Chapter 8
Molecular Mechanisms in Yeast Carbon Metabolism: Lipid Metabolism and Lipidomics

Birgit Ploier, Günther Daum and Uroš Petrovič

Abstract Lipids play several essential roles in the biology and metabolism of eukaryotic cells. In addition to their structural role as constituents of cell membranes, they have been increasingly recognized as dynamic and vital molecules, involved in a variety of cellular processes. Examples are cell signalling, membrane trafficking and influencing the stability of protein complexes in membranes. This chapter provides an overview of lipid classes and metabolic pathways in yeast. Lipid metabolism involves various organelles such as the endoplasmic reticulum (ER), mitochondria, peroxisomes and lipid droplets (LD), which will be highlighted. Specific attention is devoted to examples of recently discovered key players in yeast lipid metabolism, which illustrate our improved understanding of cells as an interconnected biological system. This chapter comprises descriptions of regulatory networks, multifunctional enzymes and lipids that serve as modulators of their own synthesis. The last part of the chapter is dedicated to the increasing numbers of biotechnological processes based on lipid metabolism. Besides the prominent model organism *Saccharomyces cerevisiae*, other predominantly oleaginous yeasts are also included.

Keywords Lipids · Yeast · Fatty acids · Phospholipids · Sterols · Triacylglycerols · Steryl esters · Non-polar lipids · Sphingolipids · Lipid droplets · Peroxisomes · Mitochondria

B. Ploier · G. Daum (✉)
Institute of Biochemistry, Graz University of Technology, Petersgasse 12/2, 8010 Graz, Austria
e-mail: guenther.daum@tugraz.at

U. Petrovič (✉)
Department of Molecular and Biomedical Sciences, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia
e-mail: uros.petrovic@ijs.si

J. Piškur and C. Compagno (eds.), *Molecular Mechanisms in Yeast Carbon Metabolism*,
DOI: 10.1007/978-3-642-55013-3_8,

Abbreviations

ABC	ATP-binding cassette
ATP	Adenosine triphosphate
CDP	Cytidinediphosphate
CL	Cardiolipin
CoA	Coenzyme A
CTP	Cytidine triphosphate
DAG	Diacylglycerol
DGPP	Diacylglycerol diphosphate
DMAPP	Dimethylallyl diphosphate
ER	Endoplasmic reticulum
ERMES	ER-mitochondria encounter structure
FA	Fatty acids
FIT	Fat storage-inducing transmembrane proteins
FPP	Farnesyl diphosphate
GGPP	Geranylgeranyl diphosphate
GPI	Glycosylphosphatidylinositol
GPP	Geranyl diphosphate
IMM	Inner mitochondrial membrane
IPC	Inositol phosphorylceramide
IPP	Isopentenyl diphosphate
LD	Lipid droplets
MAM	Mitochondria-associated membrane fraction
$M(IP)_2C$	Mannosyl (inositol phosphoryl)$_2$ ceramide
MINOS	Mitochondrial inner membrane organizing system
MIPC	Mannosylinositol phosphorylceramide
Mt	Mitochondria
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
Nu	Nucleus
OMM	Outer mitochondrial membrane
PA	Phosphatidic acid
PC	Phosphatidylcholine
PDR	Pleiotropic drug response
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
PIP	Phosphatidylinositol phosphate
PL	Phospholipids
PS	Phosphatidylserine
PUFA	Polyunsaturated FA
Px	Peroxisomes
SE	Steryl esters
SPT	Serine palmitoyltransferase complex
TG	Triacylglycerols
TORC2	Target of rapamycin complex 2
UAS_{INO}	Inositol-responsive upstream activating sequence element

8.1 Introduction

The link between central carbon metabolism and lipid synthesis is easily found: acetyl-CoA is the common precursor for all lipid biosynthetic pathways. Membrane biogenesis is, along with amino acid synthesis, one of the major consumers of acetyl-CoA and NADPH—the biosynthesis of one molecule of palmitic acid requires 8 acetyl-CoA and 14 NADPH molecules (Natter and Kohlwein 2012). However, while the understanding of connections between different metabolic routes has already reached an advanced state at the level of metabolites, the identification of regulatory mechanisms is only in its infancy.

Lipids are essential constituents of every living cell. They were long seen as primarily structural components of cellular membranes. However, lipid research over the past decades has shown that they fulfil many more vital functions that are increasingly recognized. Prominent examples are their role as regulators of energy metabolism, cell integrity and membrane-based processes such as endocytosis and vesicular trafficking (Daum et al. 1998; Souza and Pichler 2007). The accepted general definition of lipids is that they are relatively small, hydrophobic or amphiphilic molecules that are classified into eight distinct groups based on their chemical and biochemical properties: fatty acids (FA), glycerolipids, glycerophospholipids, sphingolipids, sterols and sterol derivatives, prenol lipids, glycolipids and polyketides. Altogether, more than 10,000 different lipid structures have been identified (Fahy et al. 2009). In this chapter we focus on the first five most commonly found lipid classes in yeast.

The field of lipid research has attracted more and more interest over the past decades as many lipid-associated disorders such as obesity, type-II-diabetes, insulin resistance and cardiovascular diseases have become increasing health risks in the Western world and recently also in developing countries. As the principles of lipid metabolism are well conserved between all eukaryotes and because of the many advantages of working with yeast, *Saccharomyces cerevisiae* has become a powerful model organism for lipid research. One established approach to dissect the complex network of enzymes and molecular mechanisms responsible for lipid homeostasis is the use of readily available single and multiple deletions mutants. One of the major resources that have enabled systematic studies in this direction is the repertoire of yeast deletion mutants of all non-essential genes, which have helped, in combination with different cultivation conditions, to understand the basics of lipid synthesis, storage and degradation pathways (Winzeler et al. 1999).

Many different cellular compartments are involved in lipid metabolism (Natter et al. 2005). Lipid synthesis takes place mainly in the endoplasmic reticulum (ER) and the Golgi compartment, but also lipid droplets (LD), mitochondria and peroxisomes play influential roles and will therefore be highlighted in this introduction. LD and peroxisomes, especially with respect to their role in lipid metabolism, were recently reviewed (Kohlwein et al. 2012).

LD are generally seen as a storage compartment for the non-polar lipids, triacylglycerols (TG) and steryl esters (SE). They are small spherical organelles of

approximately 400 nm in diameter consisting of a highly hydrophobic core of mainly TG, surrounded by shells of SE which are covered by a phospholipid monolayer with only a few embedded proteins (Athenstaedt et al. 1999a; Czabany et al. 2008). Proteome analysis revealed that these proteins are predominantly enzymes involved in lipid metabolism, for example TG lipases and SE hydrolases (Grillitsch et al. 2011). LD will be further described in the section on non-polar lipids.

Mitochondria are of special interest for lipid research. They provide an independent fatty acid synthesizing system (Tehlivets et al. 2007) and synthesize some phospholipids (Kuchler et al. 1986; Henry et al. 2012), but the majority of lipids are imported. Examples of autonomously formed mitochondrial lipids are phosphatidic acid (PA), cardiolipin (CL) or phosphatidylethanolamine (PE), whereas phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI) as well as sterols and sphingolipids have to be imported. Various mechanisms have been proposed for the import of lipids, such as direct membrane contact between the ER and mitochondria via the mitochondria-associated membrane (MAM) fraction, vesicular transport and the involvement of specific lipid binding and transfer proteins (Daum and Vance 1997). Mitochondria are also special regarding their lipid distribution, having an increased amount of CL and PI. The phospholipid CL comprises up to 15 %, which is very high compared to other organelles. Therefore, CL is often referred to as the typical mitochondrial phospholipid (Zinser and Daum 1995). Mitochondria are compartmentalized into four different subcompartments: the outer mitochondrial membrane, the intermembrane space and the inner mitochondrial membrane with its cristae and the matrix. A mitochondrial complex connecting the inner boundary membrane to the cristae membrane was recently identified and termed MINOS (mitochondrial inner membrane organizing system) or MitOS (mitochondrial organizing structure), and shown to be responsible for maintaining mitochondrial morphology (Hoppins et al. 2011; van der Laan et al. 2012; Zerbes et al. 2012). Most interestingly, even among the four subcompartments, lipids are not distributed randomly (Daum 1985). Therefore, intramitochondrial lipid transfer as well as the interorganelle transport of lipids is of outstanding interest. Some recent findings will be reported in the section on novel key players.

Peroxisomes deserve special attention in the description of lipid metabolism. They are spherical organelles with a diameter of about 0.1 μm, consisting of a fine granular matrix with a crystalline core, all surrounded by a single membrane. The protein content of these membranes is typically relatively low, whereas the matrix contains the highest protein concentration in eukaryotic cells with hydrogen peroxide-producing oxidase and catalase as prominent representatives (Kohlwein et al. 2012). Peroxisomes are ubiquitous and are involved in various metabolic pathways, especially detoxification processes and degradation of FA. The latter process, termed β-oxidation, makes FA available as an energy source. In contrast to mammalian cells, where β-oxidation occurs in mitochondria and peroxisomes, yeast β-oxidation takes place exclusively in peroxisomes (Poirier et al. 2006). Prior to degradation, FA have to be activated by one of six specific activators

Faa1p, Faa2p, Faa3p, Faa4p, Fat1p or Fat2p. The uptake of FA into peroxisomes can proceed by different mechanisms. Short and medium-chain length FA are thought to be taken up by diffusion, whereas long chain and very long chain FA require ABC (ATP-binding cassette) transport proteins. In yeast peroxisomes, the two ABC transporters, Pxa1p and Pxa2p, are thought to be responsible for the uptake of FA. These transporters hydrolyze FA-CoA esters prior to their entry into peroxisomes, releasing CoA into the cytoplasm, whereas FA are then re-esterified by a peroxisomal synthetase (van Roermund et al. 2012). Lipid composition of peroxisomes comprises nearly 50 % PC, 23 % PE, 16 % PI together with a remarkably high content of CL (7 %) (Zinser et al. 1991).

Recently, techniques for lipid content analysis have advanced substantially. The prerequisite for lipid analysis is usually lipid extraction into organic solvents, followed by chromatographic separation of lipid species that can then be detected by advanced spectrometric technologies. Currently, lipid research is shifting from basic molecular characterization to a global understanding of dynamic lipid regulation in the cell context. Lipids have been proposed to act as a molecular collective rather than as single molecules, best demonstrated by Guan et al. (2009) who showed that sphingolipids and sterols can interact functionally. In particular, lipidomic approaches and mathematical modelling are promising methods for interpreting lipid metabolism on a global scale (Alvarez-Vasquez et al. 2011; Santos and Riezman 2012). Lipidomics, which involves mapping all lipids of an organism or a cell, is facilitated by sophisticated mass spectrometry techniques combined with state-of-the-art data analysis software (Dennis 2009; Ejsing et al. 2009). The absolute quantification of lipids depends on internal standards which are not always available. Quantitative analysis would be particularly important in finding out how cells adapt their lipid profile to changes in the environment. Specifically, points of regulation could be identified by mathematical modelling, although this approach is still in its infancy. From the experimental point of view, however, mass spectrometry-based shotgun lipidomics has been applied to quantitatively and comprehensively asses the yeast lipidome (Ejsing et al. 2009). This approach was recently used to determine changes in the yeast lipidome under different growth conditions including growth on different carbon sources. Interestingly, different flexibilities (defined as dispersion of a given lipidomic feature across the dataset) were determined for different classes of lipids (Klose et al. 2012). The authors observed marked differences in the lipidome between growth on glucose- and non-glucose-based media.

Another hot topic of lipid research is the investigation of membrane organization by visualizing specific lipids in the cell. Visualization techniques confirmed the view that lipids and proteins are not moving freely within a membrane but that their diffusion is restricted in certain domains called rafts, which are enriched in sphingolipids and sterols (Lingwood and Simons 2010; Eggeling et al. 2009). However, the techniques are challenging, comprising high temporal and spatial super resolution microscopy and are limited by the availability of appropriate probes.

More recently, other yeast genera besides *Saccharomyces* have been attracting interest in lipid research. In particular, oleaginous yeasts, such as *Candida curvata* and *Yarrowia lipolytica*, have been shown to be industrially relevant for the sustainable production of lipids with compositions similar to those of vegetable oils and fats (Beopoulos et al. 2011). *Pichia pastoris*, an industrially highly relevant yeast especially for the expression of heterologous proteins, is another important model organism for lipid-related research, especially in organelle biology studies. Unless indicated otherwise, we will refer to *S. cerevisiae* in this chapter, but particularly in the section on biotechnological aspects other yeasts will also be mentioned.

The aim of this chapter is to provide a fundamental overview of yeast lipid metabolism, but also to point out novel findings and applications of the highly dynamic field of yeast lipid research. For detailed information beyond the scope of this chapter readers will be referred to other recent reviews (Henry et al. 2012; Jacquier and Schneiter 2012; Kohlwein et al. 2012; Natter and Kohlwein 2012; Rajakumari et al. 2008; Santos and Riezman 2012).

8.2 Lipid Classes

Lipids are divided into classes based on their structure and function. The major classes discussed in this chapter are FA, glycerophospholipids, sphingolipids, sterols and the non-polar storage lipids TG and SE. These five classes will be described with special emphasis on a basic understanding of their metabolism and function of their members. Regulatory mechanisms, especially newly identified ones, will be discussed in the section on novel key players.

