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REVIEW

Triacylglycerol lipases of the yeast

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Abstract **All eukaryotes including the yeast contain a lipid storage compartment which is named lipid particle, lipid droplet or oil body. Lipids accumulating in this subcellular fraction serve as a depot of energy and building blocks for membrane lipid synthesis. In the yeast, the major storage lipids are triacylglycerols (TGs) and steryl esters (SEs). An important step in the life cycle of these non-polar lipids is their mobilization from their site of storage and channeling of their degradation components to the appropriate metabolic pathways. A key step in this mobilization process is hydrolysis of TG and SE which is accomplished by lipases and hydrolases. In this review, we describe our recent knowledge of TG lipases from the yeast based on biochemical, molecular biological and cell biological information. We report about recent findings addressing the versatile role of TG lipases in lipid metabolism, and discuss non-polar lipid homeostasis and its newly discovered links to various cell biological processes in the yeast.**

Keywords triacylglycerol, lipase, fatty acid, acyltransferase, lipid droplet/particles, yeast

Introduction

In most types of cells, certain lipid species form large inert depots which can be mobilized upon requirement. In eukaryotic cells, storage lipids consist of variable portions of two main lipid classes, the sterols and the fatty acids. Both sterols and fatty acids are essential for cellular survival. Sterols are constituents of biological membranes where they regulate fluidity and permeability, but also participate in the control of various membrane-associated processes (Parks and Casey, 1995; Daum et al., 1998; Umebayashi and Nakano, 2003; Sharma, 2006; Turkish and Sturley, 2007). Fatty acids serve as building blocks for membrane biogenesis and also as a source of cellular energy. Excess of fatty acids and sterols, however, may become fatal for the cell because of their potential membrane disturbing effects. To circumvent this problem excess amounts of these lipids are segregated within the cell in a modified and biological inert form, namely as non-polar triacylglycerols (TG) and steryl esters (SE). Under normal conditions, TG and SE are stored in specific compartments named lipid particles (LP), lipid droplets or oil bodies.

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Upon requirement, TG and SE are mobilized from their depot, and their building blocks are channeled toward energy production and/or membrane biosynthesis. Hydrolytic reactions catalyzing mobilization of these lipids are largely conserved from bacteria to humans. Due to relevance for human pathogenicity and importance for biotechnological applications one specific class of lipid hydrolytic enzymes has gained much interest, namely the lipases. Studies with the yeast which is nowadays accepted as a reliable and most useful model system to study membrane and lipid biology contributed a lot to our present knowledge of lipolytic enzymes. In this review we will summarize the latest achievements in the field of yeast lipases focusing on biochemical characterization of these proteins, molecular properties and relevance for cell biology and cell metabolism. The reader is also referred to other recent reviews covering non-polar lipid biochemistry, molecular biology and cell biology (Athenstaedt and Daum, 2006; Turkish and Sturley, 2007; Czabany et al., 2008; Rajakumari et al., 2008)

Triacylglycerol, the substrate of lipases

"Dosis sola facit venenum" said the famous alchemist, physician and astrologer Philippus Theophrastus Aureolus Bombastus von Hohenheim, later known as Paracelsus (1493–1541). This sentence means that all substances are poisons; there is none which is not a poison. The right dose

differentiates a poison and a remedy. This statement is also true for lipids.

Lipids are a versatile group of components and essential for every cell irrespective of the kingdom of life. Despite the essential nature of lipids an excess may become harmful for the cell beyond a certain threshold. Therefore, a well balanced turnover system is important for lipid homeostasis in the cell. Under certain conditions yeast cells similar to other eukaryotic cells may accumulate free sterols or free fatty acids resulting in a situation described as lipotoxicity. Due to their chemical nature excessive amounts of these lipids can interfere in a most negative way with their environment, where they are urgently needed under normal conditions. Under certain circumstances, free fatty acids or sterols instead of serving as important membrane constituents or supplying cells with energy may cause severe defects in cell integrity and assembly of organelle membranes (Unger, 2003; Schaffer, 2003; Brookheart et al., 2009).

The yeast can store large amounts of free sterols and free fatty acids by converting them to their biological inert forms, TG and SE. These two components represent the major classes of non-polar lipids which are molecules lacking any charged groups. Due to their chemical and physical nature TG and SE are inappropriate as membrane constituents (Hunkova and Fencl, 1977; 1978; Daum and Paltauf, 1980) but have a high tendency for self assembly. These properties are the reason why non-polar constituents undergo spatial separation from membranes giving birth to a distinct organelle, the so-called lipid particles (LP) (Clausen et al., 1974; Leber et al., 1994), nowadays better known as lipid droplets, lipid bodies or oil bodies. LP are typically arranged as a highly hydrophobic core consisting of non-polar TG and SE which is surrounded by a single phospholipid monolayer with a small amount of proteins embedded. Recent biophysical analysis revealed that SE form several ordered shells below the surface phospholipid monolayer whereas TG appears to be randomly packed in the center of the particle (Czabany et al., 2008). For detailed information about the biosynthesis of TG and SE which will not be addressed in this article the reader is referred to a number of recent reviews (Wagner and Daum, 2005; Athenstaedt and Daum, 2006; Czabany et al., 2007; Rajakumari et al., 2008).

