YMR313c/TGL3 Encodes a Novel Triacylglycerol Lipase Located in Lipid Particles of Saccharomyces cerevisiae*

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Previous work from our laboratory (Athenstaedt, K., Zweytick, D., Jandrosoz, A., Kohlwein, S. D., and Daum, G. (1999) J. Bacteriol. 181, 6441–6448) showed that the gene product of YMR313c (named Tgl3p) is a component of yeast lipid particles, and deletion of this gene led to an increase in the cellular level of triacylglycerols (TAG). These observations suggested that TGL3 may encode a TAG lipase of Saccharomyces cerevisiae. Here we demonstrate by cell fractionation and by microscopic inspection of a strain bearing a Tgl3p-GFP hybrid that this polypeptide is highly enriched in the lipid particle fraction but virtually absent from other organelles. The entire TAG lipase activity of lipid particles is attributed to Tgl3p, because the activity in this organelle is completely absent in a Δtgl3 deletion mutant, whereas it is significantly enhanced in a strain overexpressing Tgl3p. A His6-tagged Tgl3p hybrid purified close to homogeneity from a yeast strain overexpressing this fusion protein exhibited high TAG lipase activity. Most importantly, experiments in vivo using the fatty acid synthesis inhibitor cerulenin demonstrated that deletion of TGL3 resulted in a decreased mobilization of TAG from lipid particles. The amino acid sequence deduced from the open reading frame YMR313c contains the consensus sequence motif GXSGXG typical for lipolytic enzymes. Otherwise, Tgl3p has no significant sequence homology to other lipases identified so far. In summary, our data identified Tgl3p as a novel yeast TAG lipase at the molecular level and by function in vivo and in vitro.

All eukaryotic organisms and also some prokaryotes of the Gram-positive genera store triacylglycerols (TAG) in intracellular lipid particles that are also named lipid bodies and lipid droplets or oil bodies, oleosomes, and spherosomes in plants. The structure of this lipid-rich compartment is similar in all cell types and is rather simple; a hydrophobic core, mainly formed of TAG and/or steryl esters (STE), is surrounded by a phospholipid monolayer in which only few proteins are embedded. Lipid particles serve as an energy source and/or as a source of fatty acids and sterols needed for membrane biogenesis, but they are also involved in the formation of specific lipophilic components, e.g. steroid hormones or prostaglandins (2–5). Utilization of TAG and STE from lipid particles requires the action of TAG lipase(s) and STE hydrolase(s). Some proteins associated with the phospholipid monolayer of lipid particles, such as mammalian perilipins or oleosins of plants, were assumed to be involved in the mobilization of the neutral lipid core of the particle by serving as a docking and/or activating protein for TAG lipases and STE hydrolases. Alternatively, such proteins were assumed to protect the stored lipids from random degradation (6–8).

Lipases are a family of enzymes with similarities in structure and function (9, 10). In particular, the active site of these proteins with a catalytic triad, which is opened or closed by a lid, has been unveiled. Some lipases exhibit high substrate specificity and regioselectivity. The subcellular localization of these enzymes varies. Lehner et al. (11) demonstrated the presence of a TAG lipase on cytosolic lipid droplets from pig liver. In contrast, Spalinger et al. (12) reported lipase activities in the cytosolic fraction of Caco2 cells but also in the apical brush border membrane and other organelles. Lipases are assumed to interact with proteins located on the lipid particle surface prior to mobilization of TAG. As an example, interaction of the cytosolic hormone-sensitive lipase with perilipins, the most abundant proteins of mammalian lipid particles, was demonstrated (6). Both hormone-sensitive lipase and perilipins are phosphorylated at the same time, and it was suggested that only phosphorylated perilipin can serve as the docking protein for the lipase.

In plants, many TAG lipases were isolated and characterized, although their subcellular localization has not been well studied. By using an antibody against animal lipases, Belguith et al. (13) detected lipases of rapeseed in different subcellular fractions isolated by differential centrifugation. During germination of maize kernel, lipases synthesized on free polyribosomes are specifically bound to oil bodies (7). Oleosins, the major proteins of oil bodies, appear to act as receptors for binding and/or activation of such lipases and to maximize the surface area of oil bodies, thus accelerating the mobilization of the storage oil from the hydrophobic core by lipases (14).