8.2.1 Fatty Acids

FA are carboxylic acids with long hydrocarbon tails and differ from each other in chain length and degree of saturation. In *S. cerevisiae*, the overall composition of FA is rather simple, the members being mainly of C18:1 (oleate), C16:1 (palmitoleate) and C16:0 (palmitate) followed by C18:0 (stearate) and minor amounts of C14:0 (myristate) and C26:0 (cerotate) (Daum et al. 1998). The composition differs in the different yeast genera. In particular, in the oleaginous yeasts such as *Y. lipolytica,* the FA composition is highly diverse, comprising longer chain lengths and, especially, more double bonds, which makes such organisms useful for the production of nutritionally valuable polyunsaturated fatty acids (PUFA) (Beopoulos et al. 2011) as will be described below.

FA fulfil many different roles in cells. Most importantly, they serve as basic molecules for the biosynthesis of complex membrane and storage lipids (Tehlivets et al. 2007). Other functions include their role as signalling molecules,

transcriptional regulators and post-translational modifiers of proteins (Nadolski and Linder 2007). One prominent example of the latter is the palmitoylation of Ras proteins, but myristate is also often added as lipid moiety (Linder and Deschenes 2004).

FA metabolism in yeast is illustrated in Fig. 8.1. There are three main sources for FA: (i) de novo synthesis (ii) uptake by specific transporters and (iii) catabolism of complex lipids (Tehlivets et al. 2007). A small proportion of FA derives from the catabolism of proteins (Tehlivets et al. 2007). Two independent pathways exist for the biosynthesis of FA, the major cytosolic pathway and the mitochondrial pathway. The former pathway involves mainly three key enzymes, encoded by *ACC1*, *FAS1* and *FAS2* (for review see Henry et al. 2012). Biosynthesis of FA starts with the carboxylation by Acc1p of acetyl-CoA to give malonyl-CoA. Acc1p possesses three different activities: it can act as a biotin carboxylase, as a biotin carboxyl-carrier protein and as a transcarboxylase. It is located on the cytoplasmic surface of the ER, contains one covalently bound biotin molecule and is essential for growth. Malonyl-CoA is metabolized by a series of reactions catalyzed by FA synthases (*FAS* genes) and elongases. *FAS1* and *FAS2* encode two different subunits of the FA synthase complex. The active FAS complex consists of six α-units and six β-units (Chirala et al. 1987). *FAS1* encodes the β-subunit, which comprises four different activities: acetyltransferase, enoyl reductase, dehydratase and malonyl-palmitoyl transferase activities. *FAS2* encodes the α-subunit that displays acyl carrier protein, 3-ketoreductase, 3-ketosynthase and phosphopantetheinyl transferase activities (reviewed by Tehlivets et al. 2007). In yeast, double bonds are introduced by a single acyl-CoA Δ9 desaturase encoded by *OLE1* (Stukey et al. 1990). Elongation is carried out predominantly by Elo1p, although elongation of very long FA, especially for sphingolipid synthesis, is catalyzed mainly by Elo2p and Elo3p. De novo synthesis of FA takes place mainly in the cytosol, whereas elongation and desaturation reactions are carried out in the ER (Tehlivets et al. 2007).

Imported FA, which can be taken up by diffusion or by transporters, can fully compensate for endogenously synthesized FA. Prerequisite for the uptake of FA is the activation of free FA with coenzyme A, which is carried out by the acyl-CoA synthetases Faa1p, Faa2p, Faa3p, Faa4p and Fat1p. These enzymes are also believed to be involved in the uptake of FA into the cell (reviewed by Black and DiRusso 2007; Henry et al. 2012). While in *S. cerevisiae* machinery for utilization of extracellular complex lipids as energy or carbon source has not been identified, oleaginous yeast species produce extracellular lipases for this purpose. The best studied model for the utilization of hydrophobic substrates such as alkanes, TG and FA is *Y. lipolytica* (reviewed by Fickers et al. 2005). *Y. lipolytica* produces surfactants when grown on lipids as the only carbon source and changes the biophysical and morphological properties of the cell surface to enable adhesion of water insoluble growth substrates. The cells produce both membrane-bound and extracellular lipases, the major one being Lip2p which catalyzes hydrolysis of TG to free FA and glycerol. Free FA are then taken up by a mechanism that is not

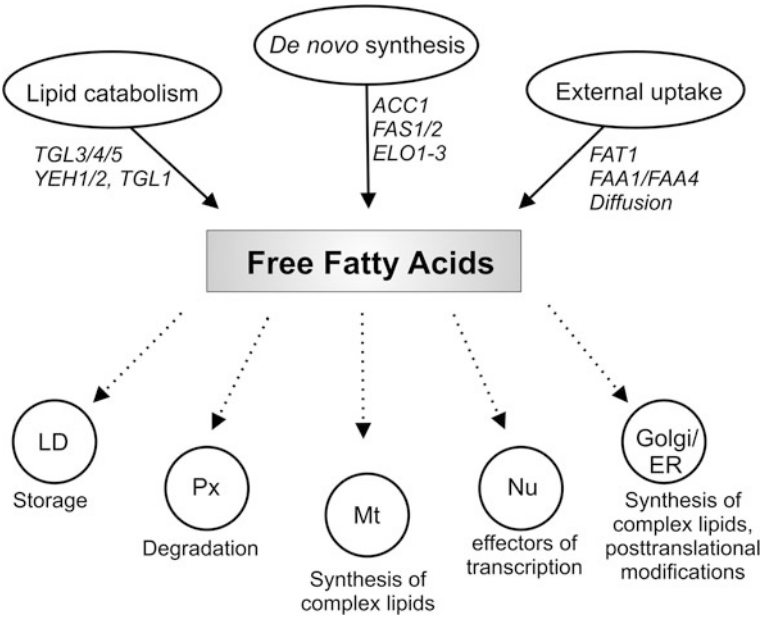

Fig. 8.1 Overview of fatty acid metabolism in yeast. FA derive mainly from three routes: catabolism of storage lipids, de novo synthesis and external uptake. They can be incorporated into storage lipids, degraded by β-oxidation to provide energy or converted into complex lipids like phospholipids or sphingolipids. FA can also act as effectors of transcription. *LD* lipid droplets, *Px* peroxisomes, *Mt* mitochondria, *Nu* nucleus, *ER* endoplasmic reticulum. For details see text

completely understood, activated by specific acyl-CoA synthetases and further metabolized similarly as described below for *S. cerevisiae*.

In general, free FA are metabolized very quickly. Elevated levels of free FA are harmful to cells because they can perturb membrane properties due to changes in fluidity. Thus, FA are either incorporated into complex lipids, i.e. PL or the storage lipids TG and SE, or they are oxidized to provide energy. Regardless of the source of free FA, the prerequisite for further conversion is activation by thioesterification with coenzyme A, which requires the action of acyl-CoA synthetases (*Faa1-4*, *Fat1*) (Black and DiRusso 2007). In mitochondria, the biosynthesis of FA is carried out by a totally different set of enzymes: Hfa1p, the mitochondrial acetyl-CoA carboxylase, catalyzes the production of malonyl-CoA which is then further processed by a different Fas complex (Hiltunen et al. 2010).

As mentioned in the introduction, catabolism of FA in yeast takes place exclusively in peroxisomes. Under standard growth conditions, the abundance of peroxisomes is quite low, but can be increased by FA supplementation to the medium (van Roermund et al. 1995). The classical β-oxidation starts with the oxidation of acyl-CoA to *trans*-2-enoyl-CoA by Fox1p (frequently called Pox1p). This reaction releases hydrogen peroxide, which is detoxified by catalase. The second step is the conversion, by Fox2p, of *trans*-2-enoyl-CoA to 3-ketoacyl-CoA.

This compound is the substrate of Fox3p, a 3-ketoacyl-CoA thiolase, which yields acetyl-CoA and a C2-shortened FA (Einerhand et al. 1991; Hiltunen et al. 1992). The route of FA directed to the site of peroxisomal β-oxidation, either via plasma membrane transport from an exogenous source, or from LD as an endogenous storage compartment, is still not completely understood.

8.2.2 Phospholipids

Phospholipids (PL) are regarded as bulk membrane constituents, since they can form lipid bilayers. They consist of a diacylglycerol backbone and a phosphate group at the *sn*-3 position that is linked to a polar head group. PL can be classified based on their different head groups. The major PL in yeast are PC, which comprises about 45 % of the total phospholipid content, PE, which makes up to 20 %, PI with 15 %, PS accounting for 5 % and CL being present at 2 % (Janssen et al. 2000; Zinser et al. 1991; Schneiter et al. 1999). However, the subcellular distribution of different phospholipids varies quantitatively and by origin. Especially, PS and CL are present just at low amounts in most organelle membranes but are major components of the plasma membrane and the inner mitochondrial membrane, respectively (Zinser and Daum 1995). In general, the lipid composition of membranes is not stochastic but characteristic of each organelle.

In addition to the role of PL as major structural components of cellular membranes, they are involved in a variety of other processes. They provide precursors for the synthesis of membranes, act as reservoirs of second messengers, conduct the lipidation of proteins for membrane association and function as molecular chaperones (reviewed by Carman and Han 2011; Dowhan and Bogdanov 2009; van Meer et al. 2008). PL can also be differentiated according to their shape, which is dictated by their head-to-tail area ratio. PC, PS and phosphatidylglycerol are cylindrically shaped since they display a head group similar to fatty acid chain area. Cylindrical PL are known to favour bilayer structures, while PE and CL, which belong to the group of non-bilayer forming PL, are conically shaped, the result of a smaller head-to-tail area (Cullis et al. 1986).

A key molecule in PL synthesis is PA (Fig. 8.2), which is also an important signalling molecule and regulator of lipid metabolism. PA derives from either glycerol-3-phosphate or dihydroxyacetone phosphate following fatty acyl-CoA dependent acyl transfer. These reactions are catalyzed by the *SCT1*- (*GAT2*) and *GPT2* (*GAT1*)-encoded glycerol-3-phosphate acyltransferases and the *SLC1*- and *ALE1*-encoded lysophospholipid acyltransferases (Athenstaedt and Daum 1997; Athenstaedt et al. 1999b; Chen et al. 2007b; Jain et al. 2007; Riekhof et al. 2007). Dihydroxyacetone phosphate is reduced by Ayr1p, which is present in LD, the ER and the mitochondrial outer membrane. PA is a branch point between the CDP-DAG (cytidinediphosphate- diacylglycerol) pathway and the formation of DAG (Athenstaedt and Daum 1999). In the first case, PA is metabolized to CDP-DAG under the catalytic action of the *CDS1*-encoded CDP-DAG synthase

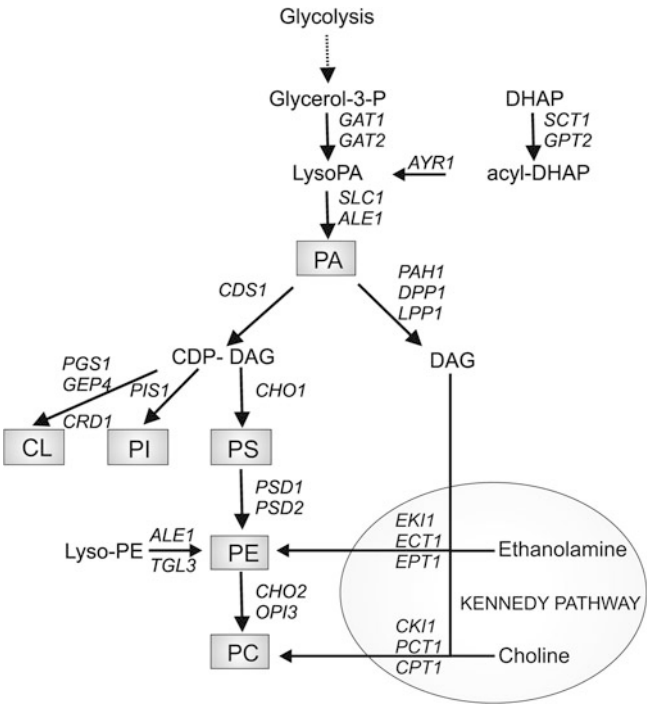

Fig. 8.2 Simplified pathway of phospholipid synthesis in the yeast *S. cerevisiae*. For details see text. *DHAP* dihydroxyacetone phosphate, *PA* phosphatidic acid, *CDP-DAG* cytidinediphosphate diacylglycerol, *DAG* diacylglycerol, *CL* cardiolipin, *PI* phosphatidylinositol, *PS* phosphatidylserine, *PE* phosphatidylethanolamine, *PC* phosphatidylcholine

(Shen et al. 1996). In the second case, the *PAH1*-encoded PA phosphatase forms DAG (Han et al. 2006). CDP-DAG and DAG are both used in the synthesis of PE and PC, but by different pathways. The first biosynthetic route is the CDP-DAG pathway, whereas in the Kennedy pathway DAG is used as a substrate for the conversion (for reviews see Carman and Han 2011; Henry et al. 2012). Both pathways are used in wild-type cells, but the CDP-DAG pathway is the major route for the synthesis of PE and PC when cells are grown in the absence of ethanolamine and choline. It starts with the conversion of CDP-DAG into PS by the ER-localized, *CHO1*-encoded PS synthase. PS is further decarboxylated to PE by two PS decarboxylases, Psd1p and Psd2p. Psd1p is localized to the inner mitochondrial membrane and accounts for the major enzymatic activity, whereas Psd2p is associated with Golgi and vacuolar membranes (Trotter and Voelker 1995; Clancey et al. 1993; Voelker 2003). PE is methylated by Cho2p and Opi3p yielding PC. PE and PC can also be obtained from exogenously supplied lysoPE and lysoPC, which can be acylated by the *ALE1*-encoded lysophospholipid acyltransferase (Riekhof and Voelker 2006; Riekhof et al. 2007). CDP-DAG can also be converted into PI by reaction with inositol catalyzed by Pis1p (Fischl and

Carman 1983). The biosynthesis of CL takes place only in mitochondria, initiated by the transfer of the phosphatidyl moiety of CDP-DAG to glycerol-3-phosphate by Pgs1p and continued by dephosphorylation of phosphatidylglycerophosphate by Gep4p (Chang et al. 1998a; Osman et al. 2010). The *CRD1*-encoded CL synthase finally produces CL (Chang et al. 1998b; Tuller et al. 1998; Jiang et al. 1997).