Besides its protective role through inactivating excessive amounts of free fatty acids TG are most efficient molecules preserving chemical energy (Sorger and Daum, 2003). For this process, TG is mobilized upon requirement. However, TG can also be used for anabolic processes. When TG is cleaved to one molecule of diacylglycerol (DG) and fatty acid, each, DG can be channeled toward the synthesis of phospholipids which are then incorporated into cellular membranes (Kurat et al., 2006). Similarly, fatty acids can be utilized for the synthesis of complex lipids. Consequently, biosynthesis and lipolysis of TG appear to be closely linked to membrane lipid biosynthesis and lipid turnover. Moreover, cleavage products of TG hydrolysis such as free fatty acids, DG or the follow-up product phosphatidic acid (PA) are important signaling molecules. Thus, balanced anabolic and catabolic reactions of TG are essential for the cell (Coleman and Lee, 2004). Recent reviews by Kohlwein (2010) and Turkish and Sturley (2007) address these problems of TG homeostasis and its regulation.

The principle of triacylglycerol lipolysis

TG hydrolases (EC 2.1.1.3) catalyzing cleavage of ester bonds in acylglycerols are enzymes present in all kingdoms of life. Although displaying a subclass of esterases, lipases are greatly different from most other hydrolytic enzymes with respect to one characteristic feature, i.e. the reaction occurs at and is restricted to the aqueous/non-aqueous interface. This phenomenon is known as interfacial activation (Verger, 1997; Schmidt and Verger, 1998). It is anticipated that conformational changes of the protein are required to expose the active site of the enzyme, and oil-in water droplets are needed to present the substrate(s) in a correct way (Vakhlu and Kour, 2006). The active center of the lipase with its catalytic triad was shown to be buried under a short helical fragment acting as a lid which can be opened and closed (Brady et al., 1990; Brzozowski et al., 1991; Kim et al., 1997). After accommodation of the substrate in the active site of the enzyme, TG is hydrolyzed resulting in the production of free fatty acids, DG and eventually monoacylglycerol (MG) and glycerol. In mammalian cells, the specificity of lipases strongly varies with respect to the substrates utilized. TG is preferentially cleaved by adipose triglyceride lipase (Zimmermann et al., 2004) also known as desnutrin (Villena et al., 2004) or phospholipase A2ε (Jenkins et al., 2004), DG by hormone-sensitive lipase (Vaughan et al., 1964; Osuga et al., 2000; Haemmerle et al., 2002) and MG by monoglyceride lipase (Zimmermann et al., 2009; Lass et al., 2011). Yeast lipases appear to be less stringent in the utilization of their substrates. It has to be noted that in principle the lipase reaction can also function in a reversed way depending on the availability of water molecules. Consequently, lipases are also able to catalyze esterification, transesterification and interesterification reactions under certain conditions (Vakhlu and Kour, 2006).

The only characteristic property shared by almost all known lipases is the presence of a serine within the highly conserved sequence motive GXSXG. In some cases such as in *Bacillus* lipases the first glycine residue is replaced by alanine. For the catalytic reaction of lipases the serine residue is essential. However, the catalytic triad of serine interacting with aspartic acid/glutamic acid and histidine was also found in the active site of α/β hydrolase fold enzymes and serine proteases (Brady et al., 1990; Winkler et al., 1990; Dartois et al., 1992; Ollis et al., 1992; Schrag and Cygler, 1997). Many TG lipases also contain a so-called patatin domain. The name patatin derives from a plant storage protein that has lipid acyl hydrolase activity (Mignery et al., 1988).

Triacylglycerol lipases of *Saccharomyces cerevisiae*

The first lipase of the yeast *Saccharomyces cerevisiae* which did not only contain the conserved lipase motif and a patatin domain but was also positively tested for lipase activity was Tgl3p (Athenstaedt et al., 1999). Tgl3p was found to be localized to LP by GFP technology, and correspondingly TG lipase activity was detected in this compartment. Tgl3p also exhibited enzymatic activity *in vitro* when purified close to homogeneity. Deletion of *TGL3* resulted in a moderate increase of the cellular TG level and hypersensitivity to the fatty acid synthase inhibitor cerulenin. The substrate specificity of Tgl3p is not much pronounced. In pulse-chase experiments *in vivo*, *tgl3Δ* mutants showed reduced utilization of TG when cells were poisoned with cerulenin. The fact that in this genetic background TG was still mobilized strongly suggested that other TG lipases were still active causing residual TG turnover.

In the following, two additional yeast TG lipases were identified. These enzymes which share 30% and 26% homology to Tgl3p, respectively, were named Tgl4p and Tgl5p (Athenstaedt and Daum, 2005; Kurat et al., 2006). Besides their sequence homology to Tgl3p they harbor the consensus motif of lipases and patatin domains. Similar to Tgl3p they comprise hydrophobic domains but no transmembrane spanning regions and are localized to the LP (Athenstaedt and Daum, 2005). Both Tgl4p and Tgl5p exhibit *in vitro* lipolytic activity, whereas *in vivo* only deletion of *TGL4* had an effect on the mobilization of TG. This was at least in part explained by the different substrate specificity of the two enzymes. Tgl4p preferentially uses TG esterified with myristic and palmitic acid as substrate, whereas Tgl5p prefers utilization of TG containing C26:0. As this very long chain fatty acid is of low abundance in yeast and specifically in yeast TG, it was not surprising that deletion of *TGL5* resulted only in a moderate overall effect. As will be described below, however, Tgl5p may play a role in the supply of very long chain fatty acids for sphingolipid biosynthesis.