TAG lipases of the yeast Saccharomyces cerevisiae are poorly characterized. Schousboe (15, 16) described a TAG lipase activity in the yeast mitochondrial fraction, but the polypeptide catalyzing this reaction was not identified. In the course of the yeast genome sequencing project, several putative yeast lipases were detected by homology to such enzymes from other organisms. Three polypeptides encoded by YDL109c, YGL144c, and YDR444w contain lipase-active site motifs (www.proteome.com), and a mutant deleted of YDL109c exhibits increased TAG levels (17). None of these open reading frames, however, was demonstrated by enzymatic analysis of the gene product to encode a TAG lipase. In addition, two proteins of unknown function named Tgl1p and Tgl2p were proposed to be yeast TAG lipases based on their homology to rat, human, and...
Pseudomonas lipases (18, 19). More recently, Cvt17p, which contains a motif conserved in esterases and lipases (20), was proposed to be a lipase involved in degradation of autophagic vesicles in the vacuole. TAG lipase activity, however, was neither demonstrated for Tgl1p nor for Tgl2p and Cvt17p.

Analysis of the most abundant yeast lipid particle proteins by mass spectrometry led to the identification of several polypeptides with unknown function (21). Because most lipid particle proteins characterized by function are directly involved in lipid metabolism, it was tempting to speculate that some of the novel lipid particle proteins may also play such roles. Computational analysis of yeast lipid particle proteins revealed that some of these polypeptides contain a lipase motif, namely gene products of TGL1, YOR059c, and YJU3. None of all lipid particle proteins identified so far contained obvious sequence homology to perilipins or oleosins. Systematic analysis of deletion mutants lacking lipid particle proteins showed that TAG levels were similar to wild-type in Δtgl1, Δyor059c, and Δyju3, but significantly increased in a strain lacking YMR313c (21). This observation led us to speculate that YMR313c may encode a protein involved in TAG mobilization from lipid particles. In the present study we provide evidence that the gene product of YMR313c is indeed a TAG lipase. This assumption is based on results obtained from in vitro and in vivo experiments performed with wild-type, a Δymr313c deletion mutant, and strains overexpressing the respective gene product.

### EXPERIMENTAL PROCEDURES

**Strains and Culture Conditions**—The haploid wild-type yeast strain *S. cerevisiae* FT1679 (MATa, ura3-52, trp1Δ63, leu2Δ21, his3Δ200) containing either the empty plasmid pYES2 (Invitrogen) or pYES2 with YMR313c inserted into the multiple cloning site, the Δgl3 mutant deleted of the open reading frame YMR313c (MATa, ura3-52, trp1Δ63, leu2Δ21, his3Δ200, Δgl3::kanMX4) (21) bearing the empty plasmid pYES2, and the wild-type strain FY1679 containing the hybrid protein of Tgl3p with the green fluorescent protein (GFP) or His6, respectively, were used throughout this study.

For the overexpression of Tgl3p, the YMR313c gene was inserted into the plasmid pYES2. The open reading frame was amplified by PCR with genomic DNA derived from a wild-type yeast strain as template using the plasmid pYES2. The open reading frame was amplified by PCR with yeast (23).

*Yeast Nitrogen Base (U. S. Biochemical Corp.), 2% galactose (Merck), and the respective amino acid supplements. Five hundred milliliters of culture medium were inoculated with 1 ml of a preculture grown aerobically for 48 h in minimal medium containing 2% glucose (Merck) as the carbon source. Growth was monitored by measuring the absorbance (A) at 600 nm.

**Isolation and Characterization of Subcellular Fractions**—Lipid particles were isolated at high purity from yeast cells grown to the early stationary phase at 30 °C in minimal medium containing 0.67% Yeast Nitrogen Base (U. S. Biochemical Corp.), 2% galactose (Merck), and the respective amino acid supplements. Five hundred milliliters of culture medium were inoculated with 1 ml of a preculture grown aerobically for 48 h in minimal medium containing 2% glucose (Merck) as the carbon source. Growth was monitored by measuring the absorbance (A) at 600 nm.