In the Kennedy pathway, exogenous ethanolamine and choline are transported into the cell by the choline/ethanolamine transporter Hnm1p. They are phosphorylated with ATP by the kinases Eki1p and Cki1p. They are then activated with CTP to form CDP-ethanolamine and CDP-choline, under the action of ethanolaminephosphate cytidylyltransferase Ect1p and cholinephosphate cytidylyltransferase Pct1p (Kennedy and Weiss 1956; Kim et al. 1999; Henry et al. 2012). PE and PC are finally formed by the *sn*-1,2-diacylglycerol ethanolaminephosphotransferase Ept1p and the cholinephosphotransferase Cpt1p catalyzing the reactions of CDP-ethanolamine and CDP-choline with DAG (Hjelmstad and Bell 1992). DAG is provided by dephosphorylation of PA by Pah1p (Fig. 8.2).

The organization of phospholipids within membranes is believed to occur via two principal mechanisms: lateral diffusion within the plane of a membrane and bidirectional, ATP-dependent movement facilitated by flippases. Intercompartmental phospholipid transport mainly occurs via vesicles and monomeric exchange (reviewed by Vehring and Pomorski 2005). These transport mechanisms do not lead to a homogeneous distribution of phospholipids. Moreover, there is increasing evidence that distinct lipid domains exist within certain cellular membranes which are called rafts (London and Brown 2000; Simons and Sampaio 2011).

The majority of PL undergoes rapid turnover and acyl-chain remodelling, which is catalyzed by specific acyltransferases, phospholipases and lipid phosphatases (reviewed in Henry et al. 2012).

8.2.3 Sterols

Sterols are important compounds in eukaryotic cells, serving as both structural and signalling molecules. Due to their rigid structure, they strongly affect membrane fluidity and permeability (Nes et al. 1993). It has been shown that yeast cells are not viable without sterols (Daum et al. 1998). They are often referred to as steroid alcohols that contain cyclopentanoperhydrophenanthrene as parent structure. The main sterol in yeast, and also the final product of sterol biosynthesis in other fungi, is ergosterol. Structural differences from the mammalian counterpart cholesterol are the double bonds between C-7,8 in the ring and C-22 in the side chain and the presence of a methyl group at C-24. The hydroxyl group at the C-3 position is the only hydrophilic component of the molecule which facilitates integration into membranes. The ergosterol biosynthetic pathway is one of the most complex biochemical pathways, comprising nearly 30 different biochemical reactions

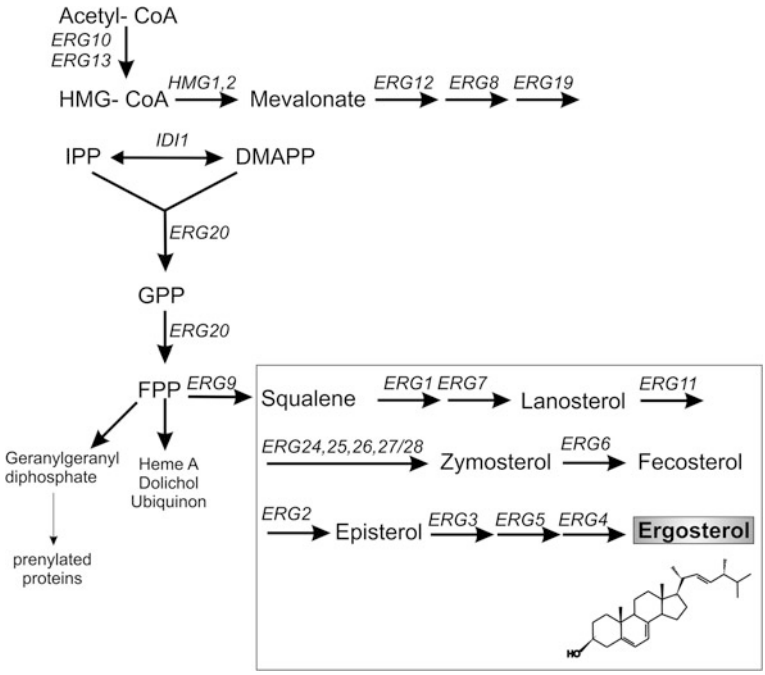

Fig. 8.3 Simplified ergosterol biosynthesis divided into the pre-squalene and post-squalene pathways, the latter being highlighted in the box. Important metabolic intermediates as well as the chemical structure of ergosterol are shown. For details see text. *CoA* Coenzyme A, *HMG* (3S)-3-hydroxy-3-methylglutaryl-CoA, *IPP* isopentenyl diphosphate, *DMAPP* dimethylallyl diphosphate, *GPP* geranyl diphosphate, *FPP* farnesyl diphosphate

catalyzed by the so-called Erg proteins (for recent reviews see Kristan and Rižner 2012; Kuranda et al. 2010; Pichler 2005). The most important steps are summarized in the following paragraph.

The ergosterol biosynthetic pathway is divided into the pre-squalene and post-squalene pathways, displayed in a much simplified scheme in Fig. 8.3. Most Erg proteins are located to the ER membrane, with the exception of Erg1p, Erg6p and Erg7p, which are localized mainly to LD (Athenstaedt et al. 1999a; Leber et al. 1994, 1998). The first steps of sterol synthesis are similar in fungi, plants and animals starting with the condensation of two acetyl-CoA molecules, catalyzed by Erg10p. This reaction yields acetoacetyl-CoA which reacts with another acetyl-CoA molecule to form (3S)-3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). In the yeast, this important intermediate is subsequently reduced to mevalonate by HMG-CoA reductases 1 and 2 (*HMG1/2*). This reaction is not only the rate-limiting step of sterol biosynthesis but also one of the major control points, since HMG-CoA reductase shows feedback inhibition by ergosterol (Bard and Downing 1981). Polakowski et al. (1998) showed that overexpression of a truncated version of Hmg1p leads to an increase in early sterol precursors. A cascade of phosphorylations and decarboxylations, also known as the mevalonate pathway, leads to

isopentenyl pyrophosphate (IPP), which is the precursor not only for squalene but also for other isoprenoids (Toth and Huwyler 1996). Isomerization of IPP to dimethylallyl pyrophosphate (DMAPP) and a subsequent head-to-tail condensation reaction of IPP and DMAPP yield geranyl pyrophosphate (GPP). These reactions are catalyzed by Idi1p and Erg20p (Anderson et al. 1989a, b; Chambon et al. 1991). Erg20p also facilitates the formation of farnesyl pyrophosphate (FPP) by adding two IPP units to DMAPP. Finally, coupling of two FPP molecules by Erg9p leads to squalene.

The first step of the post-squalene pathway is epoxidation of squalene by Erg1p. This reaction is followed by a number of complex cyclization events, catalyzed by Erg7p, that form lanosterol, which is the first intermediate with the typical sterol structure. A cascade of demethylations, desaturations and subsequent reduction events (*ERG24–ERG28*) leads to zymosterol. The reaction steps yielding zymosterol are conserved in all eukaryotic cells. It was shown that deletion of genes downstream this biosynthetic sequence leads to sterol auxotrophy, whereas cells depleted of *ERG* genes acting later in the pathway are still viable. The further methylation of zymosterol at the C-24 position by Erg6p yields fecosterol, an intermediate which is unique to yeast and other fungi. Then, Erg2p catalyzes the shift of a double bond to the C-7 position, followed by the introduction of a further double bond at the C-5 position by Erg3p. The last steps of the pathway introducing and removing double bonds (*ERG5*, *ERG4*) yield the end product, ergosterol.

Yeast cells usually synthesize sterols in excess. Since yeast is unable to degrade sterols, mechanisms of detoxification are required to avoid harmful influence on membranes. There are three main mechanisms to maintain sterol homeostasis: (i) esterification of free sterols with FA by Are1p and Are2p and storage in LD (Yang et al. 1996; Yu et al. 1996; Zweytick et al. 2000); (ii) downregulation of sterol biosynthesis; and (iii) sterol acetylation by Aft2p, which enables yeast cells to efficiently secrete excess sterols in the form of sterol acetates into the medium. The latter process is reversible and catalyzed by Say1p (Tiwari et al. 2007; Choudhary and Schneiter 2009). Acetylation of sterols has also been discussed as a possible quality control mechanism. It was suggested that sterols which do not pass a quality control cycle are acetylated and secreted. Recently, the PRY proteins (pathogen-related yeast proteins) that are involved in the secretion of acetylated sterols have been identified (Choudhary and Schneiter 2012).

Since some steps of the sterol biosynthetic pathway require oxygen, yeast becomes strictly sterol auxotroph under anaerobic conditions (Lees et al. 1995). Uptake of external sterols from the exterior is mediated by two ATP-binding cassette transporters, encoded by *AUS1* and *PDR11*, both targets of the transcriptional activator Upc2p (Crowley et al. 1998; Wilcox et al. 2002).

The intracellular sterol concentration is lowest at its place of biosynthesis, the ER and increases along the protein secretory pathway until it reaches its maximum at the plasma membrane (Zinser et al. 1993). The distribution of sterols between different cellular membranes has to be tightly regulated to maintain distinct

membrane properties such as fluidity and thickness. Intracellular sterol transport involves both vesicular and non-vesicular routes but is mainly ATP-dependent. Non-vesicular sterol transport, in addition, requires the action of carrier proteins (for review see Jacquier and Schneiter 2012). Over the last couple of years, specific yeast sterol carrier proteins have been identified, the so-called oxysterol-binding proteins homologues Osh1–7 (reviewed by Schulz and Prinz 2007). Deletion of all seven Osh proteins was found to be lethal and accompanied by a 3.5-fold increase in the cellular level of ergosterol (Schulz and Prinz 2007). Sterols can be either transported to the cell surface or sent to the *trans*-Golgi network where they associate with sphingolipids to form lipid rafts (Mesmin and Maxfield 2009). The exact role of Osh proteins in sterol transport still has to be elucidated. Georgiev et al. (2011) reported that Osh proteins act as sterol sensors and regulate the organization of sterols at the plasma membrane rather than being involved in the transport of sterols between the ER and the plasma membrane. Intracellular sterol trafficking between membranes might also be governed by Arv1p as reported by Tinkelenberg et al. (2000). Mutations of *ARV1* have been shown to render cells which are anaerobically non-viable, depend on sterol esterification and show altered intracellular sterol distribution. The balance of sterol synthesis, uptake, storage and mobilization as well as internal transport is very complex and a hot topic of lipid research.

8.2.4 Non-polar Lipids: TG and SE

TG and SE are storage lipids preserving free FA and sterols in a biologically inert form. All eukaryotic cells store excess FA in specific organelle-like compartments, often referred to as LD, lipid particles or oil bodies, used as energy depots. Yeast cells accumulate only little TG as long as they proliferate but can reach high TG levels in the stationary phase. When required, e.g. during growth or starvation, TG and SE can be mobilized to provide building blocks for membrane biosynthesis. Under these conditions the released FA are channelled into phospholipid biosynthesis (Zanghellini et al. 2008). In yeast, LD are about 400 nm in diameter and consist of a highly hydrophobic core of TG, surrounded by shells of SE and a phospholipid monolayer containing a distinct set of proteins (Czabany et al. 2008; Athenstaedt et al. 2006; Grillitsch et al. 2011; Kohlwein et al. 2012). TG are synthesized by the acyltransferases Dga1p and Lro1p, and SE by the steryl ester synthases Are1p and Are2p. All TG- and SE-synthesizing enzymes are located at the ER. Additionally, Dga1p is also found in LD. The direct precursor for TG is diacylglycerol (DAG), that can derive from different routes: (i) dephosphorylation of de novo synthesized PA, (ii) degradation of PL by phospholipases and (iii) deacylation of TG (see Fig. 8.4) (Henry et al. 2012). For synthesis of TG, DAG is acylated in the *sn*-3 position by Dga1p, Lro1p and with low efficiency by Are1p and Are2p. In *S. cerevisiae*, the acyl-CoA:diacylglycerol acyltransferase Dga1p is the most efficient TG-synthesizing enzyme. Lro1p is an acyl-CoA independent

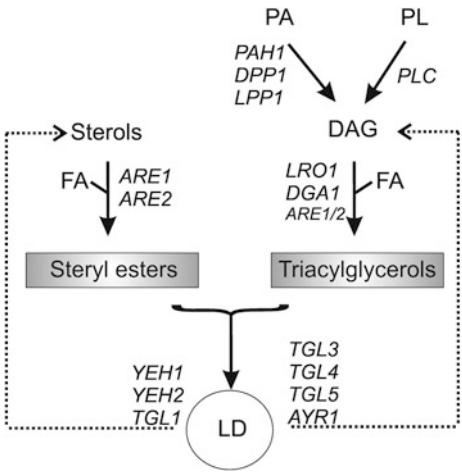

Fig. 8.4 Overview of non-polar lipid metabolism in *S. cerevisiae*. For details see text. *PA* phosphatidic acid, *PL* phospholipids, *DAG* diacylglycerol, *FA* fatty acids, *LD* lipid droplets

enzyme which uses the *sn*-2 acyl group from glycerophospholipids as cosubstrate for the acylation of DAG (Czabany et al. 2007; Rajakumari et al. 2008; Horvath et al. 2011). SE of *S. cerevisiae* are synthesized by the two acyl-CoA:cholesterol acyltransferase (ACAT) related enzymes, Are1p and Are2p (Yang et al. 1996; Yu et al. 1996). Both proteins are located in the ER and harbour multiple transmembrane domains. Are1p and Are2p are 49 % identical in sequence, but have different substrate specificities. Under standard cultivation conditions, Are2p accounts for approximately 70 % of the total SE synthase activity and esterifies preferentially ergosterol. Are1p esterifies mainly sterol intermediates with a slight preference for lanosterol and becomes particularly important under hypoxic conditions (Zweytick et al. 2000). The esterification takes place at the hydroxyl group at the C3-atom with C16:1 as the preferred fatty acid substrate followed by C18:1. Both TG and SE accumulate mainly during the stationary growth phase.

Storage of non-polar lipids would be useless without the possibility to mobilize them as required in order to provide sterols, DAG and FA for membrane synthesis and energy production. TG are mobilized by TG lipases. Currently, four LD-resident TG lipases are known, namely Tgl3p, Tgl4p, Tgl5p and Ayr1p (Athenstaed and Daum 2003, 2005; Ploier et al. 2013). SE are hydrolyzed by the three SE hydrolases Yeh1p, Yeh2p and Tgl1p (Köffel et al. 2005; Müllner et al. 2005), the highest activity being attributed to Yeh2p. The cycle of esterification of free sterols and the hydrolysis of SE are of utmost importance for a balanced level of free ergosterol (Wagner et al. 2009). Yeh1p and Tgl1p are localized to LD, whereas Yeh2p was surprisingly detected in the plasma membrane. The existence of further hydrolytic enzymes is currently under investigation (our own unpublished results). Especially, peroxisomal enzymes might be involved in the mobilization of non-polar lipids (Thoms et al. 2011; Debelyy et al. 2011). TG and SE

have long been viewed as just storage molecules, but this view has changed in recent years. TG in particular appear to be important for various cellular processes and their levels have been found to influence lipotoxicity, iron and phospholipid metabolism and cell cycle progression (Kohlwein 2010).

Non-polar lipid metabolism is inevitably connected to LD biology. Their biogenesis is still a matter of debate and different possible models have been published (Farese and Walther 2009). The most widely accepted model describes its formation at special membrane microdomains in the ER, where non-polar lipids accumulate between the two leaflets of the phospholipid bilayer until the size of the LD reaches a critical dimension (Murphy and Vance 1999; Ploegh 2007). At this stage, LD may bud off forming an independent organelle-like structure. Apart from their classical role as a storage compartment, it has to be noted that LD also participate in many other cellular processes (reviewed by Kohlwein et al. 2012). Connerth et al. (2010) described an indirect role of LD in the maintenance of membrane fluidity under environmental pressure of exogenous FA. Functions of LD unrelated to lipid turnover have also been investigated. As an example, Fei et al. (2009) reported that LD accumulated in yeast mutants with compromised protein glycosylation. The authors discussed a possible role of LD as a temporary safe depot for protein aggregates or incorrectly folded proteins. In recent studies, LD emerged as dynamic organelles through their interaction with the ER (Fei et al. 2009; Jacquier et al. 2011; Wolinski et al. 2011), peroxisomes (Binns et al. 2006), or mitochondria (Pu et al. 2011), and novel factors influencing the biogenesis and dynamics of LD were identified (Adeyo et al. 2011).

8.2.5 Sphingolipids

Sphingolipids are composed of a sphingoid base, a fatty acid and a polar head group. In yeast, the sphingoid base can be dihydrosphingosine or phytosphingosine, linked through an amide bond to a very long chain fatty acid, mostly C26:0, and O-linked to the charged head group inositol. The de novo synthesis of sphingolipids is carried out in the ER starting with the condensation of serine and palmitoyl-CoA (Fig. 8.5). This reaction is catalyzed by the serine palmitoyltransferase complex (*SPT*), which is a heterodimeric complex consisting of two major subunits, Lcb1p and Lcb2p (Nagiec et al. 1994), and one minor subunit, Tsc3p, which is necessary for full enzymatic activity (Gable et al. 2000). The product of this reaction, 3-ketodihydrosphingosine, is rapidly converted to dihydrosphingosine (also named sphinganine) by Tsc10p (Beeler et al. 1998). This product is the first sphingoid base that can be further hydroxylated by Sur2p, yielding a second sphingoid base, phytosphingosine (Grilley et al. 1998). These sphingoid bases can be either acylated to ceramides by gene products of *LIP1*, *LAG1* and *LAC1*, or phosphorylated by the sphingoid kinases encoded by *LCB4* and *LCB5* (Nagiec et al. 1998). Sphingoid base phosphates are further converted by Dpl1p to form fatty aldehydes and ethanolamine phosphates. This is the only

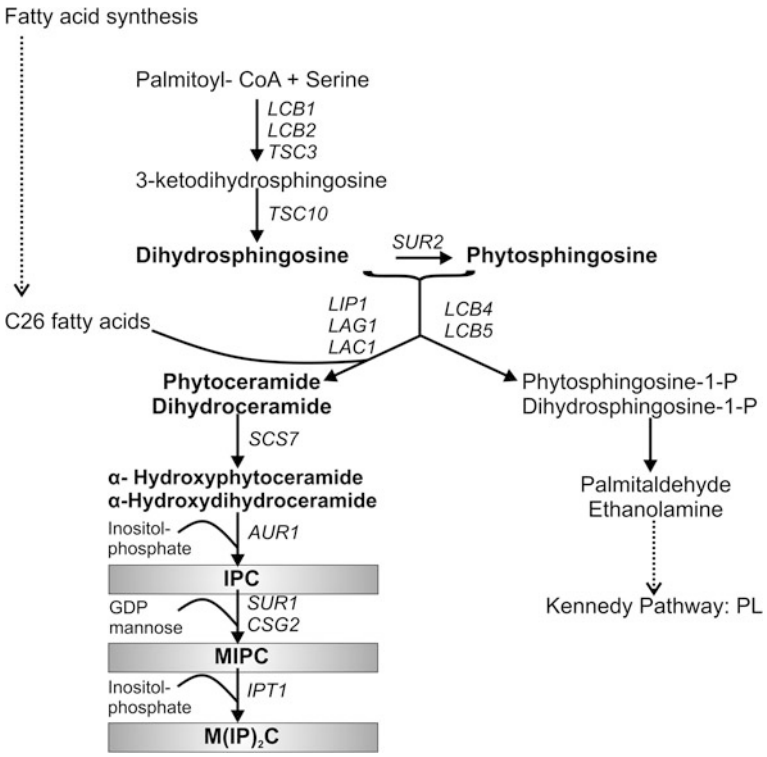

Fig. 8.5 Sphingolipid synthesis in *S. cerevisiae*. For details see text. *IPC* inositol-P-ceramide, *MIPC* mannose-IPC, *M(IP)$_2$C* mannose-(inositol-P)$_2$-ceramide

route by which sphingolipids can exit the pathway and the link of sphingolipid metabolism to the CDP-ethanolamine branch of the Kennedy pathway (Saba et al. 1997; Panwar and Moye-Rowley 2006).

If sphingolipids are not phosphorylated, both sphingoid bases can be N-acylated with C26-CoA by a ceramide synthase. Ceramide synthase comprises an ER membrane protein complex consisting of Lip1p, Lag1p and Lac1p (Schorling et al. 2001; Vallée and Riezman 2005). The two sphingoid bases and ceramides are the first products in the sphingolipid synthetic pathway. Ceramides are N-acylated sphingoid bases lacking additional head groups. They serve as substrates for the formation of complex lipids that may comprise up to 10 % of total membrane lipids. Prior to the formation of the complex sphingolipids, inositol-P-ceramide (IPC), mannose-inositol-P-ceramide (MIPC) and mannose-(inositol-P)$_2$-ceramide [M(IP)$_2$C], ceramides are α-hydroxylated by Scs7p (Haak et al. 1997; Dunn et al. 1998). Aur1p, the inositolphosphorylceramide synthase, attaches a phosphoinositol headgroup to the ceramide-yielding IPC (Nagiec et al. 1997), which is then mannosylated by Csg1p, Csg2p and Csh1p to MIPC. After mannosylation, another

inositol phosphate group is added by Ipt1p forming $M(IP)_2C$ (Beeler et al. 1997; Uemura et al. 2003; Dickson et al. 1997).

The key players of sphingolipid catabolism are encoded by *ISC1*, *YPC1* and *YDC1* (Sawai et al. 2000; Mao et al. 2000a, b). Isc1p hydrolyzes the head groups of complex sphingolipids yielding both phyto- and dihydroceramides that can be cleaved reversibly to sphingoid bases. This reaction is catalyzed by the two ceramidases, Ypc1p and Ydc1p.

Although sphingolipids fulfil many important physiological roles (Dickson et al. 2006), little is known about the regulation of cellular sphingolipid levels. Cowart and Obeid (2007) showed that there is no stringent transcriptional regulation of the key enzymes of sphingolipid metabolism. One control mechanism could be phosphorylation of the sphingoid base kinase Lcb4p by interaction of Pho85p with two of its cyclin partners, Pcl1p and Pcl2p, which leads to downregulation of Lcb4p. This effect is accompanied by a decrease in sphingoid base phosphate levels and a decrease of the cell cycle. Another study showed that ceramide synthase is regulated by casein kinase Cka2p, whose deletion resulted in a 70–75 % reduction of ceramide synthase activity (Kobayashi and Nagiec 2003). Kolaczkowski et al. (2004) found that the promoters of some sphingolipid metabolic enzymes contain a PDR (pleiotropic drug response) element for binding of the transcriptional activators Pdr1p and Pdr3p. Active PDR elements have been found in *LAC1*, *LCB2* and *SUR2*. A central element of sphingolipid regulation appears to be the interplay of Orm proteins (inhibitors of *SPT*) with Ypk1p (a kinase that inactivates Orm1p and Orm2p). These links were discovered recently (Breslow et al. 2010; Roelants et al. 2011; Sun et al. 2000) and will be discussed in the section on novel key players.

Sphingolipids are mainly found in the yeast plasma membrane where they are thought to interact with sterols to form so-called lipid rafts, also described as detergent-resistant membrane domains (Bagnat et al. 2000; Guan et al. 2009; Simons and Sampaio 2011). These domains have been proposed to constitute an important platform for certain membrane proteins, such as Pma1p, Gas1p and Gap1p (Dickson et al. 2006). The physiological role of lipid rafts has been exemplified by mis-localization of Pma1p, plasma membrane proton pump, and Gap1p, a general amino acid permease, in strains with impaired sphingolipid metabolism (Gaigg et al. 2006; Lauwers et al. 2007). The example of mis-localization of Gap1p also illustrates a functional link between sphingolipid and amino acid metabolism. In addition to their structural role, sphingolipids and their metabolites have emerged as important signalling molecules involved in endocytosis, heat stress response and cell cycle regulation (Cowart and Obeid 2007). Additionally, sphingolipids are necessary for the transport of GPI-anchored proteins from the ER to the Golgi (Skrzypek et al. 1997; Horvath et al. 1994). As mentioned above, they also influence the topology, localization, cell surface delivery and stability of important proteins, including the uracil permease Fur4p (Hearn et al. 2003), the plasma membrane ATPase Pma1p (Gaigg et al. 2005) and the vacuolar ATPase (Chung et al. 2003).

8.3 A Selection of Novel Key Players in Yeast Lipid Metabolism

A list of the major yeast lipid synthesizing and degrading enzymes is currently available, but a detailed understanding of lipid homeostasis and regulation of lipid metabolism still awaits clarification. In recent years, several new enzymes involved in lipid metabolism and related mechanisms have been identified. This development shows that the field of lipid research has become broader as links to other cellular processes became evident. To give the reader an impression of the complexity of lipid metabolism and its regulation, a few selected examples of novel insights into yeast lipid homeostasis covering different lipid species will be discussed in the following section.

8.3.1 Regulation of Phospholipid Synthesis: Inositol, PA and Opi1p

Besides acetyl-CoA, inositol is a major link of carbon metabolism to lipid metabolism. It is a carbohydrate synthesized from glucose-6-P in two steps, and is not essential under standard cultivation conditions. Inositol forms the structural component of a number of secondary messenger molecules, the inositol phosphates. In addition to its signalling role, inositol is also an important component of PI and its phosphates (PIPs), and can be regarded as the master regulator of PL biosynthesis. Inositol used for PI synthesis is either synthesized de novo or imported into the cell from the growth medium by inositol transporters encoded by *ITR1* and *ITR2* (Nikawa et al. 1991). The switch between these two possibilities is regulated by PA, which acts as an essential metabolic intermediate and a regulator of phospholipid homeostasis.

The link between inositol and PA is an effector named Opi1p (Loewen et al. 2004). As noted in a previous section, many genes involved in phospholipid biosynthesis carry a *cis*-acting, inositol-sensitive upstream activating sequence (UAS_{INO}) response element (Chen et al. 2007a). All these genes are regulated by the same transcription factors. They are activated by Ino2p and Ino4p, and repressed by Opi1p. The location of Opi1p is the key whether or not it acts as a repressor. In the absence of extracellular inositol, Opi1p is bound to the ER, together with the integral ER membrane protein Scs2p (interaction of an FFAT motif) and PA. With Opi1p in this location, genes involved in inositol synthesis are transcribed. When inositol is added to the medium, PA is consumed by conversion into PI, leading to the translocation of Opi1p to the nucleus where it represses genes carrying the UAS_{INO} element (Carman and Han 2011). This latter process is influenced by pH, because deprotonated PA is a better ligand for Opi1p than protonated PA. The intracellular pH of yeast cells is strongly dependent on the nutritional environment. During glucose starvation, it falls rapidly compromising

the binding between PA and Opi1p. This effect leads to the translocation of Opi1p to the nucleus where it acts as a repressor of phospholipid synthesis (Ktistakis 2010).

The example described above is only one among many other regulatory aspects involved in phospholipid metabolism. As another recent example, Moir et al. (2012) reported that Yft2p and Scs3p, the yeast homologues of the mammalian FIT proteins (fat storage-inducing transmembrane proteins), are required for normal ER membrane biosynthesis. It is suggested that these proteins could be candidates involved in global regulation of phospholipid metabolism. For a more detailed description of phospholipid regulatory networks and interconnections with other pathways, the reader is referred to a recent review (Carman and Han 2011).

8.3.2 Regulation of Sphingolipid Metabolism

Sphingolipid metabolism is regulated by a series of factors, Orm1p, Orm2p, Ypk1p, Slm1p, Slm2p and TORC2. Orm1p and Orm2p are evolutionarily conserved proteins that act as inhibitors of serine:palmitoyl-CoA transferase (*SPT*), encoded by *LCB1* and *LCB2*, which catalyzes the first and rate-limiting step in the de novo synthesis of sphingolipids (Fig. 8.5) (Breslow et al. 2010). Ypk1p is a serine/threonine protein kinase that inactivates Orm1p and Orm2p by phosphorylation in response to compromised sphingolipid synthesis (Roelants et al. 2011; Sun et al. 2000). Slm1p and Slm2p are phosphoinositide-binding proteins that form a complex with each other and are both phosphorylated by the TORC2 complex (Niles and Powers 2012). The interplay of these factors can be regarded as an important control mechanism for sphingolipid homeostasis, because not only do the end products of sphingolipid synthesis but also several intermediates play an essential role for the cell. The feedback loop that controls sphingolipid metabolism can be summarized as follows: Orm1p and Orm2p form a stable complex with SPT when they are dephosphorylated, repressing SPT activity. Upon sphingolipid deficiency, Orm proteins are phosphorylated by Ypk1p, which leads to their relief of SPT. Ypk1p activity is in turn controlled by phosphorylation in a TORC2-dependent manner (Raychaudhuri et al. 2012). The TORC2-dependent phosphorylation of Ypk1p requires the activation of Slm proteins. These proteins appear to sense membrane stress caused by sphingolipid depletion and react by redistribution among different membrane domains. The relocation from eisosomes is caused by the inhibition of sphingolipid synthesis, which is then followed by activation of TORC2-Ypk1 signalling (Berchtold et al. 2012).

8.3.3 Phosphatidate Phosphatase Pah1p, a Switch Point in Glycerolipid Metabolism

PAH1 encodes the enzyme phosphatidate phosphatase which has gained more and more attention, in particular because of its homology to the mammalian lipins 1 and 2, which are involved in several lipid-associated disorders in human physiology (Han et al. 2006; Reue and Brindley 2008; Reue and Dwyer 2009). Pah1p catalyzes dephosphorylation of PA, yielding DAG and P_i, in a Mg^{2+}-dependent manner. Since both the substrate and the product of this reaction are important lipid mediators, Pah1p can be regarded as a central regulator of lipid homeostasis. This enzyme is an important control point deciding whether cells produce storage lipids or phospholipids as membrane constituents (for review see Pascual and Carman 2013). Pah1p is evolutionarily conserved, since genes encoding PAP (phosphatidic acid phosphatase) enzymes have been identified in humans, mice, flies, worms and plants. The influence of Pah1p was best studied in *pah1Δ* yeast deletion strains which were severely affected at several levels of lipid homeostasis. These strains showed defects in the synthesis of TG and PL, elevation in cellular content of PA and decreased levels of DAG and TG (Fakas et al. 2011; Han et al. 2006). Moreover, the amounts of PL, FA and SE were also increased in these mutants. The importance of Pah1p is further underlined by the occurrence in a *pah1Δ* deletion strain of several phenotypic appearances such as slow growth, defects in the biogenesis and morphology of LD, aberrant expansion of the nuclear/ER membranes, FA-induced toxicity and effects in vacuole homeostasis and membrane fusion, as well as in respiratory deficiency (O'Hara et al. 2006; Adeyo et al. 2011; Fakas et al. 2011; Sasser et al. 2012). The increased amount of PL is also typical of a *pah1Δ* mutant, which could be caused by the derepression of UAS_{INO}-containing lipid synthesis genes in response to elevated PA levels (see below) (Carman and Henry 2007; Chirala et al. 1994). Recently, Dgk1p was found to be a cellular counterpart of Pah1p by its regulation of PA homeostasis (Han et al. 2008). Dgk1p is a CTP-dependent DAG kinase that catalyzes the reverse reaction of Pah1p and restores PA levels in a *pah1Δ* mutant. As unbalanced levels of PA and DAG result in many phenotypic consequences, the activity of Pah1p must be fine-tuned to maintain lipid homeostasis and normal cell physiology. Some regulatory mechanisms of Pah1p activity were reported, but all of them are very complex, occurring on different levels (Pascual and Carman 2013). Pah1p expression was found to depend on various physiological conditions such as zinc depletion or different growth phases. Regulation by lipids and nucleotides was identified as another regulatory mechanism since Pah1p activity is stimulated in response to CDP-DAG, PI and CL, whereas it is inhibited by sphingosines, phytosphingosine and sphinganine and the nucleotides ATP and CTP (Wu and Carman 1994, 1996; Wu et al. 1993). Pah1p activity and subcellular distribution are governed by the Nem1p-Spo7p protein phosphatase complex, and several kinases such as Pho85p-Pho80p, Cdc28p-cyclin B, protein kinase A and C as well as casein kinase II can act on Pah1p using phosphorylation/dephosphorylation

mechanisms; however, the fine-tuning of Pah1p still has to be examined (Choi et al. 2011; Siniossoglou et al. 1998). The action of the transmembrane protein phosphatase complex, Nem1p-Spo7p, is responsible for the recruitment of the phosphorylated form of Pah1p from the cytosol to the nuclear/ER membrane. The Nem1p-Spo7p complex dephosphorylates Pah1p, enabling a short aminoterminal amphipathic helix to anchor Pah1p, thus allowing access to its substrate PA (Pascual and Carman 2013).

8.3.4 PS Decarboxylase 1 (Psd1p)

PE belongs to the bulk PL of yeast. It can be synthesized by four different pathways, namely by (i) decarboxylation of PS through Psd1p, (ii) by decarboxylation of PS though Psd2p, (iii) by reacylation of lyso-PE by Ale1p and Tgl3p and (iv) via the CDP-ethanolamine pathway (Henry et al. 2012; Böttinger et al. 2012). These pathways account for different proportions of cellular PE. Horvath et al. (2011) reported that the CDP-ethanolamine pathway preferentially contributes to TG synthesis by providing PE as co-substrate for Lro1p catalyzed TG synthesis, indicating a close interaction between TG and PE synthesis. The main source for PE, however, is the conversion of PS into PE by Psd1p. Psd1p is encoded by a nuclear gene, synthesized on free ribosomes and imported into mitochondria, where protein maturation takes place. This processing occurs in three steps, involving the action of the mitochondrial processing peptidase (MPP), the action of Oct1p (a mitochondrial peptidase that cleaves destabilizing N-terminal residues of a subset of proteins) and autocatalytic cleavage at a highly conserved LGST motif. These processing steps yield the mature form of the enzyme that contains an α-subunit, exposed to the intermembrane space, and a β-subunit anchoring the activated protein to the inner mitochondrial membrane. Correct localization is crucial for full enzymatic activity and also for maintaining lipid homeostasis (Horvath et al. 2012). Deletion of *PSD1* leads to reduced growth on glucose, morphological changes in mitochondria, ethanolamine auxotrophy and an altered pattern of PL (Birner et al. 2001). These observations underline the importance of Psd1p in lipid homeostasis. PE levels were shown to have a tremendous impact, not only on the distribution of other lipids, but also on the function and stability of mitochondrial proteins (Böttinger et al. 2012).

8.3.5 Ups1p, ERMES and Gem1p: Components Affecting Mitochondrial Lipid Transfer

Lipid transfer between and within organelles has been an important issue for several decades but is still under intense investigation. Import of lipids into mitochondria and interaction of mitochondria with the ER are classical examples

for such studies. Recently, identification of new components provided some deeper insight into these problems.

Intramitochondrial lipid transport is important to provide substrates like PA or PS for efficient CL and PE synthesis, respectively, in the inner mitochondrial membrane. Transport of PA between the outer (OMM) and inner mitochondrial membranes (IMM) was found to be mediated by Ups1p, a protein localized to the intermembrane space (Connerth et al. 2012; Tamura et al. 2010). PA is transported in three steps starting with the binding of PA by Ups1p at the surface of the OMM. Ups1p then associates with Mdm35p to be protected against proteases before PA is released at the IMM (Potting et al. 2010). This transport is bidirectional and independent of the acyl-chain composition. Dissociation of Mdm35p from the complex is a prerequisite for PA release and facilitated by the interaction with negatively charged PL like CL. However, a very high concentration of CL prevents the detachment of Ups1p from the acceptor membrane, subsequently impairing the PA flux. This finding indicates that CL is a regulator of its own synthesis (Connerth et al. 2012). Deletion of *UPS1* leads to a decrease in Psd1p levels and causes a reduction of PE. This defect has been explained as Ups1p being responsible not only for PA transport, but also for the import of Psd1p into mitochondria. Moreover, it was shown that Ups1p also mediates the export of PE from the IMM to the OMM and promotes the conversion of PE to PC, which makes Ups1p a central regulator of phospholipid metabolism by influencing lipid traffic (Tamura et al. 2012).

A complex termed ERMES (ER mitochondria encounter structure) that tethers the ER to the OMM has been identified. This complex is composed of the five proteins Mmm1p, Mdm34p, Mdm10p, Mdm12p and Gem1p (Kornmann et al. 2009; Stroud et al. 2011). Gem1p is an OMM GTPase with a C-terminal single transmembrane segment that is exposed to the cytosol (Kornmann et al. 2011; Meisinger et al. 2007). Mmm1p, Mdm34p and Mdm12p each contain an SMP domain (synaptotagmin-like mitochondrial and lipid-binding proteins) that is involved in binding hydrophobic ligands like lipids. This arrangement suggests a possible role for the ERMES complex in lipid transport between the ER and mitochondria (Kopec et al. 2010). A transport route between these two compartments is important because the substrate of the mitochondrial Psd1p, PS, is synthesized in the ER, and PE synthesized by Psd1p in mitochondria is substrate of the ER-localized PC-synthesizing machinery. How lipid transport via ERMES may happen is controversial and still a matter of debate. Kornmann et al. (2009) reported that strains bearing mutations in the ERMES proteins showed phenotypes related to phospholipid metabolism such as decreased CL levels. However, ERMES and Gem1p have been shown not to play a direct role in the transport of PS from the ER to mitochondria. Rather, ERMES fulfils a structural role in maintaining the morphological integrity of mitochondria (Nguyen et al. 2012).

8.3.6 Squalene

Squalene is a polyunsaturated triterpene consisting of six isoprene units. It possesses several beneficial properties, e.g. as antioxidant or emollient, and has therefore become relevant for biotechnological applications (for review see Spanova and Daum 2011). As described above, squalene is an important intermediate of the sterol biosynthetic pathway. Under normal growth conditions, it is rapidly converted and therefore does not accumulate in yeast. However, under certain growth conditions or by genetic manipulations (overexpression of *HMG1/2*, *ERG1* or *ERG6*; deletion of *HEM1*) the amount of squalene can be increased (Polakowski et al. 1998; Jahnke and Klein 1983; Lorenz et al. 1989). Spanova et al. (2010) showed that under squalene-accumulating conditions this lipid is stored in LD. Unexpectedly, accumulation of squalene did not result in lipotoxic effects. In a yeast strain lacking TG and SE, which is unable to synthesize LD, squalene was found mainly in mitochondria and microsomes without causing deleterious effects. Recent reports (Spanova et al. 2012) described functions of squalene as a modulator of membrane properties affecting mainly membrane fluidity. It was shown that ER membranes become more rigid when enriched in squalene, whereas samples of plasma membranes became softer. Unlike sterols, squalene does not necessarily rigidify membranes, but modulates their dynamics in both directions. This effect could depend on the ratio of ergosterol to squalene.

8.3.7 Tgl3p, Tgl4p and Tgl5p: More Than Just Triacylglycerol Lipases?

As described in the section on non-polar lipids, Tgl3p, Tgl4p and Tgl5p are the main TG lipases of the yeast *S. cerevisiae*. Recently, Ayp1p was identified as another TG lipase with minor lipolytic activity (Ploier et al. 2013). They catalyze the cleavage of TG to DAG and FA. However, these enzymes are not only responsible for mobilization of the main storage lipids but also contribute to lipid metabolism as acyltransferases and phospholipases, which makes them novel key players in lipid metabolism (Grillitsch and Daum 2011; Rajakumari and Daum 2010a, b).

In general, lipases are a subclass of hydrolases whose catalytic activity depends on the so-called interfacial activation, which means that they act only at an aqueous/non-aqueous interface (Verger 1997). As all other lipases, the three main TG lipases of yeast, Tgl3p, Tgl4p and Tgl5p, share a common consensus sequence GXSXG, where serine is the essential residue as interaction partner of the catalytic triad aspartic acid, glutamic acid and histidine (Schrag and Cygler 1997). They also contain a patatin domain, named after a plant storage protein that possesses lipid acyl hydrolase activity (Mignery et al. 1988).

Tgl3p was the first yeast TG lipase to be identified and characterized in *S. cerevisiae* (Athenstaedt et al. 1999a; Athenstaedt and Daum 2003). Tgl4p and

Tgl5p, identified some years later, exhibit about 30 % and 26 % similarity with Tgl3p (Athenstaedt and Daum 2005; Kurat et al. 2006). Localization studies have revealed that all three TG lipases are localized to LD, although none of these three lipases show hydrophobic domains (Athenstaedt and Daum 2005; Müllner et al. 2004). In vitro, all three proteins possess lipolytic activity whereas in vivo only Tgl3p and Tgl4p mobilize TG efficiently. This finding was explained by different substrate specificities. It appears that Tgl5p accepts mainly TG-containing cerotic acid (C26:0), a fatty acid of low abundance in yeast, whereas overall effects in vivo on bulk TG hydrolysis were not observed. The main TG lipase in yeast, Tgl3p, was shown to hydrolyze TG as well as DAG, whereas substrate specificity of Tgl4p is restricted to TG (Kurat et al. 2006). A *tgl3Δtgl4Δtgl5Δ* yeast strain lacking all three TG lipases does not reveal any growth defect under standard growth conditions, although mutations in *TGL3* or *TLG4* lead to fat yeast cells that accumulate TG (Athenstaedt and Daum 2005; Kurat et al. 2006). Moreover, deletion of *TGL4* and *TGL5* leads to decreased sporulation efficiency.

Recent characterization of TG lipases has revealed novel functions of these enzymes. Protein sequences of all three TG lipases contain additional sequence motifs besides the conserved GXSXG lipase motif. Tgl3p, Tgl4p and Tgl5p harbour an acyltransferase motif (H-$(X)_4$-D), and Tgl4p was found to have in addition a phospholipase motif (GXGXXG). Further investigations revealed decreased amounts of total PL in a *tgl3Δ* deletion strain and increased amounts of PL in a *TGL3* overexpressing strain (Rajakumari et al. 2010; Rajakumari and Daum 2010a). In vitro enzyme assays showed that both Tgl3p and Tgl5p act as lysophospholipid acyltransferases with different substrate specificities. Tgl3p mainly acylates lysophosphatidylethanolamine, whereas Tgl5p prefers lysophosphatidic acid as a substrate (Rajakumari and Daum 2010a). The lipase activity of Tgl3p acts independently from the acyltransferase activity and vice versa as demonstrated by site-directed mutagenesis, inactivating either one of the two motifs. Interestingly, the sporulation defect in a *tgl3Δtgl5Δ* double mutant was still observed in a strain with mutated lipase motif but not when the acyltransferase activity was abolished.

Besides the conserved lipase motif, Tgl4p contains a (G/A)XGXXG Ca^{2+}-independent phospholipase A_2 domain. Phospholipase activity of Tgl4p was also established in vitro with PC and PE as substrates but not with PA or PS. Additionally, Tgl4p hydrolyzed SE and revealed lysophospholipid acyltransferase activity (Rajakumari and Daum 2010b). Kurat et al. (2009) reported an impact of phosphorylation of Tgl4p activity. They showed that the lipolytic activity of Tgl4p was strongly reduced when phosphorylation sites were mutated, whereas the lysophospholipid acyltransferase activity was not affected (Rajakumari and Daum 2010b). In conclusion, Tgl4p is an excellent example of a multifunctional enzyme involved in yeast lipid metabolism, which does not only hydrolyze TG and SE but also contributes to PL synthesis and membrane remodelling. Recent publications, as well as our own unpublished data, led to the conclusion that besides the currently known TG lipases, Tgl3p, Tgl4p and Tgl5p, also other TG lipases may play a role in the turnover of non-polar lipids (Debelyy et al. 2011; Thoms et al. 2008, 2011).

8.3.8 Phospholipases

Phospholipases cleave different bonds in glycerophospholipid molecules, and their physiological effects are based on the resulting products. Depending on the bond(s) cleaved, phospholipases are divided into groups A_1, A_2, B, C and D (Fig. 8.6). A thoroughly studied example of Plc1p, the canonical yeast phospholipase C, illustrates well how phospholipases activate signalling cascades by generating, in the case of Plc1p, DAG and inositol 1,4,5-triphosphate, both of which exert their intrinsic biological activity as secondary messengers (reviewed in Rebecchi and Pentyala 2000; Strahl and Thorner 2007; York 2006). However, the activity of some phospholipases appears to be restricted to metabolic functions, such as that of Pgc1p, another yeast phospholipase C, which is specific for PG hydrolysis and required for its degradation (Simocková et al. 2008). The third known yeast phospholipase C is encoded by the *ISC1* gene. It accepts phosphosphingolipids (see above) as substrates and generates phytoceramide, a signalling molecule affecting several cellular processes (reviewed in Matmati and Hannun 2008). Two phospholipases D, Spo14p and Fmp30p, have also been described in *S. cerevisiae*. The former hydrolyzes PC to choline and PA and is involved in several cellular processes including growth, secretion and regulation of *INO1* expression (Sreenivas et al. 1998), as well as sporulation (Rudge et al. 1998) and general transcription (García-López et al. 2011). Fmp30p, an IMM protein with sequence similarity to mammalian N-acylethanolamine-specific phospholipases D (Merkel et al. 2005), is also required for CL homeostasis (Kuroda et al. 2011).

Deacylating phospholipases in yeast include phospholipases B, Plb1p, Plb2p, Plb3p, Nte1p and Spo1p; phospholipases A_2, Cld1p, Tgl4p, Per1p and Bst1p; and Yor022cp, a putative phospholipase A_1. A detailed understanding of the biochemical pathways leading to the specific FA composition of PL is important, among other reasons also from the perspective of yeast-based biofuel production (see below). All deacylating phospholipases could, in principle, be involved in acyl-chain remodelling of phospholipids, but a recent study showed that, rather than phospholipases B, it is the PL:DAG acyltransferase Lro1p which provides FA for PL remodelling (Mora et al. 2012). Two other acyltransferases, Psi1p (=Cst26p) and Taz1p, play crucial roles in PL acyl-chain remodelling (reviewed by Henry et al. 2012). Plb1/2/3 proteins, on the other hand, have been proposed to be involved in biosynthesis and, together with the phospholipase D Fmp30p, in signalling through N-acylethanolamines and N-acylphosphatidylethanolamines (Merkel et al. 2005).

A special case among yeast phospholipases is Tgl4p which, as described above, is a multifunctional enzyme with reported triacylglycerol lipase, steryl ester hydrolase and Ca^{2+}-independent phospholipase A_2 activities (Rajakumari and Daum 2010b). Importantly, regulation of the activity of this protein also links lipid metabolism to cell-cycle regulation (Kurat et al. 2009). Multiple cellular processes are also affected by the activity of Per1p and Bst1p, albeit the diversity of their effects stems from the fact that these phospholipases A_2 are active on GPI-protein

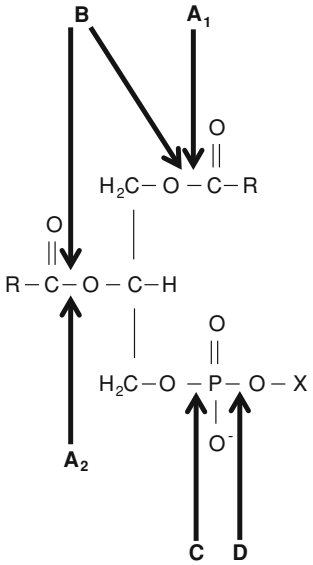

Fig. 8.6 Sites of hydrolytic action of phospholipases A_1, A_2, B, C and D on a model phospholipid molecule

anchors that enable specific localization of the proteins targeted to lipid raft regions of the plasma membrane (Fujita et al. 2006; Tanaka et al. 2004). *SPO1* is a meiosis-induced gene that encodes a phospholipase B with a role in distinct steps of sporulation, exhibiting epistasis with Spo14p phospholipase D, whereas the absence of Spo1p can be partially suppressed by overexpression of *PLB3* gene (Tevzadze et al. 2007). *CLD1* codes for a cardiolipin-specific deacetylase which, together with Taz1p, ensures the biosynthesis of mature CL (Beranek et al. 2009). Also, Nte1p seems to play an interesting role possibly regulating transcription of PL biosynthesis genes through its PC-specific phospholipase A_2 activity and subsequent modulation of Opi1p activity (see above) (Fernández-Murray et al. 2009).

8.3.9 Izh Proteins, Zinc Homeostasis and Regulation by Inositol and Fatty Acids

As described in the previous sections, regulation of inositol biosynthesis is one of the central processes in yeast lipid and general metabolism homeostasis. In this section, we will address the role of Izh (Implicated in Zinc Homeostasis) proteins, yeast homologues of the mammalian adiponectin receptors, which have recently emerged as players enabling the connection between inositol and FA metabolism and zinc homeostasis. Zinc depletion in yeast activates Zap1p transcriptional activator which affects several target genes, among them the *PIS1*-encoded PI synthase and the *DPP1*-encoded DGPP phosphatase. Zn depletion thus causes a

decrease in PE and an increase in PI concentration (Carman and Han 2011; Iwanyshyn et al. 2004). In addition, zinc depletion results in a decreased concentration of PA, thus triggering the release of Opi1p from the ER membrane and its translocation into the nucleus, where it represses expression of *CHO1* gene that encodes PS synthase by binding to and inhibition of the Ino2/4 complex (Carman and Han 2011). There are four genes in yeast, *IZH1, IZH2, IZH3* and *IZH*4 which encode proteins with sequence similarity to adiponectin receptors. In humans, adiponectin receptors mediate the antidiabetic metabolic activity of the polypeptide hormone adiponectin (Kadowaki et al. 2006). The yeast Izh2p has been confirmed as a functional homolog of adiponectin receptors in an experiment where heterologous expression of human adiponectin receptors in yeast functionally complemented Izh2p (Kupchak et al. 2007). *IZH1/2/3/4* genes were implicated to have a role in zinc metabolism after they had been identified in a screening for Zap1p targets, and were confirmed to have zinc-related phenotypes (Lyons et al. 2004). Expressions of *IZH1* and *IZH2* are directly regulated by Zap1p, and the promoters of these genes contain zinc-response elements. In addition, *IZH1, IZH2* and *IZH3* genes are regulated by exogenous FA through Oaf1p/Pip2p transcription factors that bind to oleate-response elements present in their promoters (Lyons et al. 2004). Specifically, *IZH2* expression is highly induced in cells grown in the presence of saturated FA such as myristate, and strains without this gene fail to grow normally in the presence of myristate (Karpichev et al. 2002). Transcriptome analysis of *izh2*Δ cells has revealed that a number of genes encoding proteins involved in FA metabolism and in the phosphate signalling pathway are regulated by Izh2p (Karpichev et al. 2002). Three functions of Izh proteins have been proposed by Lyons et al. (2004): (i) a role in sterol metabolism by which they would influence the permeability of the plasma membrane and consequently zinc homeostasis; (ii) a role as transporters for zinc; and (iii) a role in a zinc-independent signal transduction cascade with Zap1p as downstream target. The above results imply that, at least for Izh2p, the third possibility is the most likely one. Thus, Izh2p is emerging as a central component of a putative feedback regulatory pathway leading from FA to Zap1p activation and finally to inositol and regulation of PL biosynthesis.

8.4 Biotechnological Aspects

Lipids and their expansive roles have become increasingly recognized, resulting in a great demand for industrial high-level production of particular valuable lipid compounds. Lipid metabolism in yeasts as described above has been studied intensively and well described. Since this process is well conserved in eukaryotic cells, yeasts are ideal host systems for the biotechnological production of industrially and pharmaceutically relevant lipid compounds. *S. cerevisiae,* in particular, has been successfully applied for their production. This section describes examples, selected to illustrate the importance of lipid metabolism in biotechnology.

In this section, some of the most important approaches are described. The reader is also referred to recent reviews on these topics (such as Beopoulos et al. 2011; de Jong et al. 2012; Ruenwai et al. 2011; Uemura 2012; Veen and Lang 2004) for more details.

8.4.1 Polyunsaturated Fatty Acids

PUFA are FA with more than 16 carbon atoms in the chain that contain more than one double bond. They have multiple positive effects on human health, such as lowering the risk of heart attacks, cardiovascular diseases and cancer, and they also have major impacts on the development and improvement of retinal and brain function and on the regulation of membrane fluidity (Uemura 2012; Opekarová and Tanner 2003). Since mammals are not able to synthesize essential PUFA such as linoleic acid (C18:2n-6) or the omega-3 and omega-6 PUFA, they must be taken up from the diet. Since natural sources, such as fish oils, are limited, it is highly desirable to produce PUFA from alternative and sustainable sources. One promising option is *S. cerevisiae* or other yeasts. *S. cerevisiae,* in particular, has been shown to have a considerable potential for metabolic engineering approaches to the production of certain metabolites (Ostergaard et al. 2000).

The physiological FA composition of *S. cerevisiae* includes mainly C16:1 and C18:1 as described above in the section on FA. Since *OLE1,* that encodes a Δ9-fatty acid desaturase, is the only endogenous desaturase (Stukey et al. 1990), production of PUFA in *S. cerevisiae* requires the introduction of further desaturase and elongase genes from donor organisms such as *Mucor rouxii*, *Caenorhabditis elegans*, *Arabidopsis thaliana* or *Mortierella alpina* to produce, for example, α-linolenic acid (C18:3n-3), eicosapentaenoic acid (C20:5) and docosahexaenoic acid (C22:6) (Ruenwai et al. 2011; Uemura 2012). Combinations of multiple desaturases and elongases from various organisms were tried, but since Δ5- and Δ6-fatty acid desaturases can accept both n-3 and n-6 FA, the resulting products mostly depend on the substrate fatty acid added to the medium. Most studies used a large excess of precursor FA, yet the final yield of the PUFA produced was still low and strongly depended on cultivation conditions, such as growth media, temperature and incubation time (Uemura 2012; Misawa 2011). Construction of the complete pathway for the production of C20-PUFA, such as DGLA (dihomogamma linoleic acid) from the endogenous oleic acid, has been described by Yazawa et al. (2007). The authors cloned a Δ12-desaturase gene from *K. lactis*, and a Δ6-desaturase and the elongase *ELO1* genes from rat into *S. cerevisiae*.

One severe limitation of *S. cerevisiae* as a production host for PUFA is the low total lipid content compared to some other yeast genera. One alternative is the use of oleaginous yeasts such as *Y. lipolytica* which are characterized by their ability to accumulate lipids up to 40 % of their biomass (Beopoulos et al. 2011). *Y. lipolytica* has been applied successfully for the production of ω-3 and ω-6 PUFA such as docosahexaenoic acid, eicosapentaenoic acid and γ-linolenic acid. DuPont

de Nemours, for example, genetically engineered *Y. lipolytica* by expressing heterologous desaturases and elongases from organisms like *M. alpina* and *Fusarium moniliforme*, and by genetically inhibiting peroxisomal fatty acid degradation that produces lipids with the highest content of docosahexaenoic and eicosapentaenoic acids available (Xue et al. 2013).

8.4.2 Isoprenoids

Isoprenoids, also referred to as terpenoids, comprise a large group of naturally occurring secondary metabolites built from isoprene units, IPP (isopentenyl diphosphate) and its isomer DMAPP (dimethylallyl diphosphate). Eukaryotes synthesize IPP via the mevalonate pathway as described in the section on sterols. Head-to-tail condensation of IPP and DMAPP yields GPP, which is then converted into FPP by linkage of another molecule IPP. IPP is a branching point between GGPP (geranylgeranyl diphosphate) and the sterol pathway (see Fig. 8.3) (Pichler 2005). GPP and FPP are the precursors of monoterpenoids and sesquiterpenoids, respectively, and GGPP of diterpenes. Typically, two molecules of FPP are condensed to yield squalene, the precursor of sterols and phytoene which can be converted into carotenoids. Steroids will be discussed in the next section while the other isoprenoids are dealt with in this section.

Terpenoids comprise over 40,000 structurally different compounds. They are the largest group of natural products and have valuable properties for medical and industrial usage, especially as constituents of plant oils such as limonene, menthol and citronellol, which are used as flavours and fragrances, in their occurrence of carotenoids and as pharmaceuticals such as taxol (Misawa 2011; Chang and Keasling 2006).

S. cerevisiae does not produce monoterpenoids. Due to industrial requirement of these compounds, however, metabolic engineering approaches have been accomplished to this end (Lee et al. 2009). Herrero et al. (2008), for example, reported a recombinant wine yeast strain of *S. cerevisiae* that expresses the (*S*)-linalool synthase gene from the plant *Clarkia breweri,* and concomitantly overexpresses HMG-CoA reductase, resulting in efficient excretion of linalool reaching concentrations of 77 μg/L.

Sesquiterpenoids comprise the largest group of isoprenoids, and occur in plants and insects as pheromones and defensive agents. Because of their anti-cancer, antitumour and antibiotic properties, they are industrially important compounds (Asadollahi et al. 2010). One prominent example is artemisin, which is an effective anti-malarial drug and has been discussed as an anti-cancer agent (Firestone and Sundar 2009; Chaturvedi et al. 2010).

Several pharmaceuticals belong to the group of diterpenoids, including taxol, which is used as a potent anti-cancer agent (Wani et al. 1971). As the demand for taxol exceeds the amounts which can be isolated from its natural source *Taxus bevifolia*, heterologous production in *S. cerevisiae* by introducing parts of the 19

enzymatic step biosynthetic pathway is one alternative. Engels et al. (2008) described the production of a precursor of taxol, taxa-4(5),11(12)-diene, by expressing *Taxus chinensis* taxadiene synthase and truncated HMG-CoA reductase genes in *S. cerevisiae* together with an archaeal GGPP synthase gene from *Sulfolobus aciocaldarius*. These manipulations resulted in formation of 8.7 mg/L of taxadiene.

Carotenoids, such as β-carotene, astaxanthin and lycopene, are also isoprenoids. They are widely distributed as yellow, orange and red natural pigments in all phototrophic plants as well as in some bacteria, algae and fungi. In addition to their important physiological roles as components of the photosynthetic complex, precursors of phytohormones and chromophoric compounds of animals and plants, their anti-oxidative and photoprotective effects were proposed (Fraser and Bramley 2004). These effects are also beneficial for human health and carotenoids attracted attention as nutraceutical agents. Lycopene, for example, which occurs in tomatoes, is thought to prevent cardiovascular disease, UV-light ageing in humans and age-related macular degeneration. Carotenoid biosynthetic pathways have been introduced into *S. cerevisiae* to produce lycopene and β-carotene. The engineered strains yielded β-carotene at 5.9 mg/g dry cell weight and lycopene ate 7.8 mg/g dry cell weight (Verwaal et al. 2007; Yamano et al. 1994). For a recent review of this topic see Wriessnegger and Pichler (2013).

8.4.3 Steroids

Steroids comprise a large group of compounds with cyclopentanoperhydrophenanthrene as the common basic structure as described in the section on sterols. This group of components are roughly divided into sterols, which are steroid alcohols with a hydroxyl group in the 3-position of the A-ring, steroid hormones, steroid alkaloids and bile acids. Hundreds of distinct steroids are found in plants, animals and fungi, all of them sharing the mutual precursor squalene. They have sex-determining, growth regulating and anti-inflammatory properties and are responsible for membrane fluidity and permeability (Riad et al. 2002). The chemical synthesis is very difficult and extraction from natural sources is low-yielding and unsustainable. Therefore, the production in yeast is an appreciated alternative (Heiderpriem et al. 1992).

Several sterol intermediates are of biotechnological interest and have already found applications in industry (Donova and Egorova 2012). Lanosterol, for example, serves as an emulsifier in cosmetics, zymosterol as a precursor for cholesterol lowering substances and ergosterol itself as provitamin D2 and as a constituent of liposomal steroids used as carriers for drugs. As special pharmaceutical interest, they can serve as valuable precursors for the production of hydrocortisone and other steroid hormones like dehydroepiandosteroine, progesterone, testosterone and estrogens. The natural content of sterols in yeast is, however, too low for commercial applications and several attempts have therefore

been made to increase the total sterol content in this microorganism (Veen et al. 2003). The most successful strategies were the concomitant overexpression of *ERG1* and *ERG11* and a truncated version of *HMG1*, and the overexpression of *ERG4* and *ARE2*. The accumulation of sterols can also be promoted by the addition of ethanol into the cultivation medium by fermentation under nitrogen-limiting conditions (Sajbidor et al. 1995; Shang et al. 2006). For recent reviews of yeast metabolic engineering targeting sterol metabolism see (Wriessnegger and Pichler 2013).

8.4.4 Biofuels

Current transportation fuels are obtained mainly from fossil sources, which are not only limited but also associated with air pollution and global warming. These developments have prompted a desire for a shift from fossil fuels to biofuels. The concept of biofuels relies on the conversion of renewable resources into fuels. It comprises not only first-generation biofuels such as bioethanol and biodiesel, but also advanced biofuels such as alkanes, terpenes, short-chain alcohols and fatty acyl ethyl esters. Compared to bioethanol, the latter compounds promise energy content and combustion properties similar to those of current petroleum-based fuels (de Jong et al. 2012).

The most frequently employed microorganism to produce bioethanol is *S. cerevisiae* since it is able to hydrolyze sucrose from sugar cane into glucose and fructose at concentrations over 100 g/L, which can be converted by fermentation into ethanol. However, the availability of inexpensive fermentable sugars is limited, and re-dedicating farmland for biofuel production causes economic and ethical problems. Another limitation for ethanol as biofuel is the difficulty to distill it from fermentation broth due to its miscibility with water and its corrosive effect to storage and distribution infrastructures. As alternatives, non-food cellulose sources, including wheat straw and forest waste, can be used for the production of biofuels but, since *S. cerevisiae* is unable to convert cellulose or efficiently ferment C-5 sugars (pentoses), metabolic engineering approaches are necessary or the employment of other yeast genera (Madhavan et al. 2012).

Biodiesel is a biodegradable, non-toxic and sulphur-free alternative form of fuel, currently produced mainly by chemical transesterification of vegetable oils. One promising alternative is to use oleaginous yeasts, such as *Y. lipolytica*, *Cryptococcus curvatus* or *Lipomyces starkeyi* to produce lipids using cellulosic sugars as carbon source. These yeasts accumulate lipids at up to 40 % of their biomass, under nutrient-limiting conditions even up to 70 % (Chen et al. 2009). The microbial lipids produced show similar composition and energy values to those of vegetable oils, comprising mainly myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1, n-7), stearic (C18:0), oleic (C18:1, n-9), linoleic (C18:2, n-6) and α- linolenic (C18:3 n-3) acids, and are therefore of great commercial value for the production of sustainable biodiesel which requires C16-C18 FA (Zhao et al.

2011; Yu et al. 2011). However, from an economic point of view, the development of yeasts that produce more than 80 % lipids of their biomass would be necessary. Several engineering strategies have already been published and patented as reviewed by Beopoulos et al. (2011).

8.4.5 Flavour Compounds

Yeast biosynthesis of flavour compounds is important in fermentations of wine, beer and sake. An important group of volatile compounds produced by yeast during fermentation, which include fusel alcohols, monoterpenoids and volatile sulphur compounds, are lipid metabolism-derived acetate esters and medium-chain fatty acid (MCFA) ethyl esters (reviewed by Cordente et al. 2012). These esters are produced intracellularly by acetyl transferases from acetyl-CoA and ethanol or complex alcohols as substrates, or by acyl transferases from MCFA-CoA and ethanol as substrates. Many such esters can pass the plasma membrane and diffuse into the medium. The best studied group from the perspective of biosynthesis pathway are acetate esters. Their synthesis is catalyzed by acetyl transferases I and II, encoded by *ATF1* and *ATF2* genes (Fujii et al. 1994; Nagasawa et al. 1998). *ATF1* has been shown to be localized to LD (Verstrepen et al. 2004). Apart from volatile esters such as ethyl acetate or isoamyl acetate, *ATF1P* and *ATF2P* are also responsible for the formation of less volatile esters which add no flavour characteristics to the fermentation products. A certain amount of acetate esters are produced also in cells deleted of both *AFT* genes, indicating that additional, as yet unknown acetyl transferases may exist in yeast. Ethyl esters are the product of Eeb1p- or Eht1p-catalyzed condensation reaction between acyl-CoA and ethanol (Saerens et al. 2006). These two acyl transferases differ in their specificity towards different length of the substrate molecules and they also possess esterase activity. Similar to acetyl transferases, undiscovered acyl transferases responsible for MCFA ethyl ester biosynthesis are encoded in the yeast genome.

Understanding the physiological regulation of volatile esters biosynthesis is the prerequisite to the engineering of flavour compounds in yeast-fermented beverages. For the synthesis of acetate esters, the main regulatory step is the reaction catalyzed by acetyl transferases, whereas for MCFA ethyl ester formation, the availability of MCFA-CoA substrate is the limiting factor (Saerens et al. 2010). The amount and nature of acetyl esters could therefore be regulated by overexpression of *AFT1* or *AFT2* at different levels, possibly from different strains and therefore with different substrate specificities. The amount of MCFA ethyl esters could be controlled by modifying lipid metabolic pathways, specifically at the level of acetyl-CoA carboxylase whose activity determines the release of MCFAs from the fatty acid synthase complex (Dufour et al. 2003). Alternatively, the level of peroxisomal uptake of MCFAs may be changed, because a specific system exists for the import of this group of FA towards oxidative degradation (van Roermund et al. 2001).

For more details describing the nature and properties of yeast flavour compounds, the reader is referred to recent reviews (Saerens et al. 2010; Sumby et al. 2010; Cordente et al. 2012).

8.5 Conclusions and Perspectives

Over the last few decades, outstanding advancements have been made to identify the major enzymes involved in the pathways of lipid metabolism. Most of them are now known, covering the main cellular routes for synthesis, storage and degradation of lipid compounds. However, some gaps still remain. One intriguing open question is how cells can sense and manage their lipid composition under different environmental conditions. Investigations addressing such lipid sensors might also shed more light on the issue of how the different lipid compositions of different membranes within a single organism can be maintained. The situation gets even more complicated by the fact that enzymes of lipid synthesis are located in close vicinity to each other. To elucidate the topology of these enzymes in detail will be a challenge for the future. Other examples of unsolved problems are metabolic channelling and lipid trafficking that are just beginning to be addressed and understood. Regulation of lipid metabolism is an issue under discussion. It occurs at many different levels, and the cellular lipid composition is not only extremely dependent on growth conditions, such as nutrient availability, growth phase and pH, but also on many transcriptional control mechanisms that have been reported. Thus, the crosstalk between lipid metabolism and other cellular processes, as well as the regulatory network and interconnections of lipid metabolic pathways, will have to be studied in more detail. The elucidation of all these questions will foster the powerful role of yeast as a model organism.

Acknowledgments We thank Prof. Roger Pain and Dr. Karlheinz Grillitsch for carefully reading the manuscript. Research in G.D.'s laboratory was financially supported by the Austrian Science Fund (FWF) project W901 (DK Molecular Enzymology). The work in U.P.'s laboratory was financially supported by the Slovenian Research Agency (ARRS) grants J1-6507 and P1-0207.

References

Adeyo O, Horn PJ, Lee S, Binns DD, Chandrahas A, Chapman KD, Goodman JM (2011) The yeast lipin orthologue Pah1p is important for biogenesis of lipid droplets. J Cell Biol 192:1043–1055

Alvarez-Vasquez F, Riezman H, Hannun YA, Voit EO (2011) Mathematical modeling and validation of the ergosterol pathway in *Saccharomyces cerevisiae*. PLoS ONE 6:e28344

Anderson MS, Muehlbacher M, Street IP, Proffitt J, Poulter CD (1989a) Isopentenyl diphosphate:dimethylallyl diphosphate isomerase. An improved purification of the enzyme and isolation of the gene from *Saccharomyces cerevisiae*. J Biol Chem 264:19169–19175

Anderson MS, Yarger JG, Burck CL, Poulter CD (1989b) Farnesyl diphosphate synthetase. Molecular cloning, sequence, and expression of an essential gene from *Saccharomyces cerevisiae*. J Biol Chem 264:19176–19184

Asadollahi MA, Maury J, Schalk M, Clark A, Nielsen J (2010) Enhancement of farnesyl diphosphate pool as direct precursor of sesquiterpenes through metabolic engineering of the mevalonate pathway in *Saccharomyces cerevisiae*. Biotechnol Bioeng 106:86–96

Athenstaedt K, Daum G (1997) Biosynthesis of phosphatidic acid in lipid particles and endoplasmic reticulum of *Saccharomyces cerevisiae*. J Bacteriol 179:7611–7616

Athenstaedt K, Daum G (1999) Phosphatidic acid, a key intermediate in lipid metabolism. Eur J Biochem 266:1–16

Athenstaedt K, Daum G (2003) *YMR313c/TGL3* encodes a novel triacylglycerol lipase located in lipid particles of *Saccharomyces cerevisiae*. J Biol Chem 278:23317–23323

Athenstaedt K, Daum G (2005) Tgl4p and Tgl5p, two triacylglycerol lipases of the yeast *Saccharomyces cerevisiae* are localized to lipid particles. J Biol Chem 280:37301–37309

Athenstaedt K, Zweytick D, Jandrositz A, Kohlwein SD, Daum G (1999a) Identification and characterization of major lipid particle proteins of the yeast *Saccharomyces cerevisiae*. J Bacteriol 181:6441–6448

Athenstaedt K, Weys S, Paltauf F, Daum G (1999b) Redundant systems of phosphatidic acid biosynthesis via acylation of glycerol-3-phosphate or dihydroxyacetone phosphate in the yeast *Saccharomyces cerevisiae*. J Bacteriol 181:1458–1463

Athenstaedt K, Jolivet P, Boulard C, Zivy M, Negroni L, Nicaud J-M, Chardot T (2006) Lipid particle composition of the yeast *Yarrowia lipolytica* depends on the carbon source. Proteomics 6:1450–1459

Bagnat M, Keränen S, Shevchenko A, Shevchenko A, Simons K (2000) Lipid rafts function in biosynthetic delivery of proteins to the cell surface in yeast. Proc Natl Acad Sci USA 97:3254–3259

Bard M, Downing JF (1981) Genetic and biochemical aspects of yeast sterol regulation involving 3-hydroxy-3-methylglutaryl coenzyme A reductase. J Gen Microbiol 125:415–420

Beeler TJ, Fu D, Rivera J, Monaghan E, Gable K, Dunn TM (1997) *SUR1* (*CSG1/BCL21*), a gene necessary for growth of *Saccharomyces cerevisiae* in the presence of high Ca2+ concentrations at 37 degrees C, is required for mannosylation of inositolphosphorylceramide. Mol Gen Genet 255:570–579

Beeler T, Bacikova D, Gable K, Hopkins L, Johnson C, Slife H, 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 *csg2*Δ mutant. J Biol Chem 273:30688–30694

Beopoulos A, Nicaud J-M, Gaillardin C (2011) An overview of lipid metabolism in yeasts and its impact on biotechnological processes. Appl Microbiol Biotechnol 90:1193–1206

Beranek A, Rechberger G, Knauer H, Wolinski H, Kohlwein SD, Leber R (2009) Identification of a cardiolipin-specific phospholipase encoded by the gene *CLD1* (*YGR110W*) in yeast. J Biol Chem 284:11572–11578

Berchtold D, Piccolis M, Chiaruttini N, Riezman I, Riezman H, Roux A, Walther TC, Loewith R (2012) Plasma membrane stress induces relocalization of Slm proteins and activation of TORC2 to promote sphingolipid synthesis. Nat Cell Biol 14:542–547

Binns D, Januszewski T, Chen Y, Hill J, Markin VS, Zhao Y, Gilpin C, Chapman KD, Anderson RGW, Goodman JM (2006) An intimate collaboration between peroxisomes and lipid bodies. J Cell Biol 173:719–731

Birner R, Bürgermeister M, Schneiter R, Daum G (2001) Roles of phosphatidylethanolamine and of its several biosynthetic pathways in *Saccharomyces cerevisiae*. Mol Biol Cell 12:997–1007

Black PN, DiRusso CC (2007) Yeast acyl-CoA synthetases at the crossroads of fatty acid metabolism and regulation. Biochimica et Biophysica Acta (BBA)-Molecular Cell Biol Lipids 1771:286–298

Böttinger L, Horvath SE, Kleinschroth T, Hunte C, Daum G, Pfanner N, Becker T (2012) Phosphatidylethanolamine and cardiolipin differentially affect the stability of mitochondrial respiratory chain supercomplexes. J Mol Biol 423:677–686

Breslow DK, Collins SR, Bodenmiller B, Aebersold R, Simons K, Shevchenko A, Ejsing CS, Weissman JS (2010) Orm family proteins mediate sphingolipid homeostasis. Nature 463:1048–1053

Carman GM, Han G-S (2011) Regulation of phospholipid synthesis in the yeast *Saccharomyces cerevisiae*. Annu Rev Biochem 80:859–883

Carman GM, Henry SA (2007) Phosphatidic acid plays a central role in the transcriptional regulation of glycerophospholipid synthesis in *Saccharomyces cerevisiae*. J Biol Chem 282:37293–37297

Chambon C, Ladeveze V, Servouse M, Blanchard L, Javelot C, Vladescu B, Karst F (1991) Sterol pathway in yeast. Identification and properties of mutant strains defective in mevalonate diphosphate decarboxylase and farnesyl diphosphate synthetase. Lipids 26:633–636

Chang MCY, Keasling JD (2006) Production of isoprenoid pharmaceuticals by engineered microbes. Nat Chem Biol 2:674–681

Chang SC, Heacock PN, Clancey CJ, Dowhan W (1998a) The *PEL1* gene (renamed *PGS1*) encodes the phosphatidylglycero-phosphate synthase of *Saccharomyces cerevisiae*. J Biol Chem 273:9829–9836

Chang SC, Heacock PN, Mileykovskaya E, Voelker DR, Dowhan W (1998b) Isolation and characterization of the gene (*CLS1*) encoding cardiolipin synthase in *Saccharomyces cerevisiae*. J Biol Chem 273:14933–14941

Chaturvedi D, Goswami A, Saikia PP, Barua NC, Rao PG (2010) Artemisinin and its derivatives: a novel class of anti-malarial and anti-cancer agents. Chem Soc Rev 39:435–454

Chen M, Hancock LC, Lopes JM (2007a) Transcriptional regulation of yeast phospholipid biosynthetic genes. Biochim Biophys Acta 1771:310–321

Chen Q, Kazachkov M, Zheng Z, Zou J (2007b) The yeast acylglycerol acyltransferase *LCA1* is a key component of Lands cycle for phosphatidylcholine turnover. FEBS Lett 581:5511–5516

Chen X, Li Z, Zhang X, Hu F, Ryu DDY, Bao J (2009) Screening of oleaginous yeast strains tolerant to lignocellulose degradation compounds. Appl Biochem Biotechnol 159:591–604

Chirala SS, Kuziora MA, Spector DM, Wakil SJ (1987) Complementation of mutations and nucleotide sequence of FAS1 gene encoding beta subunit of yeast fatty acid synthase. J Biol Chem 262:4231–4240

Chirala SS, Zhong Q, Huang W, al-Feel W (1994) Analysis of *FAS3/ACC* regulatory region of *Saccharomyces cerevisiae*: identification of a functional UASINO and sequences responsible for fatty acid mediated repression. Nucleic Acids Res 22:412–418

Choi H-S, Su W-M, Morgan JM, Han G-S, Xu Z, Karanasios E, Siniossoglou S, Carman GM (2011) Phosphorylation of phosphatidate phosphatase regulates its membrane association and physiological functions in *Saccharomyces cerevisiae*: identification of SER(602), THR(723), AND SER(744) as the sites phosphorylated by *CDC28* (*CDK1*)-encoded cyclin-dependent kinase. J Biol Chem 286:1486–1498

Choudhary V, Schneiter R (2009) Monitoring sterol uptake, acetylation, and export in yeast. Methods Mol Biol 580:221–232

Choudhary V, Schneiter R (2012) Pathogen-Related Yeast (PRY) proteins and members of the CAP superfamily are secreted sterol-binding proteins. Proc Natl Acad Sci USA 109: 16882–16887

Chung J-H, Lester RL, Dickson RC (2003) Sphingolipid requirement for generation of a functional v1 component of the vacuolar ATPase. J Biol Chem 278:28872–28881

Clancey CJ, Chang SC, Dowhan W (1993) Cloning of a gene (*PSD1*) encoding phosphatidylserine decarboxylase from *Saccharomyces cerevisiae* by complementation of an Escherichia coli mutant. J Biol Chem 268:24580–24590

Connerth M, Czabany T, Wagner A, Zellnig G, Leitner E, Steyrer E, Daum G (2010) Oleate inhibits steryl ester synthesis and causes liposensitivity in yeast. J Biol Chem 285: 26832–26841

Connerth M, Tatsuta T, Haag M, Klecker T, Westermann B, Langer T (2012) Intramitochondrial transport of phosphatidic acid in yeast by a lipid transfer protein. Science 338:815–818
Cordente AG, Curtin CