amplified using genomic DNA from *S. cerevisiae* wild type as a template. Prior to ligation, the insert and the vector were cleaved by *BamH*I and *EcoR*I. Primers used for PCR are listed in Table 2.

2.3. Isolation and characterization of subcellular fractions

Yeast LD and microsomal fractions (30,000 ×g) were isolated from cells grown to the stationary growth phase as described by Leber et al. [4] and Zinser et al. [33,34]. The quality of subcellular fractions was routinely tested by Western blot analysis.

2.4. Protein analysis

Proteins of isolated fractions were precipitated with trichloroacetic acid at a final concentration of 10% and solubilized in 100 μL 0.1% SDS, 0.1% NaOH. Samples of LD were delipidated prior to protein quantification. Non-polar lipids were extracted with 2–3 volumes of diethyl ether, the organic phase was withdrawn, and the sample was dried under a stream of nitrogen. Proteins were quantified by the method of Lowry et al. [35] using bovine serum albumin (BSA) as a standard. SDS-PAGE was carried out as described by Laemmli [36] using 12.5% separation

Table 2
Primers used throughout this study.

Primer	Sequence (5′ → 3′)
Dga1HAfwd	TACGAAAATAGAGAAAAATATGGGGTACCGGATGCAGAATTGAAGATAGTTGGGTACCCATACGATGTTCCTGACTATGCGTAACGGATCCCCGGGTTAATTAA
Dga1HArev	TAAAAAATCCTTATTTATTCTAACATATTTTGTGTTTTCCAATGAATTCATTATTAATCGATGAATTCGAGCTCG
Lro1Mycfwd	GCCAATTGTCTAATTTGAGCCAGTGGGTTTCTCAGATGCCCTTCCCAATGCGGATCCCCGGGTTAATTAA
Lro1Mycrev	TCTTTTCGCTCTTTGAAATAATACACGGATGGATAGTGAGTCAATGTCGGTCATTTAATCGATGAATTCGAGCTCG
Are1Mycfwd	TTGGTGTCTGTTCAGGGCCCAGTATCATTATGACGTTGTACCTGACCTTACGGATCCCCGGGTTAATTAA
Are1Mycrev	TTGTATATCTATCAAGGGCTTGCGAGGGACACACGTGGTATGGTGGCAGTATCGATGAATTCGAGCTCG
Are2Mycfwd	TCGGTATCTGCATGGGACCAAGTGTCATGTGTACGTTGTACTTGACATTCCGGATCCCCGGGTTAATTAA
Are2Mycrev	AAAATTTACTATAAAGATTTAATAGCTCCACAGAACAGTTGCAGGATGCCATCGATGAATTCGAGCTCG
Dga1delfwd	TACATATACATAAGGAAACGCAGAGGCATACAGTTTGAACAGTCACATAAATGCGTACGCTGCAGGTCGAC
Dga1delrev	TAAAAAATCCTTATTTATTCTAACATATTTTGTGTTTTCCAATGAATTCATTATTAATCGATGAATTCGAGCTCG
Lro1delfwd	AGCCATTACAAAAGGTTCTCTACCAACGAATTCGGCGACAATCGAGTAAAAAATGCGTACGCTGCAGGTCGAC
Lro1delrev	TCTTTTCGCTCTTTGAAATAATACACGGATGGATAGTGAGTCAATGTCGGTCATTTAATCGATGAATTCGAGCTCG
Dga1Bamfwd	TATAGGATCCATGTCAGGAACATTCAATGATATAA
Dga1Ecorev	TATAGAATTCTTACCCAACTATCTTCAATTCTG
Are2Bamfwd	TATAGGATCCATGGACAAGAAGAAGGATCTACT
Are2Ecorev	TATAGAATTCTTAGAATGTCAAGTACAACGTACA

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