**Protein Analysis**—Protein was quantified by the method of Lowry et al. (30) using bovine serum albumin as a standard. Proteins were precipitated with 10% trichloroacetic acid and then solubilized in 0.1% SDS, 0.1% NaOH. Prior to protein analysis samples of the lipid particle fraction were delipidated. Non-polar lipids were extracted with 3 volumes of diethyl ether; the organic phase was withdrawn; residual solvent was removed under a stream of nitrogen; and proteins were precipitated from the aqueous phase as described above.

**SDS-PAGE**—电泳 was carried out by the method of Laemmli (31). Samples were dissociated at 37 °C to avoid hydrolysis of polypeptides that may occur at higher temperature. Western blot analysis was performed as described by Haid and Sussia (32). Immunoreactive proteins were detected by enzyme-linked immunosorbent assay using rabbit or mouse antisera as the first antibody and goat anti-rabbit or goat anti-mouse IgG, respectively, linked to peroxidase as the second antibody.

**Purification of Tgl3p-His6 of Lipid Particles by Column Chromatography**—For solubilization of lipid particle proteins, Zwittergent 3-14 (SB14) was added to lipid particles at a final concentration of 0.25 mm, and proteins were solubilized for 30 min on ice. The sample was centrifuged for 15 min at 15,000 × g at 4 °C, and solubilized proteins were separated from the floating lipid layer. The solubilized lipid particle proteins were immediately applied onto a prepared 1-ml His-TrapTM chelating column (Amersham Biosciences) using the standard protocol. Then, in brief, the column was rinsed with 5 ml of washing buffer (20 mM sodium phosphate buffer, pH 7.4; 0.5 mM EDTA; 20 µM Zwittergent 3-14), and subsequently the bound protein was eluted.
eluted with 5 ml of elution buffer (20 mM sodium phosphate buffer, pH 7.4, 0.5 M NaCl, 0.5 mM imidazole; 12.5 μM Zwittergent 3-14). Fractions of 1 ml were collected, and aliquots of 600 μl were used for measuring TAG lipase activity as described below.

**Lipid Analysis**—Lipids of whole yeast cells were extracted by the procedure of Folch et al. (33) after breaking cells with glass beads. Analysis of neutral lipids was performed as described previously (21). In brief, quantification of ergosterol and ergosteryl esters separated by TLC was carried out by densitometric scanning at 275 nm with ergosterol as a standard. TAGs were visualized on TLC plates by post-chromatographic staining and quantified by densitometric scanning at 400 nm with triolein as a standard. Fatty acids of TAGs were analyzed by gas-liquid chromatography. For this purpose, the TAG fraction isolated by TLC was subjected to methanolysis using BF3/methanol, thus converting fatty acids to their methyl esters (34). Gas-liquid chromatography analysis of fatty acid methyl esters was performed as described before (21) using a Hewlett-Packard 5890 equipped with a flame ionization detector operated at 320 °C and a capillary column (Hewlett-Packard 5, 30 m × 0.32 mm × 25 μm film thickness). Fatty acids were identified by comparison to commercial fatty acid methyl ester standards (NuCheck, Inc., Elysian, MN).

**In Vivo Mobilization of Neutral Lipids**—To measure the mobilization of neutral lipids, cells were pre-grown for 40 h in minimal medium containing 2% galactose as the carbon source. Then fresh minimal medium (2% galactose) was inoculated with the pre-grown culture to an A600 of 3, and cerulenin (final concentration 10 μM/ml) or etanerceptine (20 μM/ml), respectively, was added from ethanolic stock solutions. Control incubations contained the equivalent volume of ethanol only. At the time points indicated, 10-ml aliquots of the culture were withdrawn, and cells were harvested by centrifugation on a tabletop centrifuge. After freezing the pellet at −20 °C for at least 1 h, cells were disrupted by glass bead beating, and lipids were extracted and analyzed as described above.