Both Tgl3p and Tgl4p degrade TG, but Tgl4p appears to be more specific for this substrate than Tgl3p. *In vitro* experiments revealed that Tgl3p also catalyzed DG hydrolysis whereas Tgl4p did not exhibit hydrolytic activity with this substrate (Kurat et al., 2006). This observation is noteworthy as Tgl4p is the closest yeast ortholog of mouse adipose triglyceride lipase (ATGL) which is also highly specific for TG. It was shown that murine ATGL can restore TG mobilization in yeast mutants deleted of *TGL4* but not of *TGL3*. Lack of either one or all three yeast TG lipases did not result in an obvious growth phenotype under standard laboratory conditions (Athenstaedt and Daum, 2005). However, Kurat et al. (2006) reported that *TGL3* and *TGL4* mutations led to fat yeast cells as they lost their ability to degrade TG. Whereas addition of the fatty acid synthase inhibitor cerulenin to wild type cells resulted in rapid utilization of TG reserves from LP, no such effect was observed with mutants deleted of either *TGL3* or *TGL4*, or in a double mutant. These strains retained significant amounts of non-polar lipids in their storage form indicating a strong decay or even a disability to degrade TG. The requirement of the active serine in the lipase consensus motif of *TGL4* supported the opinion that the enzyme's function was indeed lipolysis and not that of a lipid regulator (Kurat et al., 2006).

All three yeast TG lipases described above were shown to localize constitutively to the LP indicating that translocation or regulatory processes at the expression level are not required for balanced lipolysis (Kurat et al., 2009). In a large-scale study, however, Tgl4p was identified as an *in vitro* substrate of the cyclin-dependent kinase, Cdk1/Cdc28 (Ubersax et al., 2003). Kurat et al. (2009) demonstrated that Tgl4p is indeed phosphorylated and activated by Cdk1/Cdc28. During G1/S transition of the cell cycle two phosphorylation sites, namely threonine 675 and to a lesser extent serine 890 became phosphorylated. Mutants bearing defects in proper TG mobilization showed a retarded entry into G1/S from quiescence and displayed a delay in cell-cycle progression, hence highlighting the importance of TG lipases and a direct linkage between TG catabolism and cell-cycle. Onset of Tgl4p mediated lipolysis made precursors for membrane lipid synthesis available. Without active lipolysis and deficiencies in fatty acid synthesis, cells undergo growth arrest in an unbudded state (Kurat et al., 2009).

Another yeast specific and important phenomenon of cell development is sporulation in the meiotic life cycle. When yeast cells start sporulating all three TG lipases exhibit elevated expression (Athenstaedt and Daum, 2003). Interestingly, homozygous diploid strains deleted of *TGL3* were not able to produce spores (Rajakumari and Daum, 2010a). Deletion of either *TGL4* or *TGL5* alone did not affect sporulation, whereas lack of both gene products in a homozygous double deletion strain decreased spore formation to some extent. As will be discussed below, however, the lipolytic activity of yeast TG lipases is not paramount for their role in sporulation.

Unexpected features of triacylglycerol lipases from *S. cerevisiae*

Recent investigations addressing biochemical and cell biological characterization of yeast TG lipases revealed interesting and novel aspects which broadened our view of these proteins. The findings which led us to this new facet of yeast TG lipase biology were changes in the total amount of phospholipids in *tgl3Δ* deletion or *TGL3* overexpression strains. Deletion of this gene resulted in a 34% reduction of cellular phospholipids whereas overexpression increased the total amount of phospholipids to approximately the same value (Rajakumari and Daum, 2010a; Rajakumari et al.,

2010). Phospholipids affected most were phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine. Most interestingly, *in silico* analysis of the three TG lipases revealed that Tgl3p and Tgl5p, besides their lipolytic signature motif, contained a (H-$(X)_4$-D)-motif which was reported as a well conserved domain for acyltransferases in *Escherichia coli*, yeast, plants and mouse (Heath and Rock, 1998; Dircks et al., 1999; Lewin et al., 1999; Ghosh et al., 2008). Enzymatic assays *in vitro* showed that both lipases indeed exhibited acyltransferase activity and acted as lysophospholipid acyltransferases. Tgl3p turned out to be a most efficient lysophosphatidylethanolamine acyltransferase, and Tgl5p preferentially acylated lyso-phosphatidic acid (Rajakumari and Daum, 2010a). Both lipases, Tgl3p and Tgl5p, showed a strong preference for the unsaturated co-substrate oleoyl-CoA. As a proof for the functional importance of the acyltransferase domain, site directed mutagenesis experiments were performed. Mutations of glutamate in the acyltransferase motif of *TGL3* moderately reduced the acyltransferase activity, but the exchange of histidine led to a dramatic decrease of lysophosphatidylethanolamine acylation *in vitro*. Mutations in the lipase motif did not affect the acyltransferase activity and *vice versa*, indicating that both motifs act independently of each other. Using a *tgl3* mutant defective in the lipolytic domain led to the conclusion that the protein's acyltransferase activity but not its lipolytic capacity rescued the growth delay in the log phase of *tgl3*Δ*tgl4*Δ. It was also shown that the acyltransferase activity of Tgl3p but not the lipase activity of the enzyme is indispensable for yeast sporulation (Rajakumari and Daum, 2010a).

