Review

Pia Erdbrügger and Florian Fröhlich*

The role of very long chain fatty acids in yeast physiology and human diseases

<https://doi.org/10.1515/hsz-2020-0234>

Received June 30, 2020; accepted November 2, 2020;

published online November 23, 2020

Abstract: Fatty acids (FAs) are a highly diverse class of molecules that can have variable chain length, number of double bonds and hydroxylation sites. FAs with 22 or more carbon atoms are described as very long chain fatty acids (VLCFAs). VLCFAs are synthesized in the endoplasmic reticulum (ER) through a four-step elongation cycle by membrane embedded enzymes. VLCFAs are precursors for the synthesis of sphingolipids (SLs) and glycerophospholipids. Besides their role as lipid constituents, VLCFAs are also found as precursors of lipid mediators. Mis-regulation of VLCFA metabolism can result in a variety of inherited diseases ranging from ichthyosis, to myopathies and demyelination. The enzymes for VLCFA biosynthesis are evolutionary conserved and many of the pioneering studies were performed in the model organism *Saccharomyces cerevisiae*. A growing body of evidence suggests that VLCFA metabolism is intricately regulated to maintain lipid homeostasis. In this review we will describe the metabolism of VLCFAs, how they are synthesized, transported and degraded and how these processes are regulated, focusing on *budding yeast*. We will review how lipid metabolism and membrane properties are affected by VLCFAs and which impact mutations in the biosynthetic genes have on physiology. We will also briefly describe diseases caused by mis-regulation of VLCFAs in human cells.

Keywords: fatty acid elongation; lipid homeostasis; sphingolipid; very long chain fatty acid..

Introduction

Fatty acids (FAs) are a highly diverse class of molecules that can have variable chain length, number of double bonds and hydroxylation sites. FAs are building blocks for all membrane lipids and are also used to post-translationally modify proteins. Therefore, FAs are essential molecules in all organisms. FAs with 22 or more carbon atoms are described as very long chain fatty acids (VLCFAs). This special FA sub-class is mainly incorporated into SLs and glycosylphosphatidylinositols (GPI)-anchored proteins but can also be found in other lipid classes. The abundance of VLCFAs in cells has to be tightly regulated to avoid their accumulation which affects organelle structure, function and inheritance (De Carvalho and Caramujo 2018; Schneiter and Kohlwein 1997).

In this review we will focus on VLCFA metabolism and its control mechanisms in the yeast *Saccharomyces cerevisiae*. As for many other processes, yeast as a model organism has been essential to identify the enzymes necessary for VLCFA biosynthesis, activation, regulation and degradation (Tehlivets et al. 2007; Welch and Burlingame 1973). Subsequently, evolutionary conserved homologs have been found in all higher eukaryotes and their role in human pathophysiology soon became evident (Poulos et al. 1992; Wanders et al. 1987). FA elongation was soon shown to be distinct from *de novo* FA synthesis, as enzymatic activities for both processes were retrieved in different cellular subfractions (Nugteren 1965).

VLCFAs are synthesized from the products of *de novo* FA synthesis in the endoplasmic reticulum (ER) through a four-step elongation cycle by membrane embedded enzymes. VLCFAs are mainly incorporated into SLs, but can also be found in phosphatidylinositol (PI), GPI-anchors and in storage lipids as components of lipid droplets (Olson et al. 2015a; Tehlivets et al. 2007). SLs in yeast consist of a long chain base (LCB) that is synthesized from serine and palmitoyl-CoA by the serine palmitoyltransferase (SPT). Amide linkage of a VLCFA to a LCB results in the formation of a ceramide. Subsequent addition of inositol-phosphate, mannose and a second inositol-phosphate results in the generation of the complex SLs in yeast.

*Corresponding author: Florian Fröhlich, Department of Biology/Chemistry, Molecular Membrane Biology Group, University of Osnabrück, Osnabrück, Germany; and Center of Cellular Nanoanalytics Osnabrück, Osnabrück, Germany, E-mail: florian.froehlich@uni-osnabrueck.de. <https://orcid.org/0000-0001-8307-2189>

Pia Erdbrügger, Department of Biology/Chemistry, Molecular Membrane Biology Group, University of Osnabrück, Osnabrück, Germany

VLCFAs can also be metabolized via β -oxidation in peroxisomes to generate energy in cells (Van Roermund et al. 2003). In addition, VLCFAs and VLCFA-containing lipids have been linked to endocytic sorting and trafficking and have been implicated in the generation of highly curved membranes (David et al. 1998; Obara et al. 2013; Schneider et al. 2004). Mis-regulation of VLCFAs has also been linked to oleate mediated lipotoxicity and activation of the unfolded protein response (UPR; (Micoogullari et al. 2020; Wang et al. 2018).

Technological developments were crucial to identify and understand the different VLCFA-containing molecules in cells. However, we are only just beginning to understand the cellular pathways regulating VLCFA homeostasis. Here, we will summarize the control mechanisms of VLCFA metabolism and review new insights. Because of their importance in pathophysiology, we will briefly refer to disorders caused by mis-regulation of VLCFAs in humans.

The VLCFA biosynthesis pathway

De novo synthesis of FAs starts with the conserved initial step of malonyl-CoA synthesis by carboxylation of acetyl-CoA. In yeast, this reaction is catalyzed by the acetyl-CoA carboxylase Acc1 (ACC in mammals; Roggenkamp et al. 1980). Acc1 possesses activity as a carboxyltransferase and as a biotin carboxylase and requires biotin as a co-factor (Sumper and Riepertinger 1972). Malonyl-CoA is the universal FA precursor. In eukaryotes, FA synthesis is carried out by type I fatty acid synthases (FAS) a very large multifunctional protein complex. In yeast, the FAS complex consists of the two catalytic subunits Fas1 and Fas2 and the recently identified regulatory subunit Tma17 (Lomakin et al. 2007; Schweizer and Hofmann 2004; Singh et al. 2020). All five steps of FA biosynthesis – transfer, elongation, reduction, dehydration, and another reduction – are carried out by the FAS protein complex. In a two-step transfer mechanism, the acetyl-group of acetyl-CoA is first transferred to the sulfhydryl group of a cysteine on the FAS. In parallel, a malonyl group is transferred to the 4'-phosphopantetheine of the acyl carrier protein region of the FAS. In the second step, a 4-carbon β -keto acid derivative is generated by a transfer of two carbons from the acetyl group. Finally, a three-step reaction of reduction, dehydration and reduction yields a saturated acyl chain elongated by a two-carbon unit. Constant repetition of this cycle yields a 16-carbon FA chain. The elongation cycle is terminated by hydrolysis or transesterification of palmitate to an acceptor (for a more detailed review on FA synthesis see Leibundgut et al. 2008; Schweizer and Hofmann 2004; Tehlivets et al. 2007).

VLCFA elongation follows a cycling four step process of condensation, reduction, dehydration and reduction with each step being carried out by distinct enzymes. The first and rate limiting step is the condensation of acyl-CoA with malonyl CoA and a simultaneously occurring decarboxylation to form 3-ketoacyl-CoA. The elongation up to C18 acyl-CoA in yeast is catalyzed by Elo1. The elongation of C18 to C20 or up to C26 acyl-CoA are catalyzed by the two elongases Elo2 (Fen1) and Elo3 (Sur4) while mammals have seven different elongases (ELOVL1–7). In a second step 3-ketoacyl-CoA is reduced to 3-hydroxyacyl-CoA. The reducing enzyme is Ifa38 (Ybr159w), utilizing nicotinamide adenine dinucleotide phosphate (NADPH) as a reductant (Han et al. 2002). In the third step, Phs1 dehydrates 3-hydroxyacyl-CoA to 2,3-trans-enoyl-CoA (Denic and Weissman 2007). Finally, 3-hydroxyacyl-CoA is reduced to an acyl-CoA containing two more carbon chain units than the original acyl-CoA by the 2,3-trans-enoyl-CoA reductase Tsc13 (Figure 1; Kohlwein et al. 2001).

Characterization of the VLCFA biosynthesis enzymes in yeast

The first and rate-limiting step of VLCFA elongation is the condensation reaction catalyzed by three different

Figure 1: The VLCFA biosynthesis pathway. Overview of the four-step FA elongation cycle in *S. cerevisiae*. Enzymes and co-factors are illustrated for each step. Details about the four steps - condensation, reduction, dehydration and reduction- and enzymes are described in the text. NADPH, nicotinamide adenine dinucleotide phosphate; CoA, coenzyme A.

elongases Elo1-3. The molecular mechanism of VLCFA elongation in yeast is described as an adjustable caliper (Denic and Weissman 2007). Elo1 extends C12-C16 fatty acyl-CoAs to C16-C18 FAs (Toke and Martin 1996). Elo2 utilizes these products to elongate them further up to C22 FAs and Elo3 uses saturated C18 FAs to synthesize C20-26 VLCFAs. Elo2 and Elo3 have partially overlapping activities, however the final step of C24 to C26 elongation is exclusively performed by Elo3 (Oh et al. 1997). The three *ELO* genes and their products will be described in detail below.

ELO1 was originally identified in a genetic screen for essential genes in FAS defective cells supplemented with tetradecanoic acid (C14:0). Screening for mutants that lose the ability to elongate this FA led to the identification of *ELO1* (Toke and Martin 1996). The *ELO2* and *ELO3* genes were identified based on sequence similarity to *ELO1* (Oh et al. 1997). Further characterization of the gene products showed that every elongase has a characteristic product pattern and substrate usage with little overlap between Elo2 and Elo3. Substrate specificity for each elongase was further characterized by heterologous expression of ketoacyl synthases from *C. elegans* allowing yeast to use otherwise incompatible substrates for elongation (Beaudoin et al. 2000).