**Enzymatic Analysis**—For the biosynthesis of radioactively labeled TAG, 100 ml of synthetic medium, containing 20 μCi of [14C]palmitic acid (0.4 μmol), were inoculated with 200 μl of a wild-type culture grown for 45 h. Cells were grown for 24 h and harvested, and lipids were extracted as described above. Lipids were separated by TLC using light petroleum/diethyl ether/acetic acid (70:30:2, per volume) as a solvent. Bands of TAG were scraped off the plate and extracted from the silica gel with chloroform/methanol (2:1, v/v). An aliquot of the isolated TAG was used for quantification as described above. Radioactivity was measured by liquid scintillation counting using LSC Safety (Baker) plus 5% water as a scintillation mixture.

To measure enzymatic activity of TAG lipase, 15 μg of TAG (~80,000 cpmp) was dried under a stream of nitrogen, and 150 μl of 0.1 M potassium phosphate buffer (pH 8.0) and 20 μl of a bovine serum albumin solution (20 μg/ml) were added and sonicated for 4 min in a sonicator waterbath at 30°C. Then 50 μl of 0.2 μM MgCl₂ were added, and the mixture was pre-warmed at 30 °C. Samples containing TAG lipase activity were added (25 μg of the maximum amount of protein, 600 μl of the maximum volume), and an aliquot was immediately taken as a blank. After incubation for 30 min at 30 °C, the reaction was stopped by the addition of 3 ml of chloroform/methanol (2:1, v/v), and lipids were extracted for at least 45 min at room temperature. Then the organic phase was washed once with 1 ml of 0.034% MgCl₂ and three times with 2 ml methanol/H₂O/chloroform (48:47:3, per volume) each. Lipids were separated by TLC as described above; bands were visualized with iodine vapor; TAG, fatty acids, and diacylglycerols were scraped off, and radioactivity was measured by liquid scintillation counting as described above.

**RESULTS**

During systematic analysis of yeast mutants deleted of lipid particle proteins (21), a strain lacking the gene product of YMR313c (in the following named Tgl3p) was characterized by its elevated level of cellular TAG. The primary sequence of Tgl3p revealed the consensus motif GX(SX)G, which is conserved among lipolytic enzymes (Fig. 2A). Otherwise, the polypeptide did not exhibit homology to other lipases identified so far. Analysis of the secondary structure of Tgl3p demonstrated the presence of 7 to 8 hydrophobic domains (Fig. 2B). A hydrophobic domain at the C terminus of the protein and the lack of transmembrane domains are characteristic features of lipid particle proteins as reported previously (21).

**Tgl3p Is a Major Component of Yeast Lipid Particles**—Mass spectrometric analysis (21) had identified Tgl3p as a major component of lipid particles. These studies, however, did not exclude the presence of this polypeptide in other organelles. Therefore, we compared protein patterns of subcellular fractions isolated from wild type, a Δtgl3 deletion mutant, and a strain overexpressing Tgl3p in wild-type background. As can be seen from Fig. 3, a band with an apparent molecular mass of 68 kDa present in lipid particles of wild type was absent in this fraction from Δtgl3 but highly enriched in the Tgl3p overexpressing strain. Due to the small contribution of lipid particles to total cellular protein, however, no significant changes were observed in the protein patterns of the corresponding homogenates.

To examine the possible presence of Tgl3p in other organelles, we used the subcellular distribution of a Tgl3p-GFP fusion protein (GFP) revealed the presence of the hybrid in typical lipid particle structures (Fig. 4A). The same staining pattern was observed using the lipophilic fluorescent dye Nile Red which selectively accumulates in lipid particles (Fig. 4B), thus confirming the accumulation of Tgl3p in this compartment. Similar results were obtained with a Tgl3p C-terminally tagged with GFP (data not shown). Western blot analysis of isolated organelles (Fig. 5) confirmed results obtained by fluorescence microscopy. The only signal was seen in the lipid particle fraction, whereas other organelles were devoid of immunoreactive bands. Thus, Tgl3p appears to be exclusively localized to lipid particles.