In a parallel approach, Rajakumari and Daum (2010b) discovered that enzymatic activity of Tgl4p was also not restricted to lipolysis. It was demonstrated that Tgl4p besides its lipase motif contained the conserved sequence domain (G/A)XGXXG which is characteristic of calcium-independent phospholipases A_2 (PLA_2). Cytosolic fractions from a *TGL4* overexpressing strain exhibited TG lipase activity, but also catalyzed SE and phospholipid hydrolysis. Experiments using Tgl4p purified close to homogeneity showed that this enzyme was able to cleave phosphatidylcholine and phosphatidylethanolamine, whereas phosphatidic acid and phosphatidylserine were not used as substrates. Lysophospholipase and transacylation activities of Tgl4p were excluded. Besides the newly discovered hydrolytic activities of Tgl4p it was shown that this enzyme can also act as an acyl-CoA-dependent lysophospholipid acyltransferase (LPAAT). Tgl4p utilizes several lysophospholipids as acyl acceptors but exhibits high preference for lysophosphatidic acid. Other lysophospholipids such as lysophosphatidylethanolamine, lysophosphatidylcholine, lysophosphatidylinositol and lysophosphatidylserine were also appropriate acceptors but their utilization was less efficient. Tgl4p showed the highest co-substrate specificity for oleoyl-CoA, whereas palmitoyl-CoA, stearoyl-CoA, arachidyl-CoA and myristoyl-CoA were not utilized with equivalent efficiency (Rajakumari and Daum, 2010b).

To test whether or not phosphorylation of Tgl4p (Kurat et al., 2009) had any impact on both the hydrolytic and acyltransferase activities of the enzyme phosphorylation deficient variants of Tgl4p were constructed and analyzed. Mutations in phosphorylation sites led to strong reduction of the lipolytic activity of Tgl4p, whereas LPAAT activity remained unaffected (Rajakumari and Daum, 2010b). These results served as further indication that the different enzyme activities act independently of each other. In conclusion, Tgl4p can be regarded as a multifunctional enzyme playing different roles in lipid metabolism. Besides its contribution to TG and SE turnover through its hydrolytic activities, Tgl4p supports *de novo* phospholipid synthesis by its LPA acyltransferase activity, and finally may also be involved in membrane lipid remodelling by its PLA_2 activity.

By now it has become clear that TG hydrolysis is not an isolated cellular event, but appears to have large impact on the complex network of lipid metabolism. As a further proof for this hypothesis, the involvement of TG lipases in sphingolipid metabolism was demonstrated. Rajakumari et al. (2010) showed that deletion of TG lipases led to reduced amounts of sphingolipids. The authors assumed that reduced synthesis of sphingolipids in *tgl* mutants was caused by blocking the mobilization of long chain fatty acids (LCFA) and very long chain fatty acids (VLCFA) from the TG intermediates. As possible compensation of this defect, upregulation of the two yeast fatty acyl elongases Elo1p and Elo2p which supply VLCFA to sphingolipid synthesis was considered. Indeed, in cells lacking TG lipases (Tgl3-5p) these elongases were upregulated, but the level of sphingolipids remained low. This result indicated that a compensatory mechanism by elongase upregulation was not sufficient to raise sphingolipid synthesis to wild type level.

In the course of these studies it also became evident that TG hydrolysis has a marked impact on phospholipid formation, because in *tgl* mutants levels of all glycerophospholipids were reduced (Rajakumari et al., 2010). The decreased level of phosphatidylinositol (PI) was of special interest because the inositolphosphate moiety of PI is a precursor for the sphingolipids inositolphosphoceramide and mannosyl inositolphosphoceramide (MIPC). It is likely that reduced formation of PI has a negative effect on the formation of yeast sphingolipids. Lack of TG lipases, however, did not only affect the absolute amount of phospholipids in the respective mutant strains. It was also shown that in *tgl* mutants species patterns of phospholipids were changed indicating that TG lipases may also be involved in the remodelling of phospholipids. Free FA (FFA) liberated during lipolysis may influence the composition of the acyl-CoA pool available for acylation processes in general, and DG as a degradation product of TG may serve as a direct precursor for phospholipid synthesis through the CDP-choline/ethanolamine pathway. Thus, the merry-go-round of TG synthesis and

degradation may in the end contribute significantly to the fine tuning of the cellular phospholipid composition. One additional interesting observation was that cumulative deletions of *TGL3, TGL4* and *TGL5* had no additive effects on sphingolipid and phospholipid synthesis. It may be anticipated that Tgl-proteins compensate for the loss of partner proteins and other not yet identified lipases may be activated in strains deleted of the three classical yeast TG lipases.

Yeast lipolytic enzymes different from Tgl3p, Tgl4p and Tgl5p

Prior to the discovery of the major yeast lipases described above several approaches were made to identify additional yeast lipolytic enzymes. Initial findings of this kind with *S. cerevisiae* date back to the 1970s when it was postulated that TG lipase activity was found in yeast mitochondrial fractions (Schousboe, 1976a; 1976b). At that time, however, the respective protein could not be identified. Two decades later, several candidate ORFs became of interest since computational analysis revealed either close homology to known lipases from other organisms or presence of a lipase motif (Abraham et al., 1992; Van Heusden et al., 1998; Daum et al., 1999). In Fig. 1 we summarize the sequence information of *Saccharomyces cerevisiae* TG lipases known so far.

Two of these putative lipases were Tgl1p and Tgl2p. It came as a surprise that Tgl1p was shown to exhibit SE hydrolase activity rather than TG lipase activity. In the following, Tgl1p was regarded as one of the three members of the SE hydrolase family which also includes Yeh1p and Yeh2p (Köffel et al., 2005). Nevertheless, Jandrositz et al. (2005) showed that the activity of Tgl1p was not completely restricted to SE hydrolysis, but also catalyzed cleavage of triolein at neutral pH, although at low rate.

Tgl2p showed lipolytic activity toward TG and DG with short chain fatty acids when expressed in *E.coli*, but no phenotype was observed for *tgl2Δ* deletion mutants (Van Heusden et al., 1998). Most recently, Ham et al. (2010) provided evidence that purified Tgl2p which shows sequence homology to *Pseudomonas* TG lipases indeed has lipolytic activity *in vitro* when tested with long chain TG. *In vivo* the TG level decreased after poisoning yeast cells with cerulenin, an inhibitor of fatty acid synthesis, thus supporting the expected function of Tgl2p as a lipase. Deletion of *TGL2* led to decreased mobilization of TG whereas in *TGL2* overexpressing strain TG mobilization was enhanced. In contrary to the major yeast lipases which reside to the LP fraction Tgl2p was found to be localized to mitochondria by immunofluorescence microscopy. Localization of Tgl2p in mitochondria was also verified by cell fractionation experiments. For these experiments, overexpression of the gene was needed because in wild type the abundance of the protein was very low. The physiological relevance of Tgl2p as a TG lipase of mitochondria may be scrutinized when taking into account that the substrate for the hydrolytic reaction, TG, has never been identified as a mitochondrial component. Thus, it was tempting to speculate that Tgl2p prefers other substrates as physiologic targets such as glycerophospholipids of mitochondria. As another possible substrate of Tgl2p DG was identified, whereas the enzyme was not active with MG. Control experiments using a Tgl2p variant bearing a defect in the catalytic triad (replacement of the active serine by alanine) revealed loss of the lipase activity. This result supported the assumption that Tgl2p is indeed a TG lipase (Ham et al., 2010).

So far, we described the presence of yeast lipolytic enzymes and activity in LP and mitochondria. This view has been extended by the recent finding of Thoms et al. (2008) who showed that a putative lipase named Lpx1p is present in peroxisomes. Lpx1p contains the characteristic GXSXG motif and exhibits similarity to α/β-hydrolase fold enzymes. The purified enzyme has acyl esterase, lipase and phospholipase A activities with commercially available substrates. However, TG lipase activity measured was low. As *lpx1Δ* deletion strains contain peroxisomes with altered morphology, especially abnormally vesiculated peroxisomes with membranes grossly invaginated, it was assumed that Lpx1p may play a role rather in modifying membrane phospholipids through its phospholipase A activity than in TG lipolysis (Thoms et al., 2008).

During the last few years it became evident that cells contain enzymes with completely or partially overlapping functions. Therefore, it was not surprising that recently more yeast Tgl-related proteins were identified. Heier et al. (2010) reported identification of Yju3p as the major yeast monoacylglycerol (MG) lipase. Yju3p has more than 20% sequence homology to mammalian MG lipases (MGL) (McPartland et al., 2006), contains the typical lipolytic consensus sequence GXSXG and harbors an α/β-hydrolase fold. MG lipase activity was detected in yeast lysates and in purified preparations of the enzyme. Localization experiments revealed that the protein was dually localized, namely to LP and membrane fractions. In total cell extracts of *yju3Δ*, hydrolysis of MG was reduced to 10% of wild type suggesting that Yju3p is the major MGL in yeast. It was also shown that the enzyme was specific for MG whereas DG- or TG-mimicking substrates were not accepted. *In vitro* LP preparations from a *yju3Δ* mutant exhibited a 40% reduced release of fatty acids with simultaneous 10-fold accumulation of MG. Overexpression of murine MGL in a *yju3Δ* mutant background compensated for the described effect and confirmed that Yju3p can be regarded as the functional ortholog of MGL from higher eukaryotes. Since MG lipase activity was not completely abolished in *yju3Δ* mutants, residual activity against MG esterified at the *sn*-2 position was attributed to at least one further MG lipase which still needs to be identified. The low concentration of MG even in the *yju3Δ* deletion strain was ascribed to other enzymatic reactions such as acyltransferase or phosphorylation which use MG as a

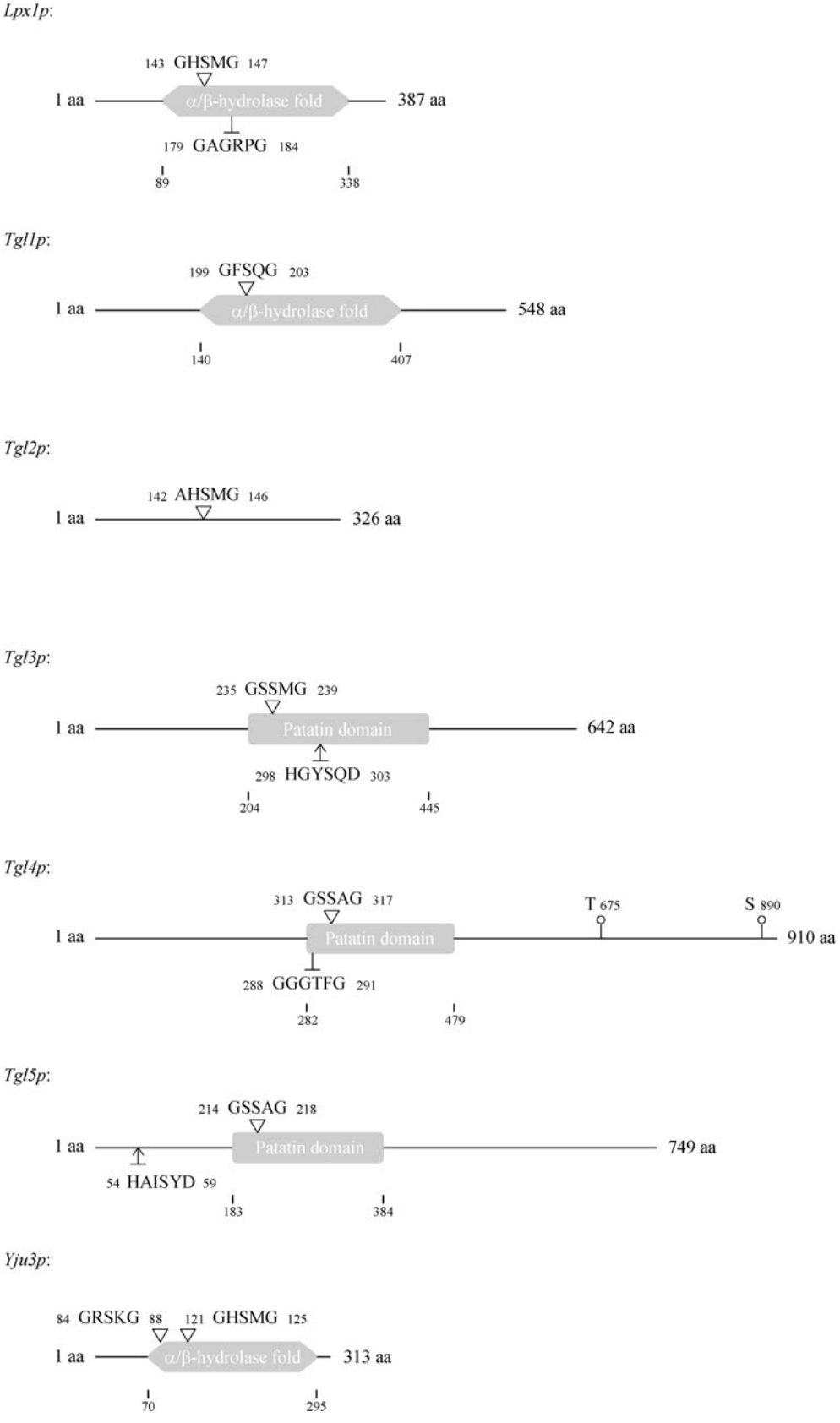

Figure 1 Sequence motifs in triacylglycerol lipases of the yeast. Protein sequences of *S. cerevisiae* TG lipases have been screened for the following conserved sequence motifs: lipase motif (A/G)XSXG marked as open triangle; phospholipase motif GXGXXG marked with line; acyltransferase motif HXXXXD marked with arrow. Patatin domain (PF01734) and α/β-hydrolase fold (PF00561) have been determined by Pfam analysis. Sites of phosphorylation experimentally proven are depicted as open circles.

substrate (Heier et al., 2010). Again, the link of non-polar lipid hydrolysis and anabolic processes became obvious.

Table 1 shows a brief summary of features of yeast TG lipases and related enzymes identified so far. Enzymatic activities of these enzymes and subcellular localization(s) of the respective proteins are included in this Table.

Lipases of *Yarrowia lipolytica*

Besides *S. cerevisiae* several other yeasts became of interest with respect to TG storage and TG lipolysis. *Yarrowia lipolytica* is the most prominent example of this kind and the best studied oleaginous or lipolytic yeast. Other members of the yeast family which utilize a wide range of hydrophobic substrates such as alkanes, fatty acids and TG are *Candida antarctica*, *Rhizopus oryzae* or *Rhizomucor miehei* (Fickers et al., 2005a). All of these yeast species produce lipases which are homologous to other TG lipases (Murzin et al., 1995). In contrast to *S. cerevisiae* which produces only intracellular lipases, *Y. lipolytica* secretes large amounts of lipolytic enzymes. Little information on intracellular TG lipases has been reported till now, since utilization of extracellular lipases had priority for biotechnological and pharmaceutical applications. However, *Y. lipolytica* contains two *TGL* orthologs whose function remained largely uncharacterized so far (Beopoulos et al., 2009). *Yarrowia* Tgl3p was identified as a component of LP but has not been further characterized by function (Athenstaedt et al., 2006).

In contrast to intracellular TG lipases, secreted lipases of *Y. lipolytica* have been extensively studied. Since TG present as a carbon source in the medium cannot pass the cell membrane, degradation is required prior to further metabolism. After cleavage of TG, free fatty acids can be taken up by the cell and utilized as a source of energy or as building blocks for complex lipids. To achieve the extracellular degradation of TG, lipases need to be secreted into the medium where hydrolysis of TG takes place (Najjar et al., 2010). Like other yeasts which produce a mixture of extracellular lipases from multiple genes (Huge-Jensen et al., 1988; Bertolini et al., 1994; Ferrer et al., 2001) *Yarrowia* contains 16 paralogues of lipase-encoding genes (Thevenieau et al., 2007). *LIP2*, *LIP4*, *LIP5* and *LIP7–LIP19* encode for members of the lipase family GL3R0084 defined by the Génolevures consortium (Beopoulos et al., 2008). Lip2p, Lip7p and Lip8p are the most prominent *Y. lipolytica* lipases which have so far been investigated best. Lip2p is the enzyme which accounts for the major extracellular lipolytic activity (Pignède et al., 2000a, 2000b). A *LIP2* knockout strain displays less than 3% lipase activity compared to wild type. Since deletion of *LIP2* did not lead to complete loss of lipolytic activity in the medium, the existence of other lipases was suggested. Indeed, *LIP7* and *LIP8* were identified as paralogues of *LIP2* and further characterized. In contrast to Lip2, which is exclusively secreted to the cellular environment, Lip7p and Lip8p are only partially secreted whereas a large portion remains associated with the cell wall. A short signal peptide of these proteins may serve as an anchor to the cell wall or to the plasma membrane (Fickers et al., 2005b). Another characteristic feature of these enzymes is their clear preference to utilize substrates ranging from medium to long chain fatty acid containing TG. Lip2p was reported to hydrolyze TG with oleoyl residues with some preference. Using *p*-nitrophenyl esters of various chain lengths it was shown that enzyme preparations of Lip7p exhibited maximum activity with caproate (C6) while Lip8p preferentially cleaved esters of caprate (C10) (Fickers et al., 2005b). Experiments exposing Lip7p and Lip8p to the surface of *S. cerevisiae* revealed that the putative signal sequences of the enzymes were necessary for maximum enzyme activities (Liu et al., 2010). It was speculated that the signal peptides assist in correct folding of the lipases and sustaining protein stability as has been shown before for pro-sequences of lipases from *R. oryzae* (Ueda, 2002).

Pathways involved in the secretion of proteins in *Yarrowia* resemble rather those of higher eukaryotes than *S. cerevisiae* (Babour et al., 2004). During secretion, the three extracellular lipases Lip2p, Lip7p and Lip8p from *Y. lipolytica* get glycosylated (Pignède et al., 2000a). Expression of *LIP7* and *LIP8* in *Pichia pastoris* also led to proper glycosylation of the respective proteins (Song et al., 2006). The importance of N-glycosylation for proper folding, assembly, secretion and catalytic activity of enzymes, especially of lipases, has already been studied before with organisms different from *Yarrowia* (Abouakil et al., 1993; Brocca et al., 2000). The requirement of glycosylation for lipase activity, however, was scrutinized since the inhibition of glycosylation resulted in correct processing of the protein (Sebban-Kreuzer et al., 2006) and non-glycosylated forms of the enzymes still had lipolytic activity (Alam et al., 2002). Yu et al. (2007) reported that after enzymatic deglycosylation Lip2p still showed 88% activity of the untreated lipase. Jolivet et al. (2007) investigated the impact of glycosylation on substrate utilization. These authors showed that inactivation of one or both glycosylation sites caused only marginal effects on lipolysis, although site-directed mutagenesis of amino acids involved in glycosylation had great influence on the catalytic activity.

When *LIP2* multi-copy strains were used for overexpression of the respective lipase, four isoforms were identified which varied in their apparent molecular masses due to different glycosylation patterns. All four isoforms showed similar activities to various substrates tested, indicating that glycosylation patterns did not affect the enzyme activity. $LIP2_A$ was found to be the major isoform coding for the smallest protein variant of 36850 Da. Lipolysis catalyzed by Lip2p leads to formation of 2-monoglycerides with a slight stereospecific preference for cleaving the ester group at the *sn-3* position of TG. The biochemical properties of the

Table 1 Survey of lipases from *Saccharomyces cerevisiae*

Gene name	Systematic name	Subcellular localization[¥]	Enzymatic activities	Substrates	Effect of		Reference
					deletion	overexpression	
LPX1	YOR084W	Peroxisomes	esterase, lipase, phospholipase A	PNB, DPG, BPC	Altered morphology of peroxisomes	–	Thoms et al., 2008
TGL1	YKL140W	Lipid particles	SE hydrolase, TG lipase	SE, triolein	–	–	Jandrositz et al., 2005
TGL2	YDR058C	Mitochondria	TG lipase	long-chain TG, DG	Decreased TG mobilization *in vivo**	Increased TG mobilization of TG *in vivo**	Ham et al., 2010
TGL3	YMR313C	Lipid particles	TG lipase, acyltransferase	TG, DG; lyso-PE and oleoyl-CoA	Increase of TG level, decreased TG mobilization *in vivo**, hypersensitivity to cerulenin; decreased level of total PL, sporulation defect	Increased level of total PL; slight decrease of TG	Athenstaedt and Daum, 2003; Rajakumari and Daum, 2010a
TGL4	YKR089C	Lipid particles	TG lipase, SE hydrolase, phospholipase A, acyltransferase	SE, TG esterified with myristic and palmitic acid	Increase of TG level, decreased TG mobilization *in vivo* in combination with *tgl5Δ*	Increased level of total PL; decrease in TG, DG, SE levels	Athenstaedt and Daum, 2005; Rajakumari and Daum, 2010b
TGL5	YOR081C	Lipid particles	TG lipase, acyltransferase	TG esterified with C26:0; lyso-PA and oleoyl-CoA	Marked increase of C26:0 TG species; decreased TG mobilization *in vivo* in combination with *tgl4Δ*; decreased level of total PL	Increased level of total PL	Athenstaedt and Daum, 2005; Rajakumari and Daum, 2010a
YJU3	YKL094W	Lipid particles, mitochondrial outer membrane, cytoplasm, endoplasmic reticulum	MG lipase	MG, preferentially esterified at *sn*-2 position	MG lipase (MGL) activity strongly reduced; moderate accumulation of MG		Heier et al., 2010

PNB, *p*-nitrophenyl butyrate; DPG,.1,2-dioleoyl-3-decanoyl-*rac*-glycerol; BPC, *bis*-BODIPY-FL C_{11}-PC; MG, monoacylglycerol; DG, diacylglycerol; TG, triacylglycerol; PL, phospholipids; *Experiment were performed in cells treated with cerulenin; [¥]data retrieved from (http://www.yeastgenome.org/23012011) and (http://ypl.tugraz.at/23012011)

enzyme are somehow similar to mammalian digestive lipases since (1) bile salts are not inhibitory, (2) high activity with long chain TG can be reached at a pH optimum of 6.0, and (3) proteins remain mostly stable at low pH and 37°C (Aloulou et al., 2007).

Expression of the major extracellular *Yarrowia* lipase Lip2p has recently been shown to be in part under control of so-called SOA proteins (specific for oleic acid) which participate in a new regulatory pathway of lipase gene expression (Desfougères et al., 2010). This finding indicates that two separate systems for the control of TG hydrolysis and free fatty acid oxidation exist. Najjar et al. (2010) performed experiments where *Y. lipolytica* was simultaneously fed with olive oil and glucose. As long as glucose was present in the culture medium, it acted as a repressor of lipase production, and lipase levels remained low until the entire glucose was consumed. In late growth stages the lipase activity was always lost. At this time point the pH reached alkaline values, and a potential proteolytic degradation by alkaline protease was suggested (Najjar et al., 2010). Bordes et al. (2010) recently reported the 1.7 Å resolution crystal structure of the *Y. lipolytica* Lip2p lipase in its closed conformation. The structure of Lip2p appears to be highly homologous to other members of the fungal lipase family. As a unique feature regarding the opening mechanism of the Lip2p lid a two-step procedure was suggested.

Yeast lipases as tools for biotechnological applications

Besides their value for fundamental investigation yeast lipases gained much interest for biotechnological applications due to their unique enzymatic properties. Here we will not discuss these aspects at length but only mention some prominent selected examples where yeast lipases served as useful tools for industrial processes.

Lipase B from *C. antarctica* is widely used as biocatalyst for the asymmetric synthesis of components. This lipase comprises a broad substrate specificity and high enantioselectivity. Reactions performed in aqueous to organic environment enable utilization of Lipase B in a wide range of applications such as transesterification and polymerization (Bornscheuer et al., 1999; 2002; Berglund, 2001; Fjerbaek et al., 2009). In contrast to most other lipases the active site of Lipase B is not protected by a lid, but the binding site for the hydrophobic substrate is directly exposed to the solvent (Martinelle et al., 1995).

Lipases from *Candida rugosa* have been extensively used in industrial applications to catalyze hydrolysis and the synthesis of ester compounds (Akoh et al., 2004). Crude forms of these lipases have been employed to produce food flavour enhancers (Domínguez de María et al., 2006). Unique features of *C. rugosa* lipase preparations are their extraordinary variation of enzymatic efficiency and regio- and stereo-specificity, because crude enzyme isolates contain a mixture of many isoenzymes (Xu et al., 2010). Another member of *Candida* species, *Candida cylindracea* became of special interest for biotechnological applications since it displays hydrolytic activity against TG without any further substrate specificity (Lotti et al., 1993).

Conclusions

Within the last decades, investigations of non-polar lipid metabolism gained much interest due to the link of lipid storage to health and disease. The yeast and especially *S. cerevisiae* turned out to be a valuable model system to extend our fundamental knowledge in this field. Many enzymes of non-polar lipid metabolism were discovered and characterized for the first time in yeast opening the view for further investigations with multicellular eukaryotic systems. Most importantly, studies with the yeast broadened our understanding of genetic and physiological links between enzymatic steps, biosynthetic and degradation pathways of non-polar lipids, and a number of other cellular processes. Despite this progress a number of questions remained open. Many regulatory aspects of non-polar lipid metabolism are still unanswered. Protein–protein interaction and protein-lipid interaction may be of special interest with that respect. Finally, detailed structural investigations of enzymes involved in non-polar lipid metabolism are still missing. Such evidence will be a key to understand the enzyme-substrate interaction, the accessibility of hydrolytic enzymes to specific substrates and the mobilization of non-polar lipids.

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