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Review

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Lipid biosynthesis in yeasts: A comparison of the lipid biosynthetic pathway between the model nonoleaginous yeast *Saccharomyces cerevisiae* and the model oleaginous yeast *Yarrowia lipolytica*

Lipid biosynthesis and its regulation have been studied mostly in the nonoleaginous yeast *Saccharomyces cerevisiae* that serves as a model for eukaryotic cells. On the other hand, the yeast *Yarrowia lipolytica* has been put forward as a model for oleaginous microorganisms because its genetics is known and tools for its genetic manipulation are becoming increasingly available. A comparison of the lipid biosynthetic pathways that function in these two microorganisms shows many similarities in key biosynthetic and regulatory steps. An example is the enzyme phosphatidic acid phosphatase that controls the synthesis of triacylglycerol (TAG) in both yeasts. Controlling the TAG synthesis is crucial for metabolic engineering efforts that aim to increase the production of microbial lipids (i.e. single cell oils) because TAG comprises the final product of these processes. At the same time the comparison reveals fundamental differences (e.g. in the generation of acetyl-CoA for lipid biosynthesis) stemming from the oleaginous nature of *Y. lipolytica*. These differences warrant more studies in *Y. lipolytica* where the biochemistry and molecular biology of oleaginicinity can be further explored.

Keywords: Lipid / Oleaginous / Phosphatidic acid phosphatase / *S. cerevisiae* / *Y. lipolytica*

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1 Introduction

The lipid biosynthetic pathway and its regulation in microorganisms have been elucidated mostly due to the studies done with the model yeast *Saccharomyces cerevisiae* [1–4]. These studies identified the genes and enzymes involved in this pathway and the regulatory mechanisms that govern the pathway [2–4]. On the other hand, studies on the regulation of the lipid biosynthetic pathway at the biochemical and genetic level in oleaginous microorganisms have lagged significantly behind, mainly because of the lack of a model organism. In this regard, the yeast *Yarrowia lipolytica* has been put forward as a model for oleaginous microorganisms [5–7]. Indeed, there is an increasing number of studies on the identification of key genes involved in lipid

biosynthesis and their regulation in *Y. lipolytica* [5, 6, 8–11], but still there are major gaps in our understanding of the pathway. To that end, it would be advantageous to utilize the large amount of knowledge in lipid biosynthesis gained with the nonoleaginous *S. cerevisiae*. This knowledge can provide valuable insights on the lipid biosynthetic pathways that operate in *Y. lipolytica* and other oleaginous microorganisms. A direct comparison of the lipid biosynthetic pathways that operate in these two model microorganisms can then be used as a basis for identifying the key metabolic and regulatory steps in *Y. lipolytica*.

Oleaginous microorganisms comprise a small group of microorganisms that is defined based on the ability of its members to accumulate large amounts of lipids in their cells, mainly in the form of triacylglycerol (TAG) [12–14]. The major function of TAG is thought to be the storage of energy and biosynthetic precursors [15], but it also plays role in the protection of cells against the toxic effects of fatty acids that are sequestered in TAG [16, 17]. In regards to the biotechnological applications of microbial lipids (i.e. single cell oils), TAG comprises the edible part of the lipids and the raw material for the making of biodiesel [18]. Therefore understanding the regulation of TAG biosynthesis in oleaginous

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Abbreviations: ACP, acyl carrier protein; CDP, cytidine diphosphate; CoA, coenzyme A; DAG, diacylglycerol; ER, endoplasmic reticulum; PAP, phosphatidic acid phosphatase; TAG, triacylglycerol

Figure 1. Lipid biosynthetic pathways and their subcellular localization (adapted from [3]). The gene names given in the figure come from *S. cerevisiae*. The pathway for generation of acetyl-CoA from citrate that operates in the cytosol of oleaginous yeasts is also shown. TAG, triacylglycerols; PI, phosphatidylinositol; PA, phosphatidic acid; CDP-DAG, CDP-diacylglycerol; DAG, diacylglycerol; Gro, glycerol; DHAP, dihydroxyacetone phosphate; PS, phosphatidylserine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PGP, phosphatidylglycerol phosphate; PMME, phosphatidylmonomethylethanolamine; PDME, phosphatidylmethylethanolamine; PC, phosphatidylcholine; Ins, inositol. Nucl, nucleus; ER, endoplasmic reticulum; Mito, mitochondria; LD, lipid droplets; G/E/V, Golgi, endosomes, vacuole; Cyt, cytoplasm; PM, plasma membrane.

microorganisms is of utmost importance for microbial lipid production.

Studies on this regulation done in *S. cerevisiae* revealed the *PAH1*-encoded phosphatidic acid phosphatase (PAP) as a key regulator of TAG biosynthesis [4, 19, 20]. PAP catalyzes the committed step in TAG biosynthesis, while the lack of *Pah1* (i.e. *pah1Δ* mutant) causes a massive decrease in TAG levels in *S. cerevisiae* and *Y. lipolytica* [9, 21]. Also, the *pah1Δ* mutation in *S. cerevisiae* is associated with severe growth phenotypes such as reduced life span [22], temperature sensitivity [23], susceptibility to fatty acid induced toxicity [17], and altered fatty acid profile of cellular lipids [17]. On the other hand, *S. cerevisiae* cells expressing hyperactive forms of *Pah1* present increased TAG levels [24]. These studies indicate that manipulation of *Pah1* activity in oleaginous microorganisms could contribute toward increasing the TAG levels in their lipids.

In this review, a comparison between the lipid biosynthetic pathways (Fig. 1) that operate in *S. cerevisiae* and in *Y. lipolytica* will be attempted. Gene–enzyme relationships will also be given when known (see Table 1). Gene names given in text by three uppercase italicized letters followed by a number are from *S. cerevisiae*, while the gene names in parentheses starting with the prefix *YALI* refer to the *Y. lipolytica* homologues. Protein names

are given by the gene name that encodes for the protein, first letter capitalized.

2 Sources of acetyl-coenzyme A

Lipid biosynthesis requires the continuous supply of fatty acids that are esterified in various lipid structures (e.g. phospholipids, TAG). This in turn requires a continuous supply of acetyl-coenzyme A (CoA) in the cytosol where fatty acid biosynthesis takes place (Fig. 2). In *S. cerevisiae*, the acetyl-CoA needed for fatty acid synthesis comes from the action of acetyl-CoA synthase on acetate that is produced by the glycolytic pathway [25, 26]. In fact, there are two acetyl-CoA synthases in *S. cerevisiae* encoded by *ACS1* and *ACS2* genes. It seems that both enzymes localize to the cytosol and the nucleus, but they are subject to different regulation: during growth on glucose, *ACS2* becomes essential while *ACS1* is repressed [25]. On the other hand, *Acs1* has a much higher affinity for acetate indicating that it is mostly involved in the glyoxylate cycle [27].

Yarrowia lipolytica seems to carry only one acetyl-CoA synthase encoded by *YALI0F05962g*, which is similar to *ACS2* of

Table 1. Gene homologies between *S. cerevisiae* and *Y. lipolytica*. Only genes that encode for key biosynthetic steps in the lipid pathway are shown