**The Δtgl3 Mutation Changes the Cellular Lipid Pattern**—Lipid analysis of total extracts from the Δtgl3 deletion strain grown on rich medium had revealed that the ratio of TAG to phospholipids was 3-fold increased compared with wild-type cells (21). This difference depends on the growth status and is less pronounced at the end of the exponential phase than in earlier growth phases. Results obtained by lipid analysis of lipid particles from Δtgl3 confirmed the view that Tgl3p contributes to TAG homeostasis in yeast, although effects observed by deletion or overexpression of Tgl3p were not dramatic (Table 2).
I). In the ∆tgl3 mutant, the ratio of TAG to protein in lipid particles was increased, whereas it was slightly decreased in the strain overexpressing Tgl3p. In contrast, the content of STE, the second major component of lipid particles, was decreased in the ∆tgl3 deletion strain and slightly increased by overexpression of Tgl3p. As a result, the total amount of neutral lipids (TAG and sterols) was higher in ∆tgl3 and lower in the Tgl3p overexpressing strain than in wild type, and the ratio of TAG to STE was decreased in the overexpressing strain and increased in the ∆tgl3 deletion strain as compared with wild type. At this point, it is noteworthy that more dramatic effects besides Tgl3p other TAG lipase isoenzymes exist in the yeast. Moreover, regulatory mechanisms unknown so far may contribute to TAG homeostasis in the yeast.

To test whether Tgl3p has a substrate specificity in vivo, we performed fatty acid analysis of the TAG fractions from wild-type and ∆tgl3 strain. As can be seen from Fig. 6, TAG of the mutant contained slightly increased amounts of very long chain fatty acids (C22:0 and C26:0) and more short chain saturated fatty acids (C14:0 and C16:0) than wild type. In contrast, the amounts of the mono-unsaturated C16:1 and C18:1 species in TAG of ∆tgl3 were slightly decreased resulting in a shift of the unsaturated fatty acid ratio from 2.03 in wild-type to 1.54 in ∆tgl3. The fatty acid pattern of TAG from the Tgl3p-overexpressing strain was similar to that of wild type (data not shown). Thus, the ∆tgl3 deletion affects moderately but significantly the fatty acid composition of TAG. Because TAG contains only a small portion of total cellular fatty acids, shifts in the fatty acid pattern of total cell extracts were not observed (21).

Tgl3p Catalyzes TAG Lipase Activity in Vitro—To measure TAG lipase activity in vitro, we established an assay that is described in detail under “Experimental Procedures.” Significant TAG lipase activity was detected in lipid particles isolated from wild type but was absent from lipid particles of ∆tgl3 (Table II). Although the amount of Tgl3p in lipid particles of the strain overexpressing this polypeptide exceeded severalfold that of wild-type (see Fig. 2), the TAG lipase activity was only slightly increased (Table II). Lipid particles of the strain expressing Tgl3p-GFP, however, exhibited significantly higher TAG lipase activity than wild type. This result indicated functionality of the hybrid but also suggested that structural modifications caused by the GFP tag may have facilitated access of the substrate to the active center of the enzyme or modified the

**TABLE I**

| Triacylglycerols and sterols in lipid particles | FY1679 (wild-type) | Overexpressant of Tgl3p | ∆tgl3 |
|-----------------------------------------------|---------------------|------------------------|-------|
| Steryl esters (STE)                           | 4.35                | 4.49                   | 3.56  |
| Triacylglycerol (TAG)                         | 8.50                | 7.82                   | 9.86  |
| Total neutral lipids                          | 12.85               | 12.31                  | 13.42 |
| TAG:STE ratio                                 | 1.95                | 1.74                   | 2.77  |

**Fig. 6.** Fatty acid composition of TAG fractions from wild-type and ∆tgl3. White bars, wild type; black bars, ∆tgl3. TAG was isolated from lipid extracts of total cells grown to the late exponential phase. Fatty acids were analyzed by gas-liquid chromatography as described under “Experimental Procedures.” Data are mean values of three independent experiments with a mean deviation of ±3%.

**Fig. 3.** Protein pattern of lipid particles from strains overexpressing Tgl3p or deleted of this polypeptide. Protein patterns of homogenate and lipid particles from wild type (a), a strain overexpressing Tgl3p (b), and the ∆tgl3 deletion mutant (c). ST, molecular mass standards.