Yeast elongases are membrane spanning proteins that form the elongation complex at the ER membrane, necessary for the evolutionary conserved process of VLCFA biosynthesis (Denic and Weissman 2007; Revardel et al. 1995). While single null mutants of an elongase are viable, *ELO2/ELO3* double mutants are lethal. In contrast to FAS mutants, elongation deficient phenotypes cannot be rescued by supplementation with exogenous VLCFAs (Rössler et al. 2003).

Single elongase mutants show distinct phenotypes (Ejsing et al. 2009). *ELO1* mutants are defective in medium to long chain elongation with more glycerophospholipid species containing 14:0 and 14:1 FAs. Interestingly, the levels of mannosyl- inositol-phosphorylceramide (IPC), inositol-phosphorylceramide (MIPC) and mannosyl-diinositol-phosphorylceramide (M(IP)₂C), the complex SL in yeast, are increased in *ELO1* mutants. This is likely the consequence of the still active Elo2 and Elo3 enzymes. In line with that, *ELO2* or *ELO3* mutants show reduced M(IP)₂C and MIPC levels and subsequent accumulation of the lipid precursors with shortened FA moieties. *ELO3* knockout cells accumulate IPC with a C22:0 FA while *elo2Δ* cells have shorter chain C16:0 and C18:0 FAs in the major IPC species (Ejsing et al. 2009). Reduced SL levels are likely a result of reduced ceramide synthesis. When VLCFA levels are low, ceramide synthesis might become rate limiting. Alternatively

the ceramide synthase has reduced activity due to the presence of a shorter, less preferred substrate, when C26 is not available (Oh et al. 1997). Defects in the *ELO2*- or *ELO3* genes have also been correlated with decreased plasma membrane (PM) ATPase activity (Garcia-Arranz et al. 1994), reduced β -glucan synthase activity (El-Sherbeini and Clemas 1995) as well as resistance to sterol synthesis inhibitors and defects in bud localization (Revardel et al. 1995). These effects are all attributed to membrane proteins that are affected due to altered membrane properties resulting from changes in the VLCFA composition (Oh et al. 1997). Over all, this highlights the importance of VLCFAs for cellular function and the complexity of the cellular lipid environment (Ejsing et al. 2009).

The molecular mechanism of how Elo proteins determine the final length of a VLCFA still remains unclear. Since an external template for VLCFA synthesis is lacking and the same group of active sites can synthesize specific products, the molecular mechanism must be included within the biosynthetic enzymes. Purification of all membrane components of the elongation complex and reconstitution into proteo-liposomes led to the identification of a key lumen-facing lysine residue and a cytosol-facing active site for Elo2 and Elo3 that allows the Elo machinery to produce VLCFAs of a defined length. With the active site as a fixed point, a caliper-like mechanism can measure the distance to the lysine residue. Presumably, the lysine residue marks the end of the hydrophobic pocket, being located at the luminal end of a transmembrane domain. Acyl chains are potentially pushed into the transmembrane region of the protein to allow the addition of two carbon units. The acyl chain is further displaced until it has reached a certain length and arrives at the lysine residue. This triggers elongation termination. Consequently, the length of the final VLCFA product is similar to the distance between the active site and the lysine (Denic and Weissman 2007). Artificially changing the lysine position results in the generation of VLCFAs of a specific length. This consequently allows genetically engineered yeast to produce altered VLCFA products that would not be generated naturally.

Ifa38 is the major 3-ketoreductase of the elongase system. The enzyme localizes to the ER and co-immunoprecipitates with Elo3 and Tsc13 as part of the elongation complex. Ifa38 mutants show a defect in 3-ketoreductase activity and has comparable phenotypes to other mutations in the elongase cycle including reduced VLCFA levels, elevated levels of medium-chain ceramides as well as elevated dihydrosphingosine (DHS) and phytosphingosine (PHS) levels. Another phenotype of the Ifa38 mutant is a reduced dehydratase activity resulting in an

accumulation of a 3-OH-acyl intermediate (Beaudoin et al. 2002; Han et al 2002). It is possible that Ifa38 is involved in the stabilization or localization of the 3-hydroxyacyl-CoA dehydratase. Alternatively, the entire elongation complex could be stabilized by Ifa38 but supporting evidence is lacking. *IFA38* is not an essential gene, suggesting the presence of proteins with similar enzymatic activity. Along that line, *Ayr1*, a bifunctional enzyme with a more promiscuous 3-ketoreductase activity potentially complements the *IFA38* knockout (Han et al. 2002).

Phs1 is the 3-hydroxyacyl-CoA dehydratase that catalyzes the third step of the elongation cycle (Figure 1; Kihara 2012). *PHS1* is an essential gene in yeast, highlighting the importance of VLCFA elongation in cellular physiology. *Phs1* was first reported as a potential regulator of LCB levels because of a strong deficiency in converting ceramides to IPC when *Phs1* levels were reduced. In addition LCBs accumulated in *PHS1* mutant cells resembling a phenotype of VLCFA metabolism mutations (Schuldiner et al. 2005). The IPC synthesis defect is most likely caused by a defect in CER or phosphatidylinositol (PI) transport from the ER to the Golgi. Interestingly, other PI transport requiring steps, such as MIPC to M(IP)₂C conversion are more affected in *PHS1* mutant cells than e.g. IPC-to-MIPC conversion. This suggests a possible connection between VLCFA synthesis and PI transport (Kihara et al. 2008).

TSC13 encodes the enoyl reductase that catalyzes the last step in the elongation cycle (Figure 1). The *Tsc13* protein family is conserved from yeast to mammals with the key glutamine 81 being conserved. A *tsc13-1* mutant allele containing a substitution of this residue with lysine is responsible for a temperature sensitive phenotype in yeast (Kohlwein et al. 2001). *TSC13* was identified in a *csg2Δ* suppressor screen for calcium sensitivity (TSC = temperature sensitive suppressors of *Csg2* mutants; Kohlwein et al. 2001). LCBs and ceramide species with shorter chain lengths accumulate in *TSC13* mutants. The *TSC13* gene is also essential for yeast viability. The protein localizes to the ER. Supporting the concept of an elongation complex, *Tsc13* co-immunoprecipitates with *Ifa38*, *Elo2* and *Elo3* (Han et al. 2002; Kohlwein et al. 2001). Interestingly, *Tsc13* is enriched in the nuclear-vacuolar junction (NVJ), a sub-domain of the ER. The molecular function of *Tsc13* at this domain remains enigmatic. Localization of *Tsc13* to the NVJ is not essential for viability and is independent of the other elongase cycle relating enzymes but depends on the *Nvj1* protein (Kohlwein et al. 2001; Pan et al. 2000). However, it cannot be excluded that its NVJ enrichment is connected to the requirement of VLCFAs in stabilizing the nuclear pore complex (NPC) (Schneider et al. 1996) or to PI containing VLCFAs present in the nuclear membrane (Schneider et al.

2004). Interestingly, yeast cells in stationary phase bud off *Tsc13* enriched vesicles into the vacuole from the nuclear membrane suggesting an involvement of *Tsc13* in nuclear envelope recycling (Schneider and Kohlwein 1997). Since *Nvj1* expression is connected to piecemeal micro autophagy of the nucleus (PMN) this further supports a connection of *Tsc13* with nuclear recycling (Kvam et al. 2005).

VLCFAs as building blocks for lipids in *Saccharomyces cerevisiae*

In *S. cerevisiae*, VLCFAs are the building blocks for SLs and glycerophospholipids. They are components of GPI-anchored proteins and can be incorporated into storage lipids (Figure 2). In the following paragraphs the role of VLCFAs in the generation of these molecules will be highlighted.

SLs are one of the three major lipid classes in all eukaryotic cells. They consist of an LCB, a FA and a polar head group. SL synthesis starts in the ER with the condensation of serine with palmitoyl-CoA to yield 3-ketodihydrosphingosine (KDHs) catalyzed by the rate-limiting enzyme SPT (Dickson et al. 2006; Olson et al. 2015b). In yeast, the SPT consist of the two catalytic

Figure 2: Schematic diagram of lipid species containing VLCFA. Shown is an overview of structures of different lipid species in *S. cerevisiae* that can contain VLCFAs. Summarizing the role of VLCFAs in cellular physiology. GPI, glycosylphosphatidylinositol; man, mannose; GlcN, *N*-acetylglucosamine; Ins, inositol; EtN, ethanol.

subunits Lcb1 and Lcb2 and the regulatory subunit Tsc3. The short lived intermediate KDHS is directly reduced to dihydrosphingosine (DHS) by Tsc10 (Beeler et al. 1998). DHS can be further hydroxylated at its C4 position by the hydroxylase Sur2. The resulting product is phytosphingosine (PHS) the major LCB in yeast cells. Yeast LCBs have varying chain lengths of 16–20 carbons (Lester and Dickson 2001). DHS and PHS are both substrates of the essential ceramide synthase in yeast. (Dickson et al. 2006; Schorling et al. 2001; Vallée and Riezman 2005). While mammalian cells have more variable ceramide FA composition, yeast cells primarily incorporate C26 VLCFAs in ceramides (Ejsing et al. 2009). Thus, VLCFAs are the primary FA of complex SLs in yeast cells. The most abundant VLCFA is 2-OH-C26:0 followed by C26:0 and small quantities of shorter FAs (Ratray et al. 1975). VLCFAs have varying hydroxylation sites. This also separates VLCFAs from shorter chain FAs that are usually not hydroxylated in wild-type cells (Dickson and Lester 2002). Hydroxylation of the ceramide VLCFA depends on the Scs7 hydroxylase/desaturase that is specific for VLCFA. Thus, Scs7 and Sur2 are both required for ceramide hydroxylation (Haak et al. 1997). Ceramides can be further linked to a polar head group via a phosphodiester bond. Usually the head group is an inositol-phosphoryl moiety. This moiety can be modified with a mannosyl group and a second inositolphosphate attached to the mannosyl making M(IP)₂C, the most abundant SL in yeast. However, IPC and MIPC are also present in detectable quantities in yeast cells (Ejsing et al. 2009; Olsen and Jantzen 2001; Steiner et al. 1969).

Complex SLs are essential molecules in all eukaryotic cells that function as structural membrane components as well as signaling molecules. In *S. cerevisiae* SLs comprise approximately 10–20% of the total membrane lipid species (Oh et al. 1997). They mainly localize to the outer leaflet of the plasma membrane but low amounts of SLs are found in the nuclear membrane (Schneiter et al. 1996). SLs are involved in signaling, secretion, apoptosis, Ca²⁺ homeostasis and linked to many other processes (Dickson and Lester 2002; Dickson et al. 2006; Schneiter 1999). Phosphorylated LCBs may also play a role in the heat stress response and growth control (Skrzypek et al. 1999). The importance of VLCFAs as components of SLs is evident in mutants unable to synthesize SLs, for example, a temperature sensitive allele of the SPT (*LCB1-100*). At the restrictive temperature *LCB1-100* cells die, unless they carry a suppressor mutation in the 1-acyl-sn-glycerol-3-phosphate acyltransferase *SLC1* (named *SLC1-1*; AGPAT1 in mammals, Schneiter et al. 2004). *LCB1-100* cells harboring the *SLC1* mutation are capable of producing C26 FA containing glycerophospholipids which structurally mimic SLs (Munn

and Riezman 1994; Zanolari et al. 2000). The cells bypass the essential requirement for ceramides by producing novel inositol glycerophospholipids that have SL like head group modifications and a VLCFA at the sn-2 position, highlighting the importance of these components.

In wild type yeast, PI is the only glycerophospholipid that contains VLCFAs. This unusual VLCFA-containing PI species comprises about 1% of total cellular PIs. It is found in the plasma membrane as well as the nuclear envelope. The C26-substitution is highly specific for the sn-1 position of the glycerol backbone. In liposomes composed of 1,2-dielaidoyl-sn-glycero-3-phosphoethanolamine (DEPE) it reduced the bilayer-to-hexagonal phase transition. This supports the idea of a possible involvement in membrane stabilization, especially of the highly curved membranes at the (NPC) (Řezanka and Sigler 2009; Schneiter et al. 2004).

In addition, VLCFAs can also be found in storage lipids at lipid droplets. Interestingly, a FA can be attached to the hydroxyl group of a ceramide by the acyl transferases Lro1 and Dga1, yielding an acyl-ceramide (Voynova et al. 2012). In addition, after hyper activation of Elo2 and a concomitant increase in VLCFAs, VLCFAs are increased in triacylglycerols (Olson et al. 2015a).

The role of VLCFAs in GPI anchor biosynthesis

Another class of VLCFA-containing molecules are the GPI-anchor containing proteins. GPIs are complex glycerophospholipids which are added post-translationally to the C-terminus of secreted proteins in the lumen of the ER. The anchoring machinery is used to attach proteins to membranes. The process of adding GPI to proteins is a complex 12 step pathway carried out by several essential enzymes. Yeast comprises about 60 GPI-anchored proteins with various functions, most of them involved in cell wall biosynthesis and stabilization (Pittet and Conzelmann 2007). The first step of GPI synthesis is the addition of *N*-acetylglucosamine (GlcNAc) to PI to yield GlcN-acyl-PI by an enzyme complex at the cytosolic surface of the ER. Next, the core carbohydrate structure of GPIs is formed by successive addition of mannose (Man) and phosphorylethanolamine (EtNH₂-P). The core structure is highly conserved among eukaryotes and synthesis follows the same basic rules. GPIs usually contain four mannoses (Man1-4) and an optional Man5. They are linked to EtNH₂-P on Man1, 2 and 3. The inositol is linked to two alternative lipid moieties, diacylglycerol (DAG) or ceramide that can be found on mature GPI anchored proteins.

While DAG classically consists of C16 to C18 FAs usually containing at least one monounsaturated fatty acyl (MUFA) chain (Ganesan et al. 2015), DAGs attached to proteins as part of a GPI anchor possess a C26:0 in sn-2. However, the lipid moieties of mature GPI-anchors of yeast contain a modified DAG possessing a C26:0 in sn-2 (Pittet and Conzelmann 2007). This process lacks mechanistic insights. The removal of the lipid moiety FA and attachment of the new VLCFA could be connected to the GPI-remodelase Gup1 (Sipos et al. 1997). In both anchor lipids, the C26 FA can be hydroxylated. However, ceramide harboring an OH-C26 are only introduced in the Golgi apparatus and not in the ER. After their biosynthesis the GPI-anchored proteins leave the ER in COPII coated vesicles and travel to the PM via the Golgi (Pittet and Conzelmann 2007). GPI anchored proteins, covalently linked to the cell wall frequently lose their lipid motif at a certain maturation stage. Cell wall attachment occurs via the remaining mannose residues of the anchor to β 1,6-glucans of the cell wall (Yin et al. 2005). The role of the VLCFA for GPI containing proteins is still unclear. Gup1 the enzymes essential for the synthesis of C26:0-containing DAG anchors, harbors a membrane bound O-acyl-transferases (MBOAT) motif and is conserved among fungi and protozoa. *gup1Δ* mutants produce normal amounts of GPI proteins but most mature GPI anchors contain PI with C16 and C18 FAs or lyso-PI. In addition, ceramide incorporation into GPI anchors is disturbed. This affects the transport of GPI proteins from the ER to the Golgi. Overall *gup1Δ* cells have fragile cell walls and defects in bipolar bud site selection, with the latter being more likely connected to another function of the Gup1 protein (Bosson et al. 2006). Additionally, ceramide synthase mutants that are not able to attach VLCFAs to LCBs show severely altered lipid remodeling of GPI-anchored proteins (Pettus et al. 2002). Otherwise the VLCFA moiety is most likely important for the correct transport of the GPI anchor protein to their destination since there are several lines of evidence connecting VLCFAs with vesicular transport. However, the molecular role of VLCFAs in GPI anchored proteins is not fully understood (David et al. 1998; Obara et al. 2013).

VLCFAs as determining factors for membrane properties

Besides their role in lipid biosynthesis and in GPI anchoring of proteins, VLCFAs have been implicated in multiple intracellular pathways based on their specific biophysical properties. Saturation and length of the yeast

VLCFAs make them unique in spanning more than one leaflet of membranes. Therefore, VLCFA cause very specific local membrane phenomena. Several studies link VLCFAs to highly curved membrane structures (David et al. 1998; Obara et al. 2013; Schneiter et al., 1996, 2004). This appears to be especially important in the endo-lysosomal pathway. For example, *VPS21* was identified as a multi-copy suppressor of VLCFA biosynthesis deficient mutants. Vps21 is a Rab GTPase (Rab5 homolog) at the endosome required for endocytic transport and sorting of vacuolar hydrolases (Horazdovsky et al. 1994; Robinson et al., 1988a, 1988b; Singer-Krüger et al. 1994). It is also required for endosomal localization of the class C core vacuole/endosome tethering (CORVET) complex (Cabrera et al. 2013; Langemeyer et al. 2020; Peplowska et al. 2007). CORVET is the tether involved in fusion of vesicles to the late endosome/ multivesicular body (MVB). Double mutants of *ELO3* and *VPS21*, or the CORVET subunit *VPS3* show synthetic lethal phenotypes (Obara et al. 2013). In addition, yeast cells lacking the elongase genes *ELO2* or *ELO3* display morphological and vacuolar defects (Chung et al. 2003; Hurst et al. 2020).

Other factors required for fusion of vesicles with their target membrane are soluble NSF attachment protein receptors (SNAREs). Snc1 and Snc2 are v-SNAREs involved in the fusion of Golgi-derived secretory vesicles with the plasma membrane. Interestingly, the temperature-sensitive growth phenotype of cells with *SNC1* and *SNC2* gene deletions can be suppressed by loss of function mutations in *ELO2* or *ELO3* (David et al. 1998). These observations indicate a role for VLCFAs in vesicular transport that cannot be substituted by long chain FAs. However, this does not explain the role of VLCFAs on a molecular level. Possibly, VLCFAs exert their essential function by supporting the highly curved membranes involved in vesicular processes. Budding and fusion of vesicles requires local generation of highly curved membranes either at the neck of the budding vesicle or at the contact site of lipid mixing during fusion. Because of their high chain length VLCFA can span both leaflets of a lipid bilayer, enabling support of highly curved membranes. Invagination for intraluminal vesicles formation at the late endosome/MVB also requires highly curved membranes, possibly explaining VLCFAs involvement in this process. Highly curved membranes might be destabilized during elevated temperatures because of increased fluidity. Along that line, the requirement for VLCFAs is increased at higher temperatures (Obara et al. 2013). Free VLCFAs are very low abundant in yeast cells. VLCFAs are mainly incorporated into SLs. Therefore, the functions described above are most likely dependent on VLCFA-containing SLs. MIPC

synthesis defective mutants *csg1Δ*, and *csh1Δ*, in combination with *vps21Δ* show growth defects at higher temperatures and impairment in vesicular transport pathways. Therefore, other parts of SLs such as the sugar head groups may also facilitate clustering of SL and therefore stabilize highly curved membranes in combination with VLCFAs (Obara et al. 2013). It is thus not surprising that SL homeostasis has been linked to endosomal transport pathways (Fröhlich et al. 2015; Voynova et al. 2015).

Additionally, VLCFAs have also been proposed to support highly curved membranes at (NPCs). The NPC spans the outer and inner membrane of the nucleus in eukaryotic cells. It is a giant protein-aqueous channel that serves as a gatekeeper by forming the site for entry and exit from the nucleus. It is anchored to the nuclear envelope by integral membrane proteins. At the NPC the nuclear membranes that are continuous with each other form a 180° turn (Wente 2000; Worman and Courvalin 2000). The connection between VLCFA and the structural integrity of the nuclear envelope is based on the observation that yeast acetyl-CoA carboxylase mutants unable to synthesize malonyl-CoA display severe perturbation of the nuclear envelope. This phenotype is independent of *de novo* synthesis of FAs and a structural role for VLCFA in stabilizing the highly curved membrane at the NPC was postulated (Schneider et al. 1996). PI containing VLCFAs present in the nuclear membrane are required for maintaining the structure and function of the yeast nuclear membrane. The absence of this rare species of VLCFA-containing glycerophospholipid might cause the alterations of the nuclear membrane in the *acc1/mtr7* mutant cells unable to synthesize VLCFA-containing PI (Schneider et al. 2004).

Finally, a recent publication has linked yeast VLCFA biosynthesis to ER membrane stress and induction of the unfolded protein response (UPR). Deletion of the VLCFA coenzyme-A synthase *Fat1* results in an increase in saturated membrane lipids in general (see below). Together these results further support the essential role of VLCFAs in membrane homeostasis (Micoogullari et al. 2020).

Yeast cells control VLCFA levels

VLCFAs are building blocks for lipid biosynthesis and affect biophysical membrane properties. Therefore, it is not surprising that cells tightly control VLCFA levels in membranes. The levels of VLCFAs are the result of several parallel pathways namely the activation, biosynthesis, regulation, transport, storage and degradation of VLCFAs (Figure 3).

Figure 3: Biosynthesis and metabolic control of VLCFA levels in *Saccharomyces cerevisiae*.

VLCFA homeostasis including synthesis, regulation, activation, storage, transport and degradation are represented graphically. Enzymes involved in these processes and their subcellular localization are included. Details about each step are given in the text. Dashed lines represent a membrane monolayer. PM, plasma membrane; ER, endoplasmic reticulum; PX, peroxisome; LD, lipid droplet; TG, triglyceride; CoA, coenzyme A.

VLCFAs have to be activated by linkage to coenzyme A (CoA). Acyl-CoA derivatives are available for incorporation into complex lipids, acylation of proteins and degradation for energy production via β -oxidation (Watkins et al. 1998). Therefore, the regulation of acyl-CoA synthesis is a key factor in lipid homeostasis. The enzymes catalyzing this process are called acyl-CoA synthetases (ACSs). ACSs are an enzymatic family that catalyze the thio-esterification of FAs with CoA (Semenkovich 1997; Watkins 1997). This two-step process is energy dependent and requires ATP and CoA. In the first step an acyl-AMP intermediate is formed by transferring an AMP from ATP to a FA. This step is driven by hydrolysis of inorganic pyrophosphate (PPi) to inorganic phosphate (2Pi). In the second step acyl-AMP reacts with CoA to form an acyl-CoA molecule and AMP is released (Hisanaga et al. 2004). Special structural features of the ACS binding pockets allow for unique activity patterns. This allows a level of flexibility to support lipid related homeostasis during cellular stress or fluctuations in nutrient availability and during various points in the cell cycle (Black and DiRusso 2007). *S. cerevisiae* has six known ACSs. They can be identified by two signature motifs, the AMP signature and the ACS signature. All of them have varying expression patterns, localization, and unique features in metabolic and regulatory pathways. They are

found in mitochondria, peroxisomes and the ER (Johnson et al. 1994; Watkins 1997). Faa1 through Faa4 are required for activation of long chain FAs (Black and DiRusso 2007). Pcs60/Fat2 is an oxalyl-CoA synthetase (Foster and Nakata 2014).

VLCFAs are activated by the Fatty acid transporter 1 (Fat1). Yeast Fat1 was identified as a FA transporter based on structural and functional similarities to the murine Fatty Acid Transport Protein 1, FATP1 (Færgeman et al. 1997). Fat1 was shown to have VLCFA-CoA synthetase activity and the same amino acid sequence motif as other ACS including a FA binding region with similar features to other VLCFA activating enzyme motifs (Johnson et al. 1994; Watkins et al. 1998). Phenylalanine 528 and leucine 669 have been identified as essential for transport activity while serine 258 and glutamate 508 are essential residues for VLCFA activation (Black and DiRusso 2007). Fat1 is highly specific for activation of FAs longer than C20. Deletion of the *FAT1* gene reduces activation of C24 FAs ~70% and leads to accumulation of VLCFAs (Choi and Martin 1999). Interestingly, Fat1 has been described as a transmembrane protein of the plasma membrane (Obermeyer et al. 2007). However, several studies have identified Fat1 as a lipid droplet protein (Athenstaedt et al. 1999; Currie et al. 2014) which is in line with our own unpublished observations. This localization would argue against a direct role in FA transport across the plasma membrane and would exclude transmembrane domains, since these are impossible to incorporate into the monolayer of lipid droplets (Kory et al. 2016).

VLCFA elongation is controlled by phosphorylation of the rate limiting enzyme

The Elo proteins catalyze the rate limiting step in VLCFA synthesis and allow for structural diversity among FA classes. So far, little is known about the regulation of VLCFA biosynthetic enzymes in yeast. The regulation of Elo2 activity appears to be tightly linked to the regulation of SPT dependent LCB synthesis. Since LCBs and VLCFAs are both substrates for ceramide synthesis it is an attractive model to also regulate VLCFA biosynthesis according to need. When SL levels at the plasma membrane are low, the target of rapamycin complex 2 (TORC2) is activated and phosphorylates the yeast Akt homologue Ypk1 (Berchtold et al. 2012; Roelants et al. 2011). This leads to phosphorylation of the Orm proteins, negative regulators of the SPT, resulting in release of the inhibition and increased SL

biosynthesis (Breslow et al. 2010; Gururaj et al. 2013; Han et al. 2010; Liu et al. 2012; Sun et al. 2012). Similarly, Elo2 regulation also responds to changes in the PM. Its phosphorylation is controlled by the plasma membrane localized Rho1 guanine nucleotide exchange factor (GEF) Rom2. Here, depletion of SL levels leads to down-regulation of Rom2-signaling and de-phosphorylation of Elo2, which in turn increases VLCFA synthesis. The regulation of TORC2 and Rom2 appear to be reciprocal. Regulation of LCBs and VLCFAs are both regulated by plasma membrane SL levels. The exact signaling cascade downstream of Rom2 remains largely elusive, but appears to involve only the initial kinases of the upstream cell wall integrity pathway. Mck1, a GSK3-like kinase is responsible for Elo2 phosphorylation. Rom2 signal is potentially transduced via Rho GTP that targets TORC1, a negative regulator of Mck1 (Lee et al. 2012). Downregulation of TORC1 therefore leads to increased Elo2 phosphorylation. Depending on different models this might activate or deactivate VLCFA synthesis (Olson et al. 2015a; Zimmermann et al. 2013). TORC1 also controls Orm inhibition and therefore SL synthesis, again supporting the concept that the coordination of VLCFA and LCB regulation is coupled (Shimobayashi et al. 2013). TORC1 down-regulation is also linked to the activation of cellular autophagy under conditions of low nutrient availability (He and Klionsky 2009). Increased VLCFA and LCB synthesis might help to provide the cell with enough membrane building blocks for autophagosome formation. VLCFA depletion also seems to constitutively activate autophagy via long chain base phosphorylation resulting in decreased survival (Zimmermann et al. 2013). The connection between all these processes is still unclear and the molecular mechanism of VLCFA regulation still needs to be determined. However, VLCFA regulation is essential for cellular survival with important implications for autophagy and cellular homeostasis (Micoogullari et al. 2020; Zimmermann et al. 2013).

VLCFA are transported between organelles

Besides VLCFA activation sufficient transport is a key factor in VLCFA homeostasis. Yeast does not import FAs greater than C20 in length. Synthesis and further metabolic consumption of VLCFAs both take place in the ER and therefore, there is little need for VLCFA transporters in the cell. Proteins delivering the acyl-CoA primers to the ER for FA elongation in yeast are the acyl-CoA binding proteins (ACBPs). ACBPs mediate cytosolic transport of acyl-CoAs

and regulate intracellular acyl-CoA levels (Hiltunen et al. 2003). VLCFA transport between membranes and across membranes is essential for energy generation via β -oxidation in peroxisomes. The enzymes required for VLCFA translocation into peroxisomes are ATP-binding cassette (ABC) 2 transporters (Van Roermund et al. 2012). ABC2 transporters have at least four domains. Two substrate binding trans-membrane domains are necessary for acyl-CoA translocation and two nucleotide binding domains provide the required energy by ATP hydrolysis (Linton and Higgins 2007). Yeast has two half-size ABC proteins: the peroxisomal ABC transporter 1 (Pxa1) and Pxa2. They form a heterodimer to build a functional transporter (Roermund et al. 2008). The Pxa1-Pxa2 complex functions as an acyl-CoA flippase. While the hydrophobic part of the FA diffuses in the membrane Pxa1-Pxa2 flip the polar CoA head from the cytoplasmic leaflet to the luminal leaflet of the peroxisomal membrane (Hettrema and Tabak 2000). Single *PXA* deletion mutants exhibit reduced β -oxidation and reduced growth on oleate containing medium. For the even stronger *pxa1 Δ* and *pxa2 Δ* double mutant phenotype a full rescue of mainly C22:0 and other VLCFA β -oxidation can be observed by expressing human ABCD2. C24:0 and C26:0 β -oxidation can be fully restored by ABCD1 expression (Van Roermund et al. 2011). It was suggested that VLCFA-CoAs are hydrolyzed by the ABC transporter during transport. The free CoA is released into the cytosol and the acyl chain is subsequently translocated into the peroxisome. In peroxisomes, VLCFAs get re-esterified by peroxisomal Fat1 (Van Roermund et al. 2012). However, this model is again in contrast to the described lipid droplet localization of Fat1. Alternatively, Fat1 could be directly involved in VLCFA transport into the peroxisome interacting with Pxa1-Pxa2 (Van Roermund et al. 2012). This raises the interesting possibility of VLCFA handover from lipid droplets to peroxisomes, potentially via a membrane contact site (Shai et al. 2016). In this model, peroxisomal accumulation of CoA can be easily prevented but also the requirement for a separate process of CoA import into the peroxisome would be necessary (Van Roermund et al. 2012).

VLCFAs are degraded in peroxisomes

Finally, intracellular VLCFA levels are also dependent on their degradation. In *S. cerevisiae* VLCFAs are exclusively degraded via β -oxidation in peroxisomes. This is in contrast to higher eukaryotes where VLCFA β -oxidation

also takes place in mitochondria (Kunau et al. 1995). Yeast can utilize FAs as an energy source. Interestingly, C24:0 and C26:0 FAs cannot be used as a sole carbon source with C22:6 being the only VLCFA meeting this requirement in yeast (Van Roermund et al. 2003). FA and VLCFA β -oxidation are mediated by three key enzymes Pox1/Fox1, Mfe2/Fox2, and Pot1/Fox3. These enzymes mediate the four-step process of FA degradation into acetyl-CoA (Figure 4). First, dehydrogenation of the activated FA introducing a double bond between the α and β carbon atom, catalyzed by Fox1. Fox 1 is the only acyl-CoA oxidase in yeast and has no substrate specificity. Therefore, it catalyzes the dehydrogenation of shorter FA as well as VLCFAs. The trans-2-enoyl-CoA intermediate is hydrated to 3-hydroxyacyl-CoA followed by another step of dehydrogenation yielding 3-ketoacyl-CoA. Both reactions are catalyzed by the multifunctional enzyme Fox2 that possesses 2-enoyl-CoA hydratase activity as well as (3R)-specific 3-hydroxy acyl-CoA dehydrogenase activity. Finally, the 3-ketoacyl-CoA thiolase Fox3 catalyzes the cleavage of 3-ketoacyl-CoA esters into a C2-shortened acyl-CoA and acetyl-CoA. The acetyl CoA is further oxidized in the tricarboxylic acid cycle providing a major source of metabolic energy. In yeast, the remaining FA is entirely degraded in the peroxisome (Hiltunen et al. 2003; Van Roermund et al. 2003).

VLCFA metabolism is connected to human pathophysiology

While this review focuses on the metabolism of VLCFAs in the model organism yeast we briefly want to highlight the role of VLCFAs in human pathologies. A number of human diseases are caused by defective VLCFA metabolism (Moser and Moser 1996). X-linked adrenoleukodystrophy (X-ALD) is characterized by an accumulation of free VLCFAs. It is caused by loss-of-function mutations in the ABCD1 transporter that imports VLCFAs into peroxisomes. These defects result in reduced peroxisomal degradation of VLCFAs and a concomitant increase in elongation (Kemp et al. 2005). VLCFAs accumulate in plasma and tissue of patients, resulting in a fatal neurodegenerative phenotype that can include childhood-onset cerebral adrenoleukodystrophy (CCALD) and adrenomyeloneuropathy (AMN) (Hama et al. 2020). AMN is the milder phenotype and is characterized by a slowly progressive axonopathy. Besides mutations in ABCD1 additional genetic or environmental factors are required to trigger brain inflammation. The phenotypic expression of the disease can vary extremely

Figure 4: The (VLC)FA β -oxidation pathway.

Overview of the four-step β -oxidation cycle in *S. cerevisiae*. Enzymes and co-factors are illustrated for each step. Details about the four steps – dehydrogenation, hydration, dehydrogenation and thiolysis – and enzymes are described in the text. NAD^+ , nicotinamide adenine dinucleotide; CoA, coenzyme A.

between patients. The correlation between genotype and different phenotypes remains unclear and does not allow to link severity of the pathology of X-ALD to any particular cellular mis-regulation. Stronger mutations can result in milder phenotypes (Wiesinger et al. 2015). Accumulating VLCFAs comprise C24:0, C26:0 and C26:1. Hexacosenoyl (26:1)-CoA is the most abundant VLCFA-CoA species in *ABCD1*-KO HeLa cells and X-ALD fibroblasts. C26:1-CoA levels are about five- to six-fold higher than 26:0-CoA but the 26:1 and 26:0 VLCFA levels are similar. Even without *ABCD1* there is still a turnover of VLCFAs indicating an *ABCD1* independent machinery that metabolizes VLCFA-CoA (Hama et al. 2020). Interestingly, deletion of the VLCFA-CoA-synthetase *FAT1* in *S. cerevisiae* results in decreased peroxisomal β -oxidation and elevated cellular VLCFA levels mimicking an X-ALD like phenotype.

Mutations in other members of the ABCD transporter family are also linked to disease. *ABCD5* deficiency causes retinal dystrophy with leukodystrophy (RDLKD) disease. RDLKD patients exhibit developmental delay as well as ataxia and spastic paraparesis. *ACBD5* deficiency leads to accumulation of VLCFAs due to impaired peroxisomal β -oxidation. Peroxisomal β -oxidation activity is reduced

for VLCFAs but not for FAs of a different length (Abu-Safieh et al. 2013; Ferdinandusse et al. 2017)

Mutations in the VLCFA elongases have also been identified to cause different diseases. Mutation of *ELOVL1* causes a disorder characterized by hypo-myelination, facial dysmorphism, and ichthyosis (Mueller et al. 2019). Mutations in *ELOVL4* or *ELOVL5* lead to spinocerebellar ataxia. *ELOVL5* is the human orthologue of *Elo2* also containing the highly conserved phosphorylation-site that is important for *Elo2* regulation (Di Gregorio et al. 2014; Hayashi et al. 2020; Olson et al. 2015a).

Future perspectives

Although the basic enzymology involved in VLCFA elongation is well worked out, many questions related to the regulation of this process, the regulation of the enzymatic machinery, the coordination with cellular lipid metabolism, and the impact of VLCFA metabolism on cellular physiology are still unresolved. What is the exact localization of the VLCFA biosynthetic machinery in cells and how does this affect activity? How are VLCFAs distributed in particular lipids and proteins and how does this affect lipid metabolism and membrane structure? Which organelles are involved in VLCFA homeostasis besides the ER? How is VLCFA synthesis linked to biological processes such as secretion and endocytosis?

For a long time, the complexity of FAs and lipid species was a major bottleneck in studying lipids. However, thanks to the important work of many groups we have overcome this limitation and mass spectrometry-based methods are easily available to study lipid and FA composition. And these methods are continuously developed and even the flux of metabolites through lipid metabolism can be analyzed in high throughput.

Another emerging technique is the determination of membrane protein complex structures by cryo-electron tomography (Gold et al. 2014). More and more complex membrane protein structures have become available in recent years which help to understand the molecular mechanisms of trans-membrane proteins. In the future, it will be interesting to see which proteins form the elongation complex for VLCFA biosynthesis. If this complex can be purified and analyzed by cryo-EM this might finally reveal the molecular mechanism of VLCFA elongation.

The molecular function of the VLCFA-CoA synthetase will also be an interesting question for the future. How does the protein localize to lipid droplets and which processes involving VLCFA are taking place here? Are lipid droplets important organelles in VLCFA metabolism and are

membrane contact sites between lipid droplets and other organelles important? Similarly, how does the localization of Tsc13 at the nuclear vacuole junction affect its activity and VLCFA biosynthesis?

S. cerevisiae has been an important model organism to understand the mechanistic details of VLCFA elongation and it will continue to do so. Elucidating the molecular mechanisms of VLCFA biosynthesis, regulation and degradation in yeast will help us to understand the complex role of VLCFAs in human pathophysiology.

Acknowledgments: We thank members of the Fröhlich lab for critical comments on the manuscript and especially Sebastian Eising for comments on the Figures. Florian Fröhlich is supported by the DFG grant FR 3647/2-2 and the SFB944 (P20). Pia Erdbrügger is supported by a fellowship from the Hans-Mühlenhoff Foundation of the University of Osnabrück.

Author contribution: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: Florian Fröhlich is supported by the DFG grant FR 3647/2-2 and the SFB944 (P20). Pia Erdbrügger is supported by a fellowship from the Hans-Mühlenhoff Foundation of the University of Osnabrück.

Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

References

- Abu-Safieh, L., Alrashed, M., Anazi, S., Alkuraya, H., Khan, A.O., Al-Owain, M., Al-Zahrani, J., Al-Abdi, L., Hashem, M., Al-Tarimi, S., et al. (2013). Autozygome-guided exome sequencing in retinal dystrophy patients reveals pathogenetic mutations and novel candidate disease genes. *Genome Res.* 23: 236–247.
- Athenstaedt, K., Zweyck, D., Jandrositz, A., Kohlwein, S.D., and Daum, G. (1999). Identification and characterization of major lipid particle proteins of the yeast *Saccharomyces cerevisiae*. *J. Bacteriol.* 181: 6441–6448.
- Beaudoin, F., Michaelson, L. V., Hey, S.J., Lewis, M.J., Shewry, P.R., Sayanova, O., and Napier, J.A. (2000). Heterologous reconstitution in yeast of the polyunsaturated fatty acid biosynthetic pathway. *Proc. Natl. Acad. Sci. U.S.A.* 97: 6421–6426.
- Beaudoin, F.R., Gable, K., Sayanova, O., Dunn, T., and Napier, J.A. (2002). A *Saccharomyces cerevisiae* gene required for heterologous fatty acid elongase activity encodes a microsomal β -keto-reductase. *J. Biol. Chem.* 277: 11481–11488.
- Beeler, T., Bacikova, D., Gable, K., Hopkins, L., Johnson, C., Slife, H., and Dunn, T. (1998). The *Saccharomyces cerevisiae* TSC10/YBR265W gene encoding 3- ketosphinganine reductase is identified in a screen for temperature-sensitive suppressors of the Ca^{2+} -sensitive *csg2Δ* mutant. *J. Biol. Chem.* 273: 30688–30694.
- Berchtold, D., Piccolis, M., Chiaruttini, N., Riezman, I., Riezman, H., Roux, A., Walther, T.C., and Loewith, R. (2012). Plasma membrane stress induces relocation of Slm proteins and activation of TORC2 to promote sphingolipid synthesis. *Nat. Cell Biol.* 14: 542–547.
- Black, P.N., and DiRusso, C.C. (2007). Yeast acyl-CoA synthetases at the crossroads of fatty acid metabolism and regulation. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1771: 286–298.
- Bosson, R., Jaquenoud, M., and Conzelmann, A. (2006). GUP1 of *Saccharomyces cerevisiae* encodes an O-acyltransferase involved in remodeling of the GPI anchor. *Mol. Biol. Cell* 17: 2636–2645.
- Breslow, D.K., Collins, S.R., Bodenmiller, B., Aebersold, R., Simons, K., Shevchenko, A., Ejsing, C.S., and Weissman, J.S. (2010). Orm family proteins mediate sphingolipid homeostasis. *Nature* 463: 1048–1053.
- Cabrera, M., Arlt, H., Epp, N., Lachmann, J., Griffith, J., Perz, A., Reggiori, F., and Ungermann, C. (2013). Functional separation of endosomal fusion factors and the class C core vacuole/endosome tethering (corvet) complex in endosome biogenesis. *J. Biol. Chem.* 288: 5166–5175.
- De Carvalho, C.C.C.R., and Caramujo, M.J. (2018). The various roles of fatty acids. *Molecules* 23. <https://doi.org/10.3390/molecules23102583>.
- Choi, J.Y., and Martin, C.E. (1999). The *Saccharomyces cerevisiae* FAT1 gene encodes an acyl-coA synthetase that is required for maintenance of very long chain fatty acid levels. *J. Biol. Chem.* 274: 4671–4683.
- Chung, J.H., Lester, R.L., and Dickson, R.C. (2003). Sphingolipid requirement for generation of a functional V1 component of the vacuolar ATPase. *J. Biol. Chem.* 278: 28872–28881.
- Currie, E., Guo, X., Christiano, R., Chitru, C., Kory, N., Harrison, K., Haas, J., Walther, T.C., and Farese, R.V. (2014). High confidence proteomic analysis of yeast LDs identifies additional droplet proteins and reveals connections to dolichol synthesis and sterol acetylation. *J. Lipid Res.* 55: 1465–1477.
- David, D., Sundarababu, S., and Gerst, J.E. (1998). Involvement of long chain fatty acid elongation in the trafficking of secretory vesicles in yeast. *J. Cell Biol.* 143: 1167–1182.
- Denic, V., and Weissman, J.S. (2007). A molecular caliper mechanism for determining very long-chain fatty acid length. *Cell* 130: 663–677.
- Dickson, R.C., and Lester, R.L. (2002). Sphingolipid functions in *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1583: 13–25.
- Dickson, R.C., Sumanasekera, C., and Lester, R.L. (2006). Functions and metabolism of sphingolipids in *Saccharomyces cerevisiae*. *Prog. Lipid Res.* 45: 447–465.
- Ejsing, C.S., Sampaio, J.L., Surendranath, V., Duchoslav, E., Ekroos, K., Klemm, R.W., Simons, K., and Shevchenko, A. (2009). Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. *Proc. Natl. Acad. Sci. U.S.A.* 106: 2136–2141.
- El-Sherbeini, M., and Cemas, J.A. (1995). Cloning and characterization of GNS1: a *Saccharomyces cerevisiae* gene involved in synthesis of 1,3- β -glucan *in vitro*. *J. Bacteriol.* 177: 3227–3234.
- Færgeman, N.J., DiRusso, C.C., Elberger, A., Knudsen, J., and Black, P.N. (1997). Disruption of the *Saccharomyces cerevisiae* homologue to the murine fatty acid transport protein impairs

- uptake and growth on long-chain fatty acids. *J. Biol. Chem.* 272: 8531–8538.
- Ferdinandusse, S., Falkenberg, K.D., Koster, J., Mooyer, P.A., Jones, R., van Roermund, C.W.T., Pizzino, A., Schrader, M., Wanders, R.J.A., Vanderver, A., et al. (2017). ACBD5 deficiency causes a defect in peroxisomal very long-chain fatty acid metabolism. *J. Med. Genet.* 54: 330–337.
- Foster, J., and Nakata, P.A. (2014). An oxalyl-CoA synthetase is important for oxalate metabolism in *Saccharomyces cerevisiae*. *FEBS Lett.* 588: 160–166.
- Fröhlich, F., Petit, C., Kory, N., Christiano, R., Hannibal-Bach, H.K., Graham, M., Liu, X., Ejsing, C.S., Farese, R.V., and Walther, T.C. (2015). The GARP complex is required for cellular sphingolipid homeostasis. *eLife* 4. <https://doi.org/10.7554/elife.08712>.
- Ganesan, S., Shabits, B.N., and Zarembek, V. (2015). Tracking diacylglycerol and phosphatidic acid pools in budding yeast. *Lipid Insights*: 75–85. <https://doi.org/10.4137/LPI.S31781>.
- Garcia-Arranz, M., Maldonado, A.M., Mazon, M.J., and Portillo, F. (1994). Transcriptional control of yeast plasma membrane H⁺-ATPase by glucose. Cloning and characterization of a new gene involved in this regulation. *J. Biol. Chem.* 269: 18076–18082.
- Gold, V.A.M., Ieva, R., Walter, A., Pfanner, N., Van Der Laan, M., and Kühlbrandt, W. (2014). Visualizing active membrane protein complexes by electron cryotomography. *Nat. Commun.* 5. <https://doi.org/10.1038/ncomms5129>.
- Di Gregorio, E., Borroni, B., Giorgio, E., Lacerenza, D., Ferrero, M., Lo Buono, N., Ragusa, N., Mancini, C., Gaussen, M., Calcia, A., et al. (2014). ELOVL5 mutations cause spinocerebellar ataxia 38. *Am. J. Hum. Genet.* 95: 209–217.
- Gururaj, C., Federman, R., and Chang, A. (2013). Orm proteins integrate multiple signals to maintain sphingolipid homeostasis. *J. Biol. Chem.* 288: 20453–20463.
- Haak, D., Gable, K., Beeler, T., and Dunn, T. (1997). Hydroxylation of *Saccharomyces cerevisiae* ceramides requires Sur2p and Scs7p. *J. Biol. Chem.* 272: 29704–29710.
- Hama, K., Fujiwara, Y., Takashima, S., Hayashi, Y., Yamashita, A., Shimozawa, N., and Yokoyama, K. (2020). Hexacosenoyl-CoA is the most abundant very long-chain acyl-CoA in ATP binding cassette transporter D1-deficient cells. *J. Lipid Res.* 61: 523–536.
- Han, G., Gable, K., Kohlwein, S.D., Beaudoin, F., Napier, J.A., and Dunn, T.M. (2002). The *Saccharomyces cerevisiae* YBR159w gene encodes the 3-ketoreductase of the microsomal fatty acid elongase. *J. Biol. Chem.* 277: 35440–35449.
- Han, S., Lone, M.A., Schneiter, R., and Chang, A. (2010). Orm1 and Orm2 are conserved endoplasmic reticulum membrane proteins regulating lipid homeostasis and protein quality control. *Proc. Natl. Acad. Sci. U.S.A.* 107: 5851–5856.
- Hayashi, Y., Yamano, M., Kono, N., Arai, H., Fujiwara, Y., and Ichi, I. (2020). Phosphorylation of Elovl5 changes its substrate preference to synthesize Mead acid in response to essential fatty acid deficiency. *BioRxiv*, 2020.01.31.929224.
- He, C., and Klionsky, D.J. (2009). Regulation mechanisms and signaling pathways of autophagy. *Annu. Rev. Genet.* 43: 67–93.
- Hettema, E.H., and Tabak, H.F. (2000). Transport of fatty acids and metabolites across the peroxisomal membrane. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1486: 18–27.
- Hiltunen, J.K., Mursula, A.M., Rottensteiner, H., Wierenga, R.K., Kastaniotis, A.J., and Gurvitz, A. (2003). The biochemistry of peroxisomal β -oxidation in the yeast *Saccharomyces cerevisiae*. *FEMS Microbiol. Rev.* 27: 35–64.
- Hisanaga, Y., Ago, H., Nakagawa, N., Hamada, K., Ida, K., Yamamoto, M., Hori, T., Arai, Y., Sugahara, M., Kuramitsu, S., et al. (2004). Structural basis of the substrate-specific two-step catalysis of long chain fatty acyl-CoA synthetase dimer. *J. Biol. Chem.* 279: 31717–31726.
- Horazdovsky, B.F., Busch, G.R., and Emr, S.D. (1994). VPS21 encodes a rab5-like GTP binding protein that is required for the sorting of yeast vacuolar proteins. *EMBO J.* 13: 1297–1309.
- Hurst, L.R., Zhang, C., Kazmirchuk, T.D.D., Rivera-kohr, D.A., Brett, C.L., Fratti, R.A. (2020). Sphingolipids with very long-chain fatty acids regulate vacuole fusion during tethering and docking. *BioRxiv*: 1–40.
- Johnson, D.R., Knoll, L.J., Levin, D.E., and Gordon, J.I. (1994). *Saccharomyces cerevisiae* contains four fatty acid activation (FAA) genes: an assessment of their role in regulating protein N-myristoylation and cellular lipid metabolism. *J. Cell Biol.* 127: 751–762.
- Kemp, S., Valianpour, F., Denis, S., Ofman, R., Sanders, R.J., Mooyer, P., Barth, P.G., and Wanders, R.J.A. (2005). Elongation of very long-chain fatty acids is enhanced in X-linked adrenoleukodystrophy. *Mol. Genet. Metabol.* 84: 144–151.
- Kihara, A. (2012). Very long-chain fatty acids: elongation, physiology and related disorders. *J. Biochem.* 152: 387–395.
- Kihara, A., Sakuraba, H., Ikeda, M., Denpoh, A., and Igarashi, Y. (2008). Membrane topology and essential amino acid residues of Phs1, a 3-hydroxyacyl-CoA dehydratase involved in very long-chain fatty acid elongation. *J. Biol. Chem.* 283: 11199–11209.
- Kohlwein, S.D., Eder, S., Oh, C.-S., Martin, C.E., Gable, K., Bacikova, D., and Dunn, T. (2001). Tsc13p is required for fatty acid elongation and localizes to a novel structure at the nuclear-vacuolar interface in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 21: 109–125.
- Kory, N., Farese, R.V., and Walther, T.C. (2016). Targeting fat: mechanisms of protein localization to lipid droplets. *Trends Cell Biol.* 26: 535–546.
- Kunau, W.H., Dommes, V., and Schulz, H. (1995). β -Oxidation of fatty acids in mitochondria, peroxisomes, and bacteria: a century of continued progress. *Prog. Lipid Res.* 34: 267–342.
- Kvam, E., Gable, K., Dunn, T.M., and Goldfarb, D.S. (2005). Targeting of Tsc13p to nucleus-vacuole junctions: a role for very-long-chain fatty acids in the biogenesis of microautophagic vesicles. *Mol. Biol. Cell* 16: 3987–3998.
- Langemeyer, L., Borchers, A.C., Herrmann, E., Füllbrunn, N., Han, Y., Perz, A., Auffarth, K., Kümmel, D., and Ungermann, C. (2020). A conserved and regulated mechanism drives endosomal Rab transition. *eLife* 9. <https://doi.org/10.7554/elife.56090>.
- Lee, J., Moir, R.D., McIntosh, K.B., and Willis, I.M.W. (2012). TOR signaling regulates ribosome and tRNA synthesis via LAMMER/Clk and GSK-3 family kinases. *Mol. Cell* 45: 836–843.
- Leibundgut, M., Maier, T., Jenni, S., and Ban, N. (2008). The multienzyme architecture of eukaryotic fatty acid synthases. *Curr. Opin. Struct. Biol.* 18: 714–725.
- Lester, R.L., and Dickson, R.C. (2001). High-performance liquid chromatography analysis of molecular species of sphingolipid-related long chain bases and long chain base phosphates in *Saccharomyces cerevisiae* after derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate. *Anal. Biochem.* 298: 283–292.

- Linton, K.J., and Higgins, C.F. (2007). Structure and function of ABC transporters: the ATP switch provides flexible control. *Pflüger's Arch. Eur. J. Physiol.* 453: 555–567.
- Liu, M., Huang, C., Polu, S.R., Schneider, R., and Chang, A. (2012). Regulation of sphingolipid synthesis through Orm1 and Orm2 in yeast. *J. Cell Sci.* 125: 2428–2435.
- Lomakin, I.B., Xiong, Y., and Steitz, T.A. (2007). The crystal structure of yeast fatty acid synthase, a cellular machine with eight active sites working together. *Cell* 129: 319–332.
- Micoogullari, Y., Basu, S.S., Ang, J., Weisshaar, N., Schmitt, N.D., Abdelmoula, W.M., Lopez, B., Agar, J.N., Agar, N., and Hanna, J. (2020). Dysregulation of very-long-chain fatty acid metabolism causes membrane saturation and induction of the unfolded protein response. *Mol. Biol. Cell* 31: 7–17.
- Moser, H.W., and Moser, A.B. (1996). Very long-chain fatty acids in diagnosis, pathogenesis, and therapy of peroxisomal disorders. *Lipids* 31: S141–S144.
- Mueller, N., Sassa, T., Morales-Gonzalez, S., Schneider, J., Salchow, D.J., Seelow, D., Knierim, E., Stenzel, W., Kihara, A., and Schuelke, M. (2019). *De novo* mutation in ELOVL1 causes ichthyosis, acanthosis nigricans, hypomyelination, spastic paraplegia, high frequency deafness and optic atrophy. *J. Med. Genet.* 56: 164–175.
- Munn, A.L., and Riezman, H. (1994). Endocytosis is required for the growth of vacuolar H⁺-ATPase-defective yeast: identification of six new END genes. *J. Cell Biol.* 127: 373–386.
- Nugteren, D.H. (1965). The enzymic chain elongation of fatty acids by rat-liver microsomes. *Biochim. Biophys. Acta Lipids Lipid. Metabol.* 106: 280–290.
- Obara, K., Kojima, R., and Kihara, A. (2013). Effects on vesicular transport pathways at the late endosome in cells with limited very long-chain fatty acids. *J. Lipid Res.* 54: 831–842.
- Obermeyer, T., Fraisl, P., DiRusso, C.C., and Black, P.N. (2007). Topology of the yeast fatty acid transport protein Fat1p: mechanistic implications for functional domains on the cytosolic surface of the plasma membrane. *J. Lipid Res.* 48: 2354–2364.
- Oh, C.S., Toke, D.A., Mandala, S., and Martin, C.E. (1997). ELO2 and ELO3, homologues of the *Saccharomyces cerevisiae* ELO1 gene, function in fatty acid elongation and are required for sphingolipid formation. *J. Biol. Chem.* 272: 17376–17384.
- Olsen, I., and Jantzen, E. (2001). Sphingolipids in bacteria and fungi. *Anaerobe* 7: 103–112.
- Olson, D.K., Fröhlich, F., Christiano, R., Hannibal-Bach, H.K., Ejlsing, C.S., and Walther, T.C. (2015a). Rom2-dependent phosphorylation of Elo2 controls the abundance of very long-chain fatty acids. *J. Biol. Chem.* 290: 4238–4247.
- Olson, D.K., Fröhlich, F., Farese, R. V., and Walther, T.C. (2015b). Taming the sphinx: mechanisms of cellular sphingolipid homeostasis. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids*.
- Pan, X., Roberts, P., Chen, Y., Kvam, E., Shulga, N., Huang, K., Lemmon, S., and Goldfarb, D.S. (2000). Nucleus-vacuole junctions in *Saccharomyces cerevisiae* are formed through the direct interaction of Vac8p with Nvj1p. *Mol. Biol. Cell* 11: 2445–2457.
- Peplowska, K., Markgraf, D.F., Ostrowicz, C.W., Bange, G., and Ungermann, C. (2007). The CORVET tethering complex interacts with the yeast Rab5 homolog Vps21 and is involved in endo-lysosomal biogenesis. *Dev. Cell* 12: 739–750.
- Pettus, B.J., Chalfant, C.E., and Hannun, Y.A. (2002). Ceramide in apoptosis: an overview and current perspectives. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1585: 114–125.
- Pittet, M., and Conzelmann, A. (2007). Biosynthesis and function of GPI proteins in the yeast *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1771: 405–420.
- Poulos, A., Beckman, K., Johnson, D.W., Paton, B.C., Robinson, B.S., Sharp, P., Usher, S., and Singh, H. (1992). Very long-chain fatty acids in peroxisomal disease. *Adv. Exp. Med. Biol.* 318: 331–340.
- Ratray, J.B.M., Schibeci, A., and Kidby, D.K. (1975). Lipids of yeasts. *Bacteriol. Rev.* 39: 197–231.
- Revardel, E., Bonneau, M., Durrens, P., and Aigle, M. (1995). Characterization of a new gene family developing pleiotropic phenotypes upon mutation in *Saccharomyces cerevisiae*. *BBA Gene Struct. Expr.* 1263: 261–265.
- Řezanka, T., and Sigler, K. (2009). Odd-numbered very-long-chain fatty acids from the microbial, animal and plant kingdoms. *Prog. Lipid Res.* 48: 206–238.
- Robinson, J.S., Klionsky, D.J., Banta, L.M., and Emr, S.D. (1988a). Protein sorting in *Saccharomyces cerevisiae*: isolation of mutants defective in the delivery and processing of multiple vacuolar hydrolases. *Mol. Cell Biol.* 8: 4936–4948.
- Robinson, J.S., Klionsky, D.J., Banta, L.M., and Emr, S.D. (1988b). Protein sorting in *Saccharomyces cerevisiae*: isolation of mutants defective in the delivery and processing of multiple vacuolar hydrolases. *Mol. Cell Biol.* 8: 4936–4948.
- Roelants, F.M., Breslow, D.K., Muir, A., Weissman, J.S., and Thorner, J. (2011). Protein kinase Ypk1 phosphorylates regulatory proteins Orm1 and Orm2 to control sphingolipid homeostasis in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A.* 108: 19222–19227.
- Roermund, C.W.T., Visser, W.F., Ijlst, L., Cruchten, A., Boek, M., Kulik, W., Waterham, H.R., and Wanders, R.J.A. (2008). The human peroxisomal ABC half transporter ALDP functions as a homodimer and accepts acyl-CoA esters. *Faseb. J.* 22: 4201–4208.
- Van Roermund, C.W.T., Waterham, H.R., Ijlst, L., and Wanders, R.J.A. (2003). Fatty acid metabolism in *Saccharomyces cerevisiae*. *Cell. Mol. Life Sci.* 60: 1838–1851.
- Van Roermund, C.W.T., Visser, W.F., Ijlst, L., Waterham, H.R., and Wanders, R.J.A. (2011). Differential substrate specificities of human ABCD1 and ABCD2 in peroxisomal fatty acid β -oxidation. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1811: 148–152.
- Van Roermund, C.W.T., Ijlst, L., Majczak, W., Waterham, H.R., Folkerts, H., Wanders, R.J.A., and Hellingwerf, K.J. (2012). Peroxisomal fatty acid uptake mechanism in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 287: 20144–20153.
- Roggenkamp, R., Numa, S., and Schweizer, E. (1980). Fatty acid-requiring mutant of *Saccharomyces cerevisiae* defective in acetyl-CoA carboxylase. *Proc. Natl. Acad. Sci. U.S.A.* 77: 1814–1817.
- Rössler, H., Rieck, C., Delong, T., Hoja, U., and Schweizer, E. (2003). Functional differentiation and selective inactivation of multiple *Saccharomyces cerevisiae* genes involved in very-long-chain fatty acid synthesis. *Mol. Genet. Genom.* 269: 290–298.
- Schneider, R. (1999). Brave little yeast, please guide us to Thebes: sphingolipid function in *S. cerevisiae*. *Bioessays* 21: 1004–1010. [https://doi.org/10.1002/\(sici\)1521-1878\(199912\)22:1<1004::aid-bies4>3.0.co;2-y](https://doi.org/10.1002/(sici)1521-1878(199912)22:1<1004::aid-bies4>3.0.co;2-y).
- Schneider, R., and Kohlwein, S.D. (1997). Organelle structure, function, and inheritance in yeast: a role for fatty acid synthesis? *Cell* 88: 431–434.

- Schneider, R., Hitomi, M., Ivessa, A.S., Fasch, E.V., Kohlwein, S.D., and Tartakoff, A.M. (1996). A yeast acetyl coenzyme A carboxylase mutant links very-long-chain fatty acid synthesis to the structure and function of the nuclear membrane-pore complex. *Mol. Cell Biol.* 16: 7161–7172.
- Schneider, R., Brügger, B., Amann, C.M., Prestwich, G.D., Epand, R.F., Zellnig, G., Wieland, F.T., and Epand, R.M. (2004). Identification and biophysical characterization of a very-long-chain-fatty-acid-substituted phosphatidylinositol in yeast subcellular membranes. *Biochem. J.* 381: 941–949.
- Schorling, S., Vallée, B., Barz, W.P., Riezman, H., and Oesterhelt, D. (2001). Lag1p and Lac1p are essential for the acyl-CoA-dependent ceramide synthase reaction in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 12: 3417–3427.
- Schuldiner, M., Collins, S.R., Thompson, N.J., Denic, V., Bhamidipati, A., Punna, T., Ihmels, J., Andrews, B., Boone, C., Greenblatt, J.F., et al. (2005). Exploration of the function and organization of the yeast early secretory pathway through an epistatic miniarray profile. *Cell* 123: 507–519.
- Schweizer, E., and Hofmann, J. (2004). Microbial type I fatty acid synthases (FAS): major players in a network of cellular FAS systems. *Microbiol. Mol. Biol. Rev.* 68: 501–517.
- Semenkovich, C.F. (1997). Regulation of fatty acid synthase (FAS). *Prog. Lipid Res.* 36: 43–53.
- Shai, N., Schuldiner, M., and Zalckvar, E. (2016). No peroxisome is an island - peroxisome contact sites. *Biochim. Biophys. Acta Mol. Cell Res.* 1863: 1061–1069.
- Shimobayashi, M., Oppliger, W., Moes, S., Jenö, P., and Hall, M.N. (2013). TORC1-regulated protein kinase Npr1 phosphorylates Orm to stimulate complex sphingolipid synthesis. *Mol. Biol. Cell* 24: 870–881.
- Singer-Krüger, B., Stenmark, H., Düsterhöft, A., Philippsen, P., Yoo, J.S., Gallwitz, D., and Zerial, M. (1994). Role of three rab5-like GTPases, Ypt51p, Ypt52p, and Ypt53p, in the endocytic and vacuolar protein sorting pathways of yeast. *J. Cell Biol.* 125: 283–298.
- Singh, K., Graf, B., Linden, A., Sautner, V., Urlaub, H., Tittmann, K., Stark, H., and Chari, A. (2020). Discovery of a regulatory subunit of the yeast fatty acid synthase. *Cell* 180: 1130–1143, e20.
- Sipos, G., Reggiori, F., Vionnet, C., and Conzelmann, A. (1997). Alternative lipid remodelling pathways for glycosylphosphatidylinositol membrane anchors in *Saccharomyces cerevisiae*. *EMBO J.* 16: 3494–3505.
- Skrzypek, M.S., Nagiec, M.M., Lester, R.L., and Dickson, R.C. (1999). Analysis of phosphorylated sphingolipid long-chain bases reveals potential roles in heat stress and growth control in *Saccharomyces*. *J. Bacteriol.* 181: 1134–1140.
- Steiner, S., Smith, S., Waechter, C.J., and Lester, R.L. (1969). Isolation and partial characterization of a major inositol-containing lipid in baker's yeast, mannosyl-diinositol, diphosphoryl-ceramide. *Proc. Natl. Acad. Sci. U.S.A.* 64: 1042–1048.
- Sumper, M., and Riepertinger, C. (1972). Structural relationship of biotin-containing enzymes. Acetyl-CoA carboxylase and pyruvate carboxylase from yeast. *Eur. J. Biochem.* 29: 237–248.
- Sun, Y., Miao, Y., Yamane, Y., Zhang, C., Shokat, K.M., Takematsu, H., Kozutsumi, Y., and Drubin, D.G. (2012). Orm protein phosphoregulation mediates transient sphingolipid biosynthesis response to heat stress via the Pkh-Ypk and Cdc55-PP2A pathways. *Mol. Biol. Cell* 23: 2388–2398.
- Tehlivets, O., Scheuringer, K., and Kohlwein, S.D. (2007). Fatty acid synthesis and elongation in yeast. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1771: 255–270.
- Toke, D.A., and Martin, C.E. (1996). Isolation and characterization of a gene affecting fatty acid elongation in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 271: 18413–18422.
- Vallée, B., and Riezman, H. (2005). Lip1p: a novel subunit of acyl-CoA ceramide synthase. *EMBO J.* 24: 730–741.
- Voynova, N.S., Vionnet, C., Ejsing, C.S., and Conzelmann, A. (2012). A novel pathway of ceramide metabolism in *Saccharomyces cerevisiae*. *Biochem. J.* 447: 103–114.
- Voynova, N.S., Roubaty, C., Vazquez, H.M., Mallela, S.K., Ejsing, C.S., and Conzelmann, A. (2015). *Saccharomyces cerevisiae* is dependent on vesicular traffic between the Golgi apparatus and the vacuole when inositolphosphorylceramide synthase aur1 is inactivated. *Eukaryot. Cell* 14: 1203–1216.
- Wanders, R.J.A., Van Roermund, C.W.T., Van Wijland, M.J.A., Schutgens, R.B.H., Heikoop, J., Van Den Bosch, H., Schram, A.W., and Tager, J.M. (1987). Peroxisomal fatty acid β -oxidation in relation to the accumulation of very long chain fatty acids in cultured skin fibroblasts from patients with Zellweger syndrome and other peroxisomal disorders. *J. Clin. Invest.* 80: 1778–1783.
- Wang, Q., Du, X., Ma, K., Shi, P., Liu, W., Sun, J., Peng, M., and Huang, Z. (2018). A critical role for very long-chain fatty acid elongases in oleic acid-mediated *Saccharomyces cerevisiae* cytotoxicity. *Microbiol. Res.* 207: 1–7.
- Watkins, P.A. (1997). Fatty acid activation. *Prog. Lipid Res.* 36: 55–83.
- Watkins, P.A., Lu, J.F., Steinberg, S.J., Gould, S.J., Smith, K.D., and Braiterman, L.T. (1998). Disruption of the *Saccharomyces cerevisiae* FAT1 gene decreases very long-chain fatty acyl-CoA synthetase activity and elevates intracellular very long-chain fatty acid concentrations. *J. Biol. Chem.* 273: 18210–18219.
- Welch, J.W., and Burlingame, A.L. (1973). Very long chain fatty acids in yeast. *J. Bacteriol.* 115: 464–466.
- Wente, S.R. (2000). Gatekeepers of nucleus. *Science* 288: 1374–1377.
- Wiesinger, C., Eichler, F.S., and Berger, J. (2015). The genetic landscape of X-linked adrenoleukodystrophy: inheritance, mutations, modifier genes, and diagnosis. *Appl. Clin. Genet.* 8: 109–121.
- Worman, H.J., and Courvalin, J.C. (2000). The inner nuclear membrane. *J. Membr. Biol.* 177: 1–11.
- Yin, Q.Y., De Groot, P.W.J., Dekker, H.L., De Jong, L., Klis, F.M., and De Koster, C.G. (2005). Comprehensive proteomic analysis of *Saccharomyces cerevisiae* cell walls: identification of proteins covalently attached via glycosylphosphatidylinositol remnants or mild alkali-sensitive linkages. *J. Biol. Chem.* 280: 20894–20901.
- Zanolari, B., Friant, S., Funato, K., Sütterlin, C., Stevenson, B.J., and Riezman, H. (2000). Sphingoid base synthesis requirement for endocytosis in *Saccharomyces cerevisiae*. *EMBO J.* 19: 2824–2833.
- Zimmermann, C., Santos, A., Gable, K., Epstein, S., Gururaj, C., Chymkowitch, P., Pultz, D., Rødkær, S. V., Clay, L., Bjørås, M., et al. (2013). TORC1 inhibits GSK3-mediated Elo2 phosphorylation to regulate very long chain fatty acid synthesis and autophagy. *Cell Rep.* 5: 1036–1046.