<i>S. cerevisiae</i> gene	<i>Y. lipolytica</i> ortholog	Function
ACS1	None	Acetyl-CoA synthetase
None	YALI0E34793g	ATP:citrate lyase subunit (<i>ACL1</i>)
None	YALI0D24431g	ATP:citrate lyase subunit (<i>ACL2</i>)
ACC1	YALI0C11407g	Acetyl-CoA carboxylase
FAS1	YALI0B15059g	Fatty acid synthase subunit
FAS2	YALI0B19382g	Fatty acid synthase subunit
OLE1	YALI0C05951g	$\Delta 9$ Fatty acid desaturase
None	YALI0B10153g	$\Delta 12$ Fatty acid desaturase
SCT1	YALI0C00209g	Gro3P acyltransferase
GPT2	None	Gro3P acyltransferase
SLC1	YALI0E18964g	1-Acyl-sn-Gro3P acyltransferase
ALE1	YALI0F19514g	1-Acyl-sn-Gro3P acyltransferase
CDS1	YALI0E14443g	CDP-DAG synthase
PI51	YALI0F20328g	Phosphatidylinositol synthase
CHO1	YALI0D08514g	Phosphatidylserine synthase
PSD1	YALI0D21604g	Phosphatidylserine decarboxylase
PSD2	None	Phosphatidylserine decarboxylase
CHO2	YALI0E06061g	Phosphatidylethanolamine methyltransferase
OPI3	YALI0E12441g	Phospholipid methyltransferase
PAH1	YALI0D27016g	Phosphatidic acid phosphatase
DGA1	YALI0E32769g	Acyl-CoA diacylglycerol O-acyltransferase
None	YALI0D07986g	Acyl-CoA diacylglycerol O-acyltransferase (<i>DGA2</i>)
LRO1	YALI0E16797g	Phospholipid:diacylglycerol acyltransferase
ARE1	YALI0F06578g	Acyl-CoA:sterol O-acyltransferase
ARE2	<i>DGA2</i> (see above)	Acyl-CoA:sterol O-acyltransferase
APP1	YALI0D02233g	Phosphatidic acid phosphatase
DPP1, LPP1 ^{a)}	YALI0C11297g YALI0B14531g	Lipid phosphatases

^{a)} It is not clear which of the two *Y. lipolytica* homologues corresponds to DPP1 and which to LPP1.

S. cerevisiae. In *Y. lipolytica*, however, most of the acetyl-CoA required for fatty acid synthesis is coming from ATP:citrate lyase (encoded by *ACL1* and *ACL2* genes). ATP:citrate lyase is found only in oleaginous yeasts and is localized in the cytosol where it cleaves the citrate that comes out of the mitochondria to oxaloacetate and acetyl-CoA [28]. This then means that a continuous efflux of citrate from the mitochondria to the cytosol is required to sustain fatty acid biosynthesis. This efflux is the result of the downregulation of the mitochondrial isocitrate dehydrogenase (YALI0E05137g, YALI0D06303g), which converts citrate to isocitrate [14]. Downregulation of this enzymatic activity causes the accumulation of citrate in the mitochondria which, after reaching a critical concentration, effluxes in exchange for cytosolic maleate. The isocitrate dehydrogenase of oleaginous microorganisms is unique in that its activity depends absolutely on AMP [12, 29]. The intracellular concentration of this nucleotide decreases when nitrogen levels in the cell drop, as is the case for cells grown in nitrogen limiting media [12]. This decrease in AMP concentration is attributed to the action of the enzyme AMP deaminase (YALI0E11495g), which cleaves AMP to inosine

monophosphate and ammonium ions. These ammonium ions can then be used to satisfy the cell needs for nitrogen, e.g. for amino acid biosynthesis. The decrease in AMP levels would then cause a drop in isocitrate dehydrogenase activity leading to the accumulation of citrate in the mitochondria and its subsequent efflux to the cytosol.

3 Sources of NADPH

Besides the need for a supply of carbon in the form of acetyl-CoA, fatty acid biosynthesis requires significant amounts of reducing energy that is provided by NADPH. The reaction catalyzed by malic enzyme has been put forward as the major source of NADPH for lipid biosynthesis in oleaginous microorganisms [30–32]. This malic enzyme activity presents dual localization in the cytosol and the mitochondria; only the cytosolic form though is thought to contribute to lipid biosynthesis [33]. Evidence for this contribution comes from metabolic engineering experiments where overexpression of the cytosolic malic enzyme in *Mucor circinelloides* caused a substantial increase in lipid content [31]. *Yarrowia lipolytica*, however, does not contain a cytosolic malic enzyme, but it contains a mitochondrial malic enzyme encoded by YALI0E18634g [10, 34]. Overexpression of the mitochondrial malic enzyme in *Y. lipolytica* did not cause any increase in the lipid content, indicating that this activity does not contribute to the NADPH pool used for fatty acid biosynthesis [34]. In *Y. lipolytica*, the required NADPH is probably supplied by the oxidative pentose phosphate pathway [11]. The enzymatic reactions involved in NADPH biosynthesis would then probably be the ones catalyzed by glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase [33].

4 Fatty acid biosynthesis

Fatty acid biosynthesis takes place mainly in the cytosol in a series of reactions that convert the biosynthetic precursor acetyl-CoA to long chain fatty acids (i.e. fatty acids that contain more than 14 carbon atoms). The first step of the conversion of acetyl-CoA to fatty acids is its carboxylation to malonyl-CoA, a reaction catalyzed by the *ACC1*-encoded acetyl-CoA carboxylase (YALI0C11407g). *Acc1* is a biotin-dependent enzyme that catalyzes an irreversible reaction that is the committed step in fatty acid biosynthesis [35]. The next steps in the fatty acid biosynthetic pathway are catalyzed by the fatty acid synthase (*Fas*) complex that is made of two subunits: *Fas1* (YALI0B15059g) and *Fas2* (YALI0B19382g). The *Fas* complex is localized in the cytosol and catalyzes a series of reactions that convert the malonyl-CoA (formed by *Acc1*) to long chain esters of CoA. This series starts with the condensation of malonyl-CoA with acetyl-CoA to form 3-keto-acyl-ACP (acyl carrier protein) that is then reduced to 3-hydroxyacyl-ACP [36]. This reduction is followed by a dehydration reaction that produces 2,3-*trans*-enoyl-ACP, which is then reduced to butyryl-ACP. This series of reactions results in the addition of two carbon atoms in the growing acyl-ACP chain that originates from malonyl-CoA. The series is repeated several times, which results in the formation of saturated acyl chains made of 16 (C16:0) or 18 (C18:0) carbon atoms. These acyl

Figure 2. Pathways for generation of acetyl-CoA for fatty acid biosynthesis in *S. cerevisiae* and *Y. lipolytica*. In *S. cerevisiae*, acetyl-CoA in the cytosol is generated by the action of acetyl-CoA synthase (Acs1) on acetate derived from glycolysis. In *Y. lipolytica* and other oleaginous microorganisms that are under nutrient limitation the acetyl-CoA in the cytosol is generated by the action of ATP:citrate lyase (Acl1/Acl2) on citrate that comes from the mitochondria (see Section 2 for a detailed description of the pathway). Abbreviations as in Fig. 1.

chains are then transferred to CoA giving rise to palmitoyl-CoA (C16:0) and stearoyl-CoA (C18:0), respectively. Palmitoyl-CoA and stearoyl-CoA can be desaturated by the Δ^9 desaturase, which is encoded by *OLE1* (YALI0C05951g), to form palmitoleyl-CoA (C16:1) or oleoyl-CoA (C18:1) [37,38]. *Saccharomyces cerevisiae* does not contain any other fatty acid desaturase enzymes and thus it only synthesizes monounsaturated fatty acids (i.e. C16:1 and C18:1) [37]. On the other hand, *Y. lipolytica* synthesizes significant amounts of linoleic acid (C18:2) that comes from the desaturation of oleic acid (C18:1) by the Δ^{12} fatty acid desaturase (YALI0B10153g). In contrast to the reactions of fatty acid biosynthesis that take place in the cytosol, desaturation reactions take place in the endoplasmic reticulum (ER) where the corresponding enzymes are localized.

The overall stoichiometry for fatty acid biosynthesis from acetyl-CoA, assuming that palmitate is the final product, is:

5 Lipid biosynthetic pathway—key enzymes

The acyl-CoAs that are generated from fatty acid synthase are channeled to the lipid biosynthetic pathway to become part of the cellular lipids (e.g. phospholipids, TAG). The lipid biosynthetic pathway has been studied extensively in *S. cerevisiae* mainly because the genetics of this yeast is well known and this has allowed for the identification and characterization of most of the genes involved in this pathway [1–3]. The biosynthetic precursor of lipids is glycerol-3-phosphate (Gro3P) that derives from the glycolytic pathway. Actually, overexpression of the Gro3P dehydrogenase *GPD1* that converts dihydroxyacetone phosphate produced from glycolysis to Gro3P increased lipid accumulation, which indicates that Gro3P plays role in the regulation of lipid biosynthesis [39]. The *de novo* lipid synthesis starts with the acylation of Gro3P by the *SCT1*- (YALI0C00209g) and

GPT2-encoded glycerol-3-phosphate acyltransferases to yield lysophosphatidic acid, which is acylated to phosphatidic acid by the *SLC1*- (YALI0E18964g) and *ALE1* (YALI0F19514g) encoded lysophospholipid acyltransferases [40]. Phosphatidic acid sits at the branching point between the cytidine diphosphate diacylglycerol (CDP-DAG) pathway, which synthesizes the membrane phospholipids, and the pathway for the synthesis of the major storage lipid TAG.

5.1 The CDP-DAG pathway

The first step in the CDP-DAG pathway is the synthesis of the liponucleotide CDP-DAG from phosphatidic acid and CTP, catalyzed by the *CDS*-encoded CDP-DAG synthase (YALI0E14443g). The next steps take place in the ER where CDP-DAG is partitioned to phosphatidylinositol, catalyzed by the *PIS1*-encoded phosphatidylinositol synthase (YALI0F20328g), or to phosphatidylserine, catalyzed by the *CHO1*-encoded phosphatidylserine synthase (YALI0D08514g). *Pis1* converts CDP-DAG to phosphatidylinositol, while *Cho1* converts CDP-DAG to phosphatidylserine. Phosphatidylserine then translocates to the mitochondria where the major phosphatidylserine decarboxylase encoded by *PSD1* (YALI0D21604g) resides. *Psd1* localizes in the inner mitochondrial membrane where it catalyzes the decarboxylation of phosphatidylserine to phosphatidylethanolamine. In addition to *Psd1*, there is a minor phosphatidylserine decarboxylase activity in *S. cerevisiae*, which is encoded by *PSD2* and this activity is found in the Golgi and vacuole [41]. In the next steps of the CDP-DAG pathway, phosphatidylethanolamine is converted to phosphatidylcholine through a series of sequential methylation reactions. This series of reactions is catalyzed by two enzymes: the first methylation reaction that converts phosphatidylethanolamine to phosphatidyl-N-monomethylethanolamine is catalyzed by the *CHO2*-encoded phosphatidylethanolamine methyltransferase (YALI0E06061g), while the next two steps are

catalyzed by the *OPI3*-encoded phospholipid methyltransferase (YALI0E12441g). *Opi3* catalyzes the methylation of phosphatidyl-N-monomethylethanolamine to phosphatidyl-N,N-dimethylethanolamine and the conversion of the latter to phosphatidylcholine, the final product of the pathway.

5.2 TAG biosynthesis

As discussed in the previous section, phosphatidic acid can either be channeled to phospholipid biosynthesis through the CDP-DAG pathway (described in 5.1) or to TAG biosynthesis. The latter pathway starts with the dephosphorylation of phosphatidic acid to DAG, a reaction catalyzed by the *PAH1*-encoded PAP [23, 42]. This reaction supplies the bulk of DAG required for TAG biosynthesis [17, 43]. Indeed, recent studies indicate that *Pah1* is a major regulator of lipid biosynthesis [4] and affects the growth and physiology of cells [22]. Because of its essential role in regulating lipid biosynthesis, *Pah1* will be discussed in detail in the next section.

The DAG that is generated from the action of *Pah1* on phosphatidic acid is acylated by acyl-CoA diacylglycerol O-acyltransferase (DGAT) to form TAG [44]. The major DGAT activity is encoded by *DGA1* in *S. cerevisiae* [45], while in *Y. lipolytica* there are two genes that encode for DGAT activity, namely *DGA1* (YALI0E32769g) [6] and *DGA2* (YALI0D07986g) [8]. However, as in *S. cerevisiae*, *Dga1* is responsible for the majority of TAG synthesis in *Y. lipolytica* [8]. In both yeasts, *Dga1* shows a dual localization in the lipid particles and the ER [46, 47], while nothing is known about the localization of *Dga2* in *Y. lipolytica*.

DGAT has been the target of several efforts aimed to increase the lipid content in both yeasts. In *S. cerevisiae*, overexpression of *DGA1* caused a twofold increase in lipid content [48]. This increase is even more pronounced when *DGA1* is overexpressed in cells lacking the transcription factor *Snf2* (i.e. *snf2Δ* mutant) which is involved in the regulation of phospholipid synthesis enzymes [48]. Subsequent studies revealed that the activation of *DGA1* in the *snf2Δ* background was due to proteolytic cleavage at the N-terminal region of *Dga1* [49]. Indeed, overexpression of the truncated form of *Dga1* in *S. cerevisiae* increases the lipid content of the cells to 45%, making these cells oleaginous [50]. On the other hand, overexpression of *DGA1* in *Y. lipolytica* increased lipid production fourfold [51].

The DGAT reaction accounts for the bulk of TAG synthesis in both yeasts, but a separate acyl-CoA-independent pathway exists, which uses the *sn*-2 acyl group of phosphatidylcholine instead of acyl-CoA as the acyl donor. This acylation reaction is catalyzed by the *LRO1*-encoded phospholipid:diacylglycerol acyltransferase (YALI0E16797g) activity, which is found in both yeasts [6, 52]. The *Lro1* reaction contributes to TAG biosynthesis mostly during exponential phase, while the *Dga1* reaction is responsible for most of the TAG produced during the stationary phase [6, 45]. Deletion of both genes in *S. cerevisiae* (i.e. *dga1Δlro1Δ* strain) abolished more than 90% of TAG biosynthesis in both exponential and stationary growth phases [45]. The residual minor DAG acylating activity in *S. cerevisiae* has been attributed to the acyl-CoA:sterol O-acyltransferases encoded by *ARE1* and *ARE2*, the major function of which is the synthesis of sterol esters [53]. Indeed, the *S. cerevisiae* quadruple mutant *dga1Δlro1*

Δ are1Δare2Δ does not contain any TAG or sterol esters, but is viable and does not exhibit any significant growth defects [53]. On the other hand, deletion of both *DGA1* and *LRO1* in *Y. lipolytica* decreases the amount of TAG by only 70% in stationary phase [6]. This is because of the presence of an active *DGA2* (the *ARE2* homologue—YALI0D07986g) in the *dga1Δlro1Δ* strain, which contributes to the acylation of DAG [8]. *Yarrowia lipolytica* also contains an *ARE1* (YALI0F06578g) gene that seems to be involved in TAG biosynthesis [8]. As in *S. cerevisiae*, deletion of all four genes (i.e. *dga1Δdga2Δlro1Δare1Δ*) in *Y. lipolytica* abolishes TAG synthesis, while the quadruple mutant does not show any growth defects [8].

6 The role of PAP enzymes in TAG biosynthesis

The role of the PAP reaction in the biosynthesis of TAG in *S. cerevisiae* was first reported by Hosaka and Yamashita in 1984 [54]. Subsequent studies revealed that the activity reported back in 1984 was actually due to four different gene products (i.e. *PAH1*, *APP1*, *DPPI*, *LPP1*) [4, 55]. Of those four, *Pah1* (YALI0D27016g) has been established as the major activity responsible for TAG biosynthesis [21, 23]. Indeed, deletion of the *PAH1* gene (i.e. *pah1Δ*) decreases cellular TAG levels by more than 90% in stationary phase cells of *S. cerevisiae* [23]. Similar results were obtained with the *Y. lipolytica* *pah1Δ* mutant [9], although this mutant was grown on oleic acid media and not glucose like in *S. cerevisiae*. This massive decrease in TAG levels in both yeasts, caused by the deletion of *PAH1*, points to the regulatory role *Pah1* activity plays in TAG biosynthesis in both oleaginous and nonoleaginous microorganisms. Along with the decrease in TAG levels, the *pah1Δ* mutation causes a great increase in the phospholipid levels, which causes the expansion of the ER membrane, a phenotype characteristic of the *pah1Δ* mutant [9, 21]. Also, *pah1Δ* cells are temperature sensitive [21] and exhibit a reduced chronological life span [22]. Lack of *Pah1* causes a decrease in lipid droplet number and this is thought to be due to the low availability of DAG produced from the *Pah1*-catalyzed reaction [56]. On the other hand, overexpression of the *Pah1* activity leads to cell death [24, 42]. These severe phenotypes caused by the deregulation of *Pah1* activity indicate that this activity should be tightly regulated in the cells.

The regulation of *Pah1* activity has been studied in detail in *S. cerevisiae* while no studies have been done with the *Y. lipolytica* enzyme. *Pah1* belongs to a group of enzymes known as Mg^{2+} -dependent PAPs and bears the DXDX(T/V) catalytic motif in its HAD-like domain, which is characteristic for these enzymes [23]. *Pah1* localizes mostly in the cytosol but translocates to the ER, where the substrate phosphatidic acid resides, to catalyze its reaction [57]. In *S. cerevisiae*, *Pah1* activity increases during growth and culminates at the stationary phase where TAG biosynthesis increases [20], which is in line with the regulatory role of *Pah1* in TAG biosynthesis. The *Pah1* activity is regulated at different levels in the cell. Protein abundance is regulated by proteasome-mediated degradation, which takes place mostly in stationary phase [58]. The degradation of the *Pah1* protein in stationary phase is thought as a protective mechanism against the

detrimental effects of increased Pah1 activity [58]. The activity itself is regulated by the phosphorylation of the Pah1 protein by various kinases and these phosphorylations decrease the enzymatic activity [24, 42, 59]. The Nem1-Spo7 phosphatase counteracts this effect by dephosphorylating Pah1 thus making it more active [42]. The tight regulation of Pah1 activity by the mechanisms described ensures that the cell is protected from the detrimental effects of the Pah1 deregulation.

The action of Pah1 on phosphatidic acid is counteracted by the *DGK1*-encoded DAG kinase, which converts the DAG generated from Pah1 back to phosphatidic acid [60]. Dgk1 of *S. cerevisiae* is unique in that it uses CTP instead of ATP as the phosphate donor for the phosphorylation of DAG [60, 61]. Deletion of *DGK1* in the *pah1Δ* background (i.e. *pah1Δdkg1Δ*) rescues the phenotypes caused by the *pah1Δ* mutation [60, 61], which means that Pah1 and Dgk1 work in concert to regulate the levels of phosphatidic acid. Also, lack of Dgk1 activity (i.e. *dkg1Δ*) causes delayed resumption of growth in *S. cerevisiae* cells exiting the stationary phase [62]. This is probably because Dgk1 converts the DAG generated from the TAG hydrolysis that takes place in cells exiting stationary phase, to phosphatidic acid that is being used to synthesize membrane phospholipids needed for rapid growth initiation [62].

Besides *PAH1*, there are three more genes in *S. cerevisiae* that encode for PAP activity, these are *DPP1* [63], *LPP1* [64], and *APP1* [55]. App1 (YALI0D02233g) is the newest member of these enzymes. App1 is also an Mg^{2+} -dependent PAP enzyme that is found in cortical actin patches, which are thought to be involved in endocytosis [55, 65]. The physiological role of App1 is not clear: it is known that it does not contribute to TAG biosynthesis, but it could play role in endocytosis [55]. Dpp1 and Lpp1 (YALI0C11297g/YALI0B14531g) are lipid phosphatase enzymes that are not specific to phosphatidic acid; instead, they also catalyze the dephosphorylation of lipid compounds, such as DAG pyrophosphate and lysophosphatidic acid. These enzymes are integral membrane proteins that do not require Mg^{2+} for activity and are not involved in TAG biosynthesis [4].

7 Global regulators of lipid biosynthesis

The regulation of the lipid biosynthetic pathway by global regulators has drawn much attention recently because these are thought as obvious targets for manipulating the pathway toward increasing lipid production. Snf1 belongs to the SNF1/AMPK protein kinase family, the members of which have been implicated in energy homeostasis through monitoring of cellular ATP levels [66]. Deletion of *SNF1* in *S. cerevisiae* causes inositol auxotrophy by decreasing the expression of the *INO1* gene, a major regulator of phospholipid biosynthesis [67]. In *Y. lipolytica*, deletion of *SNF1* gene caused the cells to accumulate lipids in the presence of nitrogen, resulting in a constitutive lipid accumulation phenotype [66]. This was due to, at least in part, the increased expression of some genes of the lipid biosynthetic pathway, indicating that Snf1 might also act as a transcription factor [66].

Interestingly, in *S. cerevisiae* the transcription factor Mig1 is a target for Snf1 phosphorylation, which takes place in response to low glucose levels and causes Mig1 to translocate from the

nucleus to the cytoplasm [68, 69]. In the nucleus, Mig1 represses the transcription of several genes so its translocation to the cytoplasm relieves that repression. In *Y. lipolytica*, disruption of *MIG1* increases the lipid content of cells and this is accompanied by an increase in the number of lipid droplets [70]. Also, the disruption of *MIG1* causes an increase in the expression of several genes involved in lipid biosynthesis, such as *ACL1* and *ACL2*, while it increases the expression levels of acyl-CoA oxidases, encoded by *POX1-6* genes, which are involved in lipid degradation. The transcriptional regulation exerted by Mig1 is probably mediated by a (G/C)(C/T)G motif present in the promoter of these genes (e.g. *ACL1*, *POX1*, *POX5*) that Mig1 recognizes and binds to [70].

Mga2 has been sought as a target for genetic manipulation since its overproduction has been linked to an upregulation of TAG synthesis, which is accompanied by an increase in size of lipid droplets [71]. In *S. cerevisiae*, Mga2 induces the transcription of the *OLE1*-encoded desaturase gene under hypoxic conditions or when cells are shifted to low temperatures (i.e. 10° C) [72]. Also in *S. cerevisiae*, disruption of *MGA2* causes a reduction of C16:1 and an increase in C18:1 levels, which is consistent with a role of Mga2 in the regulation of *OLE1* transcription [73]. *Yarrowia lipolytica* strains that express a mutant form of Mga2 that carries a single amino acid substitution (i.e. G643R) present increased lipogenesis, as indicated by Nile Red staining [74]. Analysis of the fatty acid profile of the *mga2Δ* and *MGA2*(G643R) mutants showed a shift toward higher unsaturation exhibited by an increase in both C16:1 and C18:1 levels, which is different than the trend seen in the *S. cerevisiae mga2Δ* mutant [74]. The expression of *MGA2*(G643R) in the *mga2Δ* mutant of *Y. lipolytica* caused a transcriptional rewiring presented by changes in the expression levels of many genes, but more importantly it increased the expression of genes of the fatty acid biosynthetic pathway and at the same time decreased the expression of genes involved in fatty acid metabolism [74]. Taken together, these results suggest that Mga2 is a potential target for lipid overproduction strategies.

8 Metabolic engineering of *Y. lipolytica* for production of microbial lipids

Some of the latest developments on microbial lipids focus on the use of metabolic engineering for the production of food grade lipids that can be used as dietary supplements. Indeed, there is a growing demand for lipids that contain high amounts of polyunsaturated fatty acids because these lipids can protect against heart disease and have been shown to decrease triglyceride levels in patients with severe hypertriglyceridemia [75]. Currently, the major source of these lipids are fish oils that present several drawbacks such as limited availability because of decreasing fish numbers and contamination with heavy metals ingested by the fish [76, 77]. Alternative sources are needed and certainly microbial lipids can fill this gap [18, 78, 79]. To that end, several metabolic engineering efforts have been made for producing polyunsaturated lipids in large scale and these efforts culminated with the development of a process for the production

of lipids rich in eicosapentanoic acid from engineered *Y. lipolytica* cells [75]. However, most of the current work in *Y. lipolytica* focuses on increasing overall lipid production by metabolic engineering [80]. Some of the recent advances in the field will be discussed next.

As mentioned in Section 5.2, *DGA1* has drawn much attention as a target for metabolic engineering because of its pivotal role in TAG biosynthesis. For example, increased coexpression of *DGA1* and *ACC1* caused a fivefold increase in the lipid content of cells [51]. When this strategy is combined with an overexpression of the *OLE1* gene, it causes a further increase of the lipid content by 25% [81]. Interestingly, *OLE1* overexpression increases glucose tolerance and the growth rate of cells, and these improvements lead to a significantly enhanced productivity and decreased the fermentation time [81]. The mechanism by which *OLE1* overexpression leads to increased lipid biosynthesis seems to be the alleviation of the repression of *Acc1* exerted by saturated fatty acids (i.e. stearic and palmitic acids). Indeed, *OLE1* overexpression increases the unsaturated fatty acid content at the expense of saturated fatty acids by converting stearyl-CoA to oleyl-CoA, which is used for phospholipid synthesis during exponential phase and TAG synthesis during stationary phase [81]. A total rewiring of the lipid biosynthetic pathway that included several target genes involved in lipid biosynthesis and degradation, and leucine and uracil biosynthesis was reported. In this work, overexpression of *DGA1* along with the deletion of *PEX10*, which prevents peroxisome biogenesis, and *MFE1*, which prevents β -oxidation, led to a lipid content of 74% [82]. Further cultivation of the strain in a bioreactor under well-controlled conditions increased the lipid content to 88%, the highest reported for *Y. lipolytica* [82].

Pyruvate carboxylase catalyzes the conversion of pyruvic acid to oxalic acid, which then enters the citric acid cycle. Since citric acid is the precursor of acetyl-CoA that is used for fatty biosynthesis in oleaginous microorganisms, pyruvate carboxylase manipulation could affect lipid biosynthesis. In *Y. lipolytica*, pyruvate carboxylase is encoded by a single gene named *PYC1* (YALI0C24101g) [83]. Heterologous expression of *PYC1* from *Pichia guilliermondii* in *Y. lipolytica* increased the lipid content of cells, while simultaneous overexpression of *ACL1* further increased the lipid content [84]. The mutant strain expressing both *PYC1* and *ACL1* contained about 50% lipids, which represents a significant enhancement over the original strain.

9 Biotechnological applications of yeast lipids

The composition of lipids produced from wild-type yeast strains is similar to the bulk plant oils and thus these lipids have been considered as raw material for the production of the so called third-generation biodiesel [18]. This biodiesel is produced from waste materials, such as raw glycerol, which can be converted to yeast lipids [85–87]. Despite the metabolic engineering efforts reported above, the cost for the production of yeast lipids is still high, more than double the cost for the production of the same amount of plant oils. This means that the economic viability of the yeast biodiesel is uncertain. A case has been made though that besides the costs, one should consider the environmental

implications of using plant oils for the making of biodiesel [88, 89]. Indeed, the demand for oils for human consumption is increasing, which means that more arable land will need to be allocated for the production of edible oils. This also means that the production of more plant-derived biodiesel would compete with edible oils for land and resources [88]. In this scenario, yeast lipids may play role as viable alternatives to plant lipids. However, yeast lipids would also have to compete with algal lipids, which seem to be the preferred raw material for biodiesel [90]. Yeasts, however, could achieve higher growth rates and higher biomass production than algae, which means that they can achieve higher biodiesel productivities.

An alternative to biodiesel is the production of high-value lipids that contain polyunsaturated fatty acids for human nutrition [14, 76, 91]. Indeed, these lipids command high prices so this could make up for the increased production costs [76]. This route had not been possible for yeast lipids before the advent of metabolic engineering, which allowed for the manipulation of the fatty acid profile of yeast lipids. Actually, wild-type *Y. lipolytica* lipids that are made mostly of monounsaturated (i.e. oleic) and diunsaturated (i.e. linoleic) fatty acids can be converted to polyunsaturated oils with the heterologous expression of the proper genes. This task though is far from trivial. A combined heterologous expression of several desaturases and elongases is needed for yeast cells to be able to synthesize polyunsaturated fatty acids. This pathway was engineered in *Y. lipolytica* that was now able to produce the highly unsaturated long chain fatty acid, eicosapentanoic acid (EPA - C20:5) [75]. The engineered *Y. lipolytica* strain was constructed by DuPont that developed a process for the commercial production of this oil [75]. The oil got out on the market in 2010 under the name New Harvest and was advertised as vegetarian eicosapentanoic acid that helps support a healthy heart and positive mood. The actual volume sold is not known, but it seems that this oil is currently out of the market. Nevertheless, this production process gave the proof of concept that *Y. lipolytica* can be engineered to produce polyunsaturated fatty acids. Should a need arise in the future, *Y. lipolytica* can be used as a platform to produce a variety of polyunsaturated fatty acids.

Polyunsaturated fatty acids have been the main targets of microbial lipid biotechnology but some commercial opportunities may exist for some specialty fatty acids. One group of fatty acids that might become important is conjugated fatty acids and that is because of their health benefits [92–95]. Actually, conjugated linoleic acids are thought to fight obesity by decreasing energy intake and increasing the mobilization of fat along with fatty acid oxidation [94]. Since the natural sources of conjugated linoleic acids are limited, biotechnological approaches have been sought for the production of these fatty acids. Heterologous expression of the linoleic acid isomerase gene from *Propionibacterium acnes* along with the delta 12-desaturase gene from *Mortierella alpina* in *Y. lipolytica* yielded an engineered strain capable of producing *trans*-10, *cis*-12-conjugated linoleic acid [96, 97]. Supplementation of the *Y. lipolytica* culture medium with soybean oil, which contains high amounts of linoleic acid, further increased the conjugated linoleic acid yields [97]. Although this process has not made it to large scale yet, it shows promise for the production of conjugated linoleic acids that could be used as dietary supplements.

Besides dietary applications, fatty acids and their derivatives have been used as raw materials for the oleochemical industry. For example, ricinoleic acid produced from castor oil has a variety of applications that include its use as precursor for the synthesis of biodegradable polymers, cosmetics, and pharmaceuticals. The fluctuation of castor oil prices and the toxicity of the seed against humans has led to the development of alternative production processes. Heterologous expression in *Y. lipolytica* of the delta 12-hydroxylase enzyme from the castor bean plant *Ricinus communis* or from the fungus *Claviceps purpurea* (a known producer of ricinoleic acid) along with the deletion of delta 12-desaturase that competes for the oleic acid substrate yielded a strain that could produce ricinoleic acid, although at very low amounts [98]. Further engineering of the strain by overexpressing of the *LRO1*-encoded acyltransferase increased the content of ricinoleic acid in lipids to more than 40% of the total fatty acids [98]. However, the overall ricinoleic acid yields were still low indicating the need for further genetic manipulation to increase the yields.

As lipids continue to be on the spotlight of biomedical research our knowledge of their biological functions and health benefits continues to increase. This new knowledge may identify novel targets for biotechnology or reinvigorate the interest in well-known production processes that are currently suspended. As new lipid products are sought and old processes are revisited, the use of the metabolic engineering tools developed in the last decade would drive the field of lipid biotechnology.

10 Concluding remarks

Oleaginous microorganisms are better poised to provide information on the regulation of lipid accumulation since their lipid biosynthetic machinery is geared toward TAG biosynthesis. However, the studies done on the regulation of the genes and enzymes of the lipid biosynthetic pathway in oleaginous microorganisms are far less than those done in the nonoleaginous *S. cerevisiae*. Such studies are warranted by the interest in the regulation of the lipid biosynthetic pathway as it relates to lipid accumulation and the biotechnological production of lipids. For example, studies on the regulation of *Pah1* activity in *Y. lipolytica* would shed more light on the regulation of the lipid biosynthetic pathway in this yeast and might identify targets for metabolic engineering.

Most studies on metabolic engineering for lipid production focus on targets identified by previous work on the regulation of lipid biosynthesis. These targets include genes involved in the biosynthesis of fatty acids (e.g. *ACC1*, *ACL1*) and genes involved in lipid biosynthesis (e.g. *DGA1*). The strategies presented here underline a common theme that is the coordinated manipulation of more than one gene is necessary to substantially increase lipid production. At the same time, these studies showcase the feasibility of rewiring the cell metabolic machinery toward lipid synthesis. Indeed, the nonoleaginous *S. cerevisiae* was engineered to become oleaginous and accumulate lipids, and the low accumulating *Y. lipolytica* (i.e. wild-type strains accumulate about 30% lipids) was engineered to accumulate 90% lipids in its biomass. Further understanding of the regulation of the lipid biosynthetic machinery will certainly reveal novel targets for manipulation.

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11 References

- [1] Carman, G. M., Han, G.-S., Regulation of phospholipid synthesis in yeast. *J. Lipid Res.* 2009, 50, S69–S73.
- [2] Carman, G. M., Han, G.-S., Regulation of phospholipid synthesis in the yeast *Saccharomyces cerevisiae*. *Annu. Rev. Biochem.* 2011, 80, 859–883.
- [3] Henry, S. A., Kohlwein, S. D., Carman, G. M., Metabolism and regulation of glycerolipids in the yeast *Saccharomyces cerevisiae*. *Genetics* 2012, 190, 317–349.
- [4] Pascual, F., Carman, G. M., Phosphatidate phosphatase, a key regulator of lipid homeostasis. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 2013, 1831, 514–522.
- [5] Beopoulos, A., Mrozova, Z., Thevenieau, F., Le Dall, M. T. et al., Control of lipid accumulation in the yeast *Yarrowia lipolytica*. *Appl. Environ. Microbiol.* 2008, 74, 7779–7789.
- [6] Athenstaedt, K., YALI0E32769g (*DGA1*) and YALI0E16797g (*LRO1*) encode major triacylglycerol synthases of the yeast *Yarrowia lipolytica*. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 2011, 1811, 587–596.
- [7] Koch, B., Schmidt, C., Daum, G., Storage lipids of yeasts: a survey of nonpolar lipid metabolism in *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Yarrowia lipolytica*. *FEMS Microbiol. Rev.* 2014, 38, 892–915.
- [8] Beopoulos, A., Haddouche, R., Kabran, P., Dulermo, T. et al., Identification and characterization of *DGA2*, an acyltransferase of the *DGAT1* acyl-CoA:diacylglycerol acyltransferase family in the oleaginous yeast *Yarrowia lipolytica*. New insights into the storage lipid metabolism of oleaginous yeasts. *Appl. Microbiol. Biotechnol.* 2012, 93, 1523–1537.
- [9] Guerfal, M., Claes, K., Knittelfelder, O., De Rycke, R. et al., Enhanced membrane protein expression by engineering increased intracellular membrane production. *Microb. Cell Fact.* 2013, 12, 1–10.
- [10] Zhang, H., Zhang, L., Chen, H., Chen, Y. et al., Regulatory properties of malic enzyme in the oleaginous yeast, *Yarrowia lipolytica*, and its non-involvement in lipid accumulation. *Biotechnol. Lett.* 2013, 35, 2091–2098.
- [11] Wasylenko, T. M., Ahn, W. S., Stephanopoulos, G., The oxidative pentose phosphate pathway is the primary source of NADPH for lipid overproduction from glucose in *Yarrowia lipolytica*. *Metab. Eng.* 2015, 30, 27–39.
- [12] Botham, P., Ratledge, C., A biochemical explanation for lipid accumulation in *Candida* 107 and other oleaginous microorganisms. *J. Gen. Microbiol.* 1979, 114, 361–375.
- [13] Ratledge, C., Regulation of lipid accumulation in oleaginous microorganisms. *Biochem. Soc. Trans.* 2002, 30, 1047–1050.
- [14] Ratledge, C., Wynn, J., The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. *Adv. Appl. Microbiol.* 2002, 51, 1–51.

- [15] Wagner, A., Daum, G., Formation and mobilization of neutral lipids in the yeast *Saccharomyces cerevisiae*. *Biochem. Soc. Trans.* 2005, 33, 1174–1177.
- [16] Garbarino, J., Padamsee, M., Wilcox, L., Oelkers, P. M. et al., Sterol and diacylglycerol acyltransferase deficiency triggers fatty acid-mediated cell death. *J. Biol. Chem.* 2009, 284, 30994–31005.
- [17] Fakas, S., Qiu, Y., Dixon, J. L., Han, G.-S. et al., Phosphatidate phosphatase activity plays key role in protection against fatty acid-induced toxicity in yeast. *J. Biol. Chem.* 2011, 286, 29074–29085.
- [18] Papanikolaou, S., Aggelis, G., Lipids of oleaginous yeasts. Part II: Technology and potential applications. *Eur. J. Lipid Sci. Technol.* 2011, 113, 1052–1073.
- [19] Soto-Cardalda, A., Fakas, S., Pascual, F., Choi, H.-S. et al., Phosphatidate phosphatase plays role in zinc-mediated regulation of phospholipid synthesis in yeast. *J. Biol. Chem.* 2012, 287, 968–977.
- [20] Pascual, F., Soto-Cardalda, A., Carman, G. M., *PAH1*-encoded phosphatidate phosphatase plays a role in the growth phase- and inositol-mediated regulation of lipid synthesis in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 2013, 288, 35781–35792.
- [21] Han, G.-S., Siniosoglou, S., Carman, G. M., The cellular functions of the yeast lipin homolog *Pah1p* are dependent on its phosphatidate phosphatase activity. *J. Biol. Chem.* 2007, 282, 37026–37035.
- [22] Park, Y., Han, G.-S., Mileykovskaya, E., Garrett, T. A. et al., Altered lipid synthesis by lack of yeast *Pah1* phosphatidate phosphatase reduces chronological life span. *J. Biol. Chem.* 2015, 290, 25382–25394.
- [23] Han, G.-S., Wu, W.-I., Carman, G. M., The *Saccharomyces cerevisiae* lipin homolog is a Mg^{2+} -dependent phosphatidate phosphatase enzyme. *J. Biol. Chem.* 2006, 281, 9210–9218.
- [24] Choi, H. S., Su, W.-M., Morgan, J. M., Han, G.-S. et al., Phosphorylation of phosphatidate phosphatase regulates its membrane association and physiological functions in *Saccharomyces cerevisiae* Identification of $SER602$, THR^{723} , and SER^{744} as the sites phosphorylated by *CDC28* (*CDK1*)-encoded cyclin-dependent kinase. *J. Biol. Chem.* 2011, 286, 1486–1498.
- [25] Galdieri, L., Zhang, T., Rogerson, D., Lleshi, R. et al., Protein acetylation and acetyl coenzyme A metabolism in budding yeast. *Eukaryot. Cell* 2014, 13, 1472–1483.
- [26] Krivoruchko, A., Zhang, Y., Siewers, V., Chen, Y. et al., Microbial acetyl-CoA metabolism and metabolic engineering. *Metab. Eng.* 2015, 28, 28–42.
- [27] van den Berg, M. A., de Jong-Gubbels, P., Kortland, C. J., van Dijken, J. P. et al., The two acetyl-coenzyme A synthetases of *Saccharomyces cerevisiae* differ with respect to kinetic properties and transcriptional regulation. *J. Biol. Chem.* 1996, 271, 28953–28959.
- [28] Dulerio, T., Lazar, Z., Dulerio, R., Rakicka, M. et al., Analysis of ATP-citrate lyase and malic enzyme mutants of *Yarrowia lipolytica* points out the importance of mannitol metabolism in fatty acid synthesis. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 2015, 1851, 1107–1117.
- [29] Evans, C. T., Scragg, A. H., Ratledge, C., Regulation of citrate efflux from mitochondria oleaginous and non-oleaginous yeasts by adenine nucleotides. *Eur. J. Biochem.* 1983, 132, 609–615.
- [30] Wynn, J., Ratledge, C., Malic enzyme is a major source of NADPH for lipid accumulation by *Aspergillus nidulans*. *Microbiology* 1997, 143, 253–257.
- [31] Zhang, Y., Adams, I. P., Ratledge, C., Malic enzyme: the controlling activity for lipid production? Overexpression of malic enzyme in *Mucor circinelloides* leads to a 2.5-fold increase in lipid accumulation. *Microbiology* 2007, 153, 2013–2025.
- [32] Wynn, J., Hamid, A., Ratledge, C., The role of malic enzyme in the regulation of lipid accumulation in filamentous fungi. *Microbiology* 1999, 145, 1911–1917.
- [33] Ratledge, C., The role of malic enzyme as the provider of NADPH in oleaginous microorganisms: a reappraisal and unsolved problems. *Biotechnol. Lett.* 2014, 36, 1557–1568.
- [34] Beopoulos, A., Cescut, J., Haddouche, R., Uribealarea, J.-L. et al., *Yarrowia lipolytica* as a model for bio-oil production. *Prog. Lipid Res.* 2009, 48, 375–387.
- [35] Hasslacher, M., Ivessa, A. S., Paltauf, F., Kohlwein, S. D., Acetyl-CoA carboxylase from yeast is an essential enzyme and is regulated by factors that control phospholipid metabolism. *J. Biol. Chem.* 1993, 268, 10946–10952.
- [36] Tehlivets, O., Scheuringer, K., Kohlwein, S. D., Fatty acid synthesis and elongation in yeast. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 2007, 1771, 255–270.
- [37] Martin, C. E., Oh, C.-S., Jiang, Y., Regulation of long chain unsaturated fatty acid synthesis in yeast. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 2007, 1771, 271–285.
- [38] Stuke, J. E., McDonough, V. M., Martin, C. E., Isolation and characterization of *OLE1*, a gene affecting fatty acid desaturation from *Saccharomyces cerevisiae*. *J. Biol. Chem.* 1989, 264, 16537–16544.
- [39] Dulerio, T., Nicaud, J.-M., Involvement of the G3P shuttle and β -oxidation pathway in the control of TAG synthesis and lipid accumulation in *Yarrowia lipolytica*. *Metab. Eng.* 2011, 13, 482–491.
- [40] Carman, G. M., Henry, S. A., Phosphatidic acid plays a central role in the transcriptional regulation of glycerophospholipid synthesis in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 2007, 282, 37293–37297.
- [41] Trotter, P. J., Voelker, D. R., Identification of a non-mitochondrial phosphatidylserine decarboxylase activity (*PSD2*) in the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* 1995, 270, 6062–6070.
- [42] O'Hara, L., Han, G.-S., Peak-Chew, S., Grimsey, N. et al., Control of phospholipid synthesis by phosphorylation of the yeast lipin *Pah1p/Smp2p* Mg^{2+} -dependent phosphatidate phosphatase. *J. Biol. Chem.* 2006, 281, 34537–34548.
- [43] Carman, G. M., Han, G.-S., Phosphatidic acid phosphatase, a key enzyme in the regulation of lipid synthesis. *J. Biol. Chem.* 2009, 284, 2593–2597.
- [44] Sorger, D., Daum, G., Triacylglycerol biosynthesis in yeast. *Appl. Microbiol. Biotechnol.* 2003, 61, 289–299.
- [45] Oelkers, P., Cromley, D., Padamsee, M., Sturley, S., The *DGA1* gene determines a second triglyceride synthetic pathway in yeast. *J. Biol. Chem.* 2002, 277, 8877–8881.
- [46] Sorger, D., Daum, G., Synthesis of triacylglycerols by the acyl-coenzyme A:diacyl-glycerol acyltransferase *Dga1p* in lipid particles of the yeast *Saccharomyces cerevisiae*. *J. Bacteriol.* 2002, 184, 519–524.

- [47] Athenstaedt, K., Jolivet, P., Boulard, C., Zivy, M. et al., Lipid particle composition of the yeast *Yarrowia lipolytica* depends on the carbon source. *Proteomics* 2006, 6, 1450–1459.
- [48] Kamisaka, Y., Tomita, N., Kimura, K., Kainou, K. et al., *DGA1* (diacylglycerol acyltransferase gene) overexpression and leucine biosynthesis significantly increase lipid accumulation in the $\Delta snf2$ disruptant of *Saccharomyces cerevisiae*. *Biochem. J.* 2007, 408, 61–68.
- [49] Kamisaka, Y., Kimura, K., Uemura, H., Shibakami, M., Activation of diacylglycerol acyltransferase expressed in *Saccharomyces cerevisiae*: overexpression of Dga1p lacking the N-terminal region in the $\Delta snf2$ disruptant produces a significant increase in its enzyme activity. *Appl. Microbiol. Biotechnol.* 2010, 88, 105–115.
- [50] Kamisaka, Y., Kimura, K., Uemura, H., Yamaoka, M., Overexpression of the active diacylglycerol acyltransferase variant transforms *Saccharomyces cerevisiae* into an oleaginous yeast. *Appl. Microbiol. Biotechnol.* 2013, 97, 7345–7355.
- [51] Tai, M., Stephanopoulos, G., Engineering the push and pull of lipid biosynthesis in oleaginous yeast *Yarrowia lipolytica* for biofuel production. *Metab. Eng.* 2013, 15, 1–9.
- [52] Oelkers, P., Tinkelenberg, A., Erdeniz, N., Cromley, D. et al., A lecithin cholesterol acyltransferase-like gene mediates diacylglycerol esterification in yeast. *J. Biol. Chem.* 2000, 275, 15609–15612.
- [53] Petschnigg, J., Wolinski, H., Kolb, D., Zellnig, G. et al., Good fat, essential cellular requirements for triacylglycerol synthesis to maintain membrane homeostasis in yeast. *J. Biol. Chem.* 2009, 284, 30981–30993.
- [54] Hosaka, K., Yamashita, S., Regulatory role of phosphatidate phosphatase in triacylglycerol synthesis of *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* 1984, 796, 110–117.
- [55] Chae, M., Han, G.-S., Carman, G. M., The *Saccharomyces cerevisiae* actin patch protein App1p is a phosphatidate phosphatase enzyme. *J. Biol. Chem.* 2012, 287, 40186–40196.
- [56] Adeyo, O., Horn, P. J., Lee, S., Binns, D. D. et al., The yeast lipin orthologue Pah1p is important for biogenesis of lipid droplets. *J. Cell Biol.* 2011, 192, 1043–1055.
- [57] Karanasios, E., Han, G.-S., Xu, Z., Carman, G. M. et al., A phosphorylation-regulated amphipathic helix controls the membrane translocation and function of the yeast phosphatidate phosphatase. *Proc. Natl. Acad. Sci. USA* 2010, 107, 17539–17544.
- [58] Pascual, F., Hsieh, L.-S., Soto-Cardalda, A., Carman, G. M., Yeast Pah1p phosphatidate phosphatase is regulated by proteasome-mediated degradation. *J. Biol. Chem.* 2014, 289, 9811–9822.
- [59] Su, W.-M., Han, G.-S., Carman, G. M., Cross-talk phosphorylations by protein kinase C and Pho85p-Pho80p protein kinase regulate Pah1p phosphatidate phosphatase abundance in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 2014, 289, 18818–18830.
- [60] Han, G.-S., O'Hara, L., Carman, G. M., Siniosoglou, S., An unconventional diacylglycerol kinase that regulates phospholipid synthesis and nuclear membrane growth. *J. Biol. Chem.* 2008, 283, 20433–20442.
- [61] Han, G.-S., O'Hara, L., Siniosoglou, S., Carman, G. M., Characterization of the yeast *DGK1*-encoded CTP-dependent diacylglycerol kinase. *J. Biol. Chem.* 2008, 283, 20443–20453.
- [62] Fakas, S., Konstantinou, C., Carman, G. M., *DGK1*-encoded diacylglycerol kinase activity is required for phospholipid synthesis during growth resumption from stationary phase in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 2011, 286, 1464–1474.
- [63] Toke, D. A., Bennett, W. L., Dillon, D. A., Wu, W.-I. et al., Isolation and characterization of the *Saccharomyces cerevisiae* *DPP1* gene encoding diacylglycerol pyrophosphate phosphatase. *J. Biol. Chem.* 1998, 273, 3278–3284.
- [64] Toke, D. A., Bennett, W. L., Oshiro, J., Wu, W.-I. et al., Isolation and characterization of the *Saccharomyces cerevisiae* *LPP1* gene encoding a Mg^{2+} -independent phosphatidate phosphatase. *J. Biol. Chem.* 1998, 273, 14331–14338.
- [65] Chae, M., Carman, G. M., Characterization of the yeast actin patch protein App1p phosphatidate phosphatase. *J. Biol. Chem.* 2013, 288, 6427–6437.
- [66] Seip, J., Jackson, R., He, H., Zhu, Q. et al., Snf1 is a regulator of lipid accumulation in *Yarrowia lipolytica*. *Appl. Environ. Microbiol.* 2013, 79, 7360–7370.
- [67] Shirra, M. K., Patton-Vogt, J., Ulrich, A., Liuta-Tehlivets, O. et al., Inhibition of acetyl coenzyme A carboxylase activity restores expression of the *ino1* gene in a *snf1* mutant strain of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 2001, 21, 5710–5722.
- [68] De Vit, M. J., Waddle, J., Johnston, M., Regulated nuclear translocation of the Mig1 glucose repressor. *Mol. Biol. Cell* 1997, 8, 1603–1618.
- [69] Treitel, M. A., Kuchin, S., Carlson, M., Snf1 protein kinase regulates phosphorylation of the Mig1 repressor in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 1998, 18, 6273–6280.
- [70] Wang, Z.-P., Xu, H.-M., Wang, G.-Y., Chi, Z. et al., Disruption of the *MIG1* gene enhances lipid biosynthesis in the oleaginous yeast *Yarrowia lipolytica* ACA-DC 50109. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 2013, 1831, 675–682.
- [71] Kaliszewski, P., Szkopińska, A., Ferreira, T., Świeżewska, E. et al., Rsp5p ubiquitin ligase and the transcriptional activators Spt23p and Mga2p are involved in co-regulation of biosynthesis of end products of the mevalonate pathway and triacylglycerol in yeast *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 2008, 1781, 627–634.
- [72] Nakagawa, Y., Sakumoto, N., Kaneko, Y., Harashima, S., Mga2p is a putative sensor for low temperature and oxygen to induce *OLE1* transcription in *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.* 2002, 291, 707–713.
- [73] Chellappa, R., Kandasamy, P., Oh, C.-S., Jiang, Y. et al., The membrane proteins, Spt23p and Mga2p, play distinct roles in the activation of *Saccharomyces cerevisiae* *OLE1* gene expression. *J. Biol. Chem.* 2001, 276, 43548–43556.
- [74] Liu, L., Markham, K., Blazeck, J., Zhou, N. et al., Surveying the lipogenesis landscape in *Yarrowia lipolytica* through understanding the function of a Mga2p regulatory protein mutant. *Metab. Eng.* 2015, 31, 102–111.
- [75] Xue, Z., Sharpe, P. L., Hong, S.-P., Yadav, N. S. et al., Production of omega-3 eicosapentaenoic acid by metabolic engineering of *Yarrowia lipolytica*. *Nat. Biotechnol.* 2013, 31, 734–740.
- [76] Ratledge, C., Microbial oils: an introductory overview of current status and future prospects. *OCL* 2013, 20, D602.
- [77] Bellou, S., Triantaphyllidou, I.-E., Aggeli, D., Elazzazy, A. M. et al., Microbial oils as food additives: recent approaches for improving microbial oil production and its polyunsaturated fatty acid content. *Curr. Opin. Biotechnol.* 2016, 37, 24–35.

- [78] Fakas, S., Bellou, S., Makri, A., Aggelis, G., Single cell oil and gamma-linolenic acid production by *Thamnidium elegans* grown on raw glycerol, in: Aggelis, G. (Ed.), *Microbial Conversions of Raw Glycerol*, Nova Science Publishers, New York 2009, pp. 85–96.
- [79] Fakas, S., Papanikolaou, S., Galiotou-Panayotou, M., Komaitis, M. et al., Biochemistry and biotechnology of single cell oil, in: Pandey, A., Larroche, C. (Eds.), *New Horizons in Biotechnology*, Asiatech Publishers, Delhi, India 2009; pp. 38–60.
- [80] Liu, H.-H., Ji, X.-J., Huang, H., Biotechnological applications of *Yarrowia lipolytica*: Past, present and future. *Biotechnol. Adv.* 2015, 33, 1522–1546.
- [81] Qiao, K., Imam Abidi, S. H., Liu, H., Zhang, H. et al., Engineering lipid overproduction in the oleaginous yeast *Yarrowia lipolytica*. *Metab. Eng.* 2015, 29, 56–65.
- [82] Blazeck, J., Hill, A., Liu, L., Knight, R. et al., Harnessing *Yarrowia lipolytica* lipogenesis to create a platform for lipid and biofuel production. *Nat. Commun.* 2014, 5, 3131.
- [83] Flores, C.-L., Gancedo, C., *Yarrowia lipolytica* mutants devoid of pyruvate carboxylase activity show an unusual growth phenotype. *Eukaryot. Cell* 2005, 4, 356–364.
- [84] Wang, G.-Y., Zhang, Y., Chi, Z., Liu, G.-L. et al., Role of pyruvate carboxylase in accumulation of intracellular lipid of the oleaginous yeast *Yarrowia lipolytica* ACA-DC 50109. *Appl. Microbiol. Biotechnol.* 2015, 99, 1637–1645.
- [85] Papanikolaou, S., Aggelis, G., Lipid production by *Yarrowia lipolytica* growing on industrial glycerol in a single-stage continuous culture. *Bioresour. Technol.* 2002, 82, 43–49.
- [86] Papanikolaou, S., Chevalot, I., Komaitis, M., Marc, I. et al., Single cell oil production by *Yarrowia lipolytica* growing on an industrial derivative of animal fat in batch cultures. *Appl. Microbiol. Biotechnol.* 2002, 58, 308–312.
- [87] Papanikolaou, S., Fakas, S., Fick, M., Chevalot, I. et al., Biotechnological valorisation of raw glycerol discharged after biodiesel (fatty acid methyl-esters) manufacturing process: production of 1,3-propanediol, citric acid and single cell oil. *Biomass Bioenergy* 2008, 32, 60–71.
- [88] Zhang, Z., O'Hara, I. M., Mundree, S., Gao, B. et al., Biofuels from food processing wastes. *Curr. Opin. Biotechnol.* 2016, 38, 97–105.
- [89] Sitepu, I. R., Garay, L. A., Sestric, R., Levin, D. et al., Oleaginous yeasts for biodiesel: Current and future trends in biology and production. *Biotechnol. Adv.* 2014, 32, 1336–1360.
- [90] Bellou, S., Baeshen, M. N., Elazzazy, A. M., Aggeli, D. et al., Microalgal lipids biochemistry and biotechnological perspectives. *Biotechnol. Adv.* 2014, 32, 1476–1493.
- [91] Ratledge, C., Single cell oils for the 21st century, in: Cohen, Z., Ratledge, C. (Eds.), *Single Cell Oils*, AOCS Press, Champaign, IL 2010, pp. 1–20.
- [92] Churruga, I., Fernandez-Quintela, A., Portillo, M. P., Conjugated linoleic acid isomers: Differences in metabolism and biological effects. *Biofactors* 2009, 35, 105–111.
- [93] Lee, Y., Thompson, J. T., De Lera, A. R., Heuvel, J. P. V., Isomer-specific effects of conjugated linoleic acid on gene expression in RAW 264.7. *J. Nutr. Biochem.* 2009, 20.
- [94] Kennedy, A., Martinez, K., Schmidt, S., Mandrup, S. et al., Antiobesity mechanisms of action of conjugated linoleic acid. *J. Nutr. Biochem.* 2010, 21, 171–179.
- [95] Crumb, D. J., Vatter, D. A., Conjugated linoleic acid (CLA)—An overview. *Int. J. Appl. Res. Nat. Prod.* 2011, 4.
- [96] Zhang, B. X., Rong, C. C., Chen, H. Q., Song, Y. D. et al., De novo synthesis of trans-10, cis-12 conjugated linoleic acid in oleaginous yeast *Yarrowia lipolytica*. *Microb. Cell Fact.* 2012, 11.
- [97] Zhang, B., Chen, H., Li, M., Gu, Z. et al., Genetic engineering of *Yarrowia lipolytica* for enhanced production of trans-10, cis-12 conjugated linoleic acid. *Microb. Cell Fact.* 2013, 12, 1–8.
- [98] Béopoulous, A., Verbeke, J., Bordes, F., Guicherd, M. et al., Metabolic engineering for ricinoleic acid production in the oleaginous yeast *Yarrowia lipolytica*. *Appl. Microbiol. Biotechnol.* 2014, 98, 251–262.