**Fig. 4.** Localization of Tgl3p-GFP by fluorescence microscopy. A, GFP fluorescence; B, cells stained with Nile Red; C, differential interference contrast optics (transmission). Size bar, 10 μm.

**Fig. 5.** Western blot analysis of organelles from a strain bearing an N-terminal fusion of GFP to Tgl3p. 1, vacuoles; 2, lipid particles; 3, 30,000 × g microsomes; 4, 40,000 × g microsomes; 5, 100,000 × g microsomes; 6, cytosol; 7, mitochondria. Each lane contained 10 μg of total protein. Polypeptides were detected by using a monospecific mouse antiserum against GFP.

**Fig. 2.** A strain overexpressing Tgl3p-GFP, however, exhibited significantly higher TAG lipase activity than wild type. This result indicated functionality of the hybrid but also suggested that structural modifications caused by the GFP tag may have facilitated access of the substrate to the active center of the enzyme or modified the
particles in vitro Tgl3p measured the structural gene for a yeast TAG lipase. This enzyme exhibited high specific activity thus confirming that TGL3 is over that of wild type. Most importantly, the isolated protein derived from the sample buffer.

A Tgl3p-His6 fusion protein was constructed, isolated by affinity chromatography, and tested for its enzymatic activity. As can be seen from Fig. 7, the Tgl3p-His6 fusion protein was significantly overexpressed in the transformant overexpressing Tgl3p-His6; lane 3, Tgl3p-His6 isolated by affinity chromatography. The asterisk indicates the position of the artifact band derived from the sample buffer.

Deletion of TGL3 Abolishes TAG Mobilization from Lipid Particles—To examine whether the TAG lipase activity of Tgl3p is a TAG lipase, we could not exclude the possibility that this polypeptide acts as an activator/regulator of the lipase reaction. To solve this problem, a Tgl3p-His6 fusion protein was constructed, isolated by chromatography, and analyzed by SDS-PAGE as described under “Experimental Procedures.” Lane ST, molecular mass standards; lane 1, lipid particles from wild type; lane 2, lipid particles from a transformant overexpressing Tgl3p-His6, lane 3, Tgl3p-His6 isolated by affinity chromatography. The asterisk indicates the position of the artifact band derived from the sample buffer.

Deletion of TGL3 Abolishes TAG Mobilization from Lipid Particles—To examine whether the TAG lipase activity of Tgl3p measured in vitro is also relevant in vivo, we followed mobilization of cellular TAG in the presence of cerulenin, an inhibitor of fatty acid synthesis in yeast (35). As shown in Fig. 8A, Δtg13 was more sensitive to cerulenin than wild type. Incubation of wild-type cells with cerulenin led to almost complete depletion of stored TAG within 5 h (Fig. 8B). In contrast, the control with untreated wild-type cells showed a decrease of the TAG level within 3 h followed by a steady increase. This effect is due to the shift of cells to fresh medium. During the same period of observation, the level of TAG in Δtg13 decreased only slowly more or less independently of the presence of the inhibitor. This minor decrease indicates that Tgl3p is of major relevance for the mobilization of TAG depots in vivo but also shows that besides Tgl3p other TAG lipases appear to be active in the yeast. The hypersensitivity of Δtg13 to cerulenin may be caused by the failure of this mutant to mobilize TAG depots under conditions of fatty acid depletion. Cells overexpressing Tgl3p or harboring Tgl3p-GFP mobilized TAG with almost the same rate as wild type upon cerulenin treatment (data not shown). Thus, the lipase activity of Tgl3p is not the limiting factor for TAG mobilization in wild type.

Even though STE did not accumulate in Δtg13 (see Table I) and measurements in vivo and in vitro (see Fig. 8 and Table II) indicated that Tgl3p is a TAG lipase, we tested the possibility whether Tgl3p might be involved in unspecific mobilization of neutral lipids from lipid particles in vivo. Such a function was ascribed to perilipins of mammalian cells (for a review see Ref. 1). The yeast Tgl3p, however, has no such properties. When terbinafine, an inhibitor of squalene epoxidase (Erg1p) (36), was added to Δtg13, STE of lipid particles were mobilized at wild-type rates, and no hypersensitivity of the mutant against this drug was observed (Fig. 9).
Previous studies from our laboratory had revealed that in a yeast strain bearing a deletion of YMR313c/TGL3, the amount of TGL3 was significantly increased compared with wild type (21). This observation may be interpreted in three different ways: Tgl3p could act as (i) a bona fide TAG lipase; (ii) an auxiliary factor of a TAG lipase, e.g., a docking protein for a cystolic lipase; or (iii) a negative regulator of TAG synthesis. To distinguish between these possibilities and to determine the function of this protein, we performed in vitro and in vivo experiments with wild type, Δtgl3, and a Tgl3p overexpressing strain. The results of these experiments revealed that Tgl3p (i) is involved in TAG catabolism in vivo, (ii) exhibits TAG lipase activity in highly purified lipid particles, and (iii) is enzymatically active in vitro when purified close to homogeneity. Thus, all data presented here clearly support the view that Tgl3p is a TAG lipase and the first enzyme of this kind from S. cerevisiae identified by function at the molecular level. Although Tgl3p does not show homology to other known lipases, the consensus sequence GXSXG of lipolytic enzymes present in Tgl3p is in line with its identification as a lipase. Most recently, a lipase from Candida parapsilosis was identified, which also contains the consensus motif but lacks similarity with other lipases (37).

How does accumulation of TAG affect cell growth? In the Δtgl3 mutant the moderate increase of the TAG level does not lead to major phenotypic alterations (21) except the hypersensitivity to cerulenin (see Fig. 8A). In contrast, a dramatic accumulation of TAG in humans results in severe defects as observed with patients suffering from a metabolic disorder named neutral lipid storage disease (38). In cultured neutral lipid storage disease fibroblasts TAG with long acyl chains (C_{12} and longer) remained undegraded, whereas short/medium chain (C_4 to C_10) TAG species and endogenous STE were mobilized at a normal rate (39). This result is reminiscent of the slight substrate specificity of the yeast Tgl3p in vivo (see Fig. 6).

Localization of Tgl3p in lipid particles raised the question as to the coordinate formation, storage, and mobilization/degradation of TAG. How can Tgl3p be prevented from permanent lysis of TAG stored in lipid particles? Although we cannot yet answer this question at the molecular level, we can speculate about regulatory aspects by comparing the yeast system to other experimental systems. Wiggins and Gibbons (40) reported that about 70% of newly synthesized TAG in the rat liver is subjected to a lipolysis/re-esterification cycle before being secreted in the form of very low density lipoproteins. Yang et al. (41) reported that a minimum of 60% of the very low density lipoprotein-TAG is derived from the stored TAG by lipolysis/re-esterification. Lehner et al. (11) showed that a TAG lipase is associated with the cytoplasmic TAG droplet in pig liver and that the ontogeny of the TAG lipase seems to be coordinated with very low density lipoprotein secretion by the liver. Similar mechanisms of lipolysis/re-esterification may also exist in the yeast. Studies from our laboratory revealed that yeast lipid particles, besides TAG lipase activity, also harbor a TAG synthase (42). Thus, a lipolysis/re-esterification cycle may also exist in yeast lipid particles. Regulation in vivo through presently unknown mechanisms may favor one or the other direction of this enzymatic step leading either to accumulation or mobilization of TAG depots. Depending on the requirement for fatty acids, stores of TAG may be utilized to a greater or lesser extent.

Even though the in vivo experiment shown in Fig. 8 using the inhibitor cerulenin revealed that cleavage of TAG strongly depends on Tgl3p, the moderate decrease of the TAG content observed in Δtgl3 suggests the existence of additional TAG lipases with minor enzymatic activities in yeast. Another indication for the presence of additional TAG lipase activities was the observation that the fatty acid pattern of TAG from the Δtgl3 deletion mutant was different from that of wild type (see Fig. 6) and of strains deleted of two homologues of Tgl3p. A common feature of Tgl3p and its homologues is their increased expression during sporulation. Systematic analysis of the neutral lipid patterns of single and multiple deletion strains will show whether the TGL3 homologues are also involved in TAG mobilization. Furthermore, these investigations may elucidate a possible role of this gene family during yeast spore formation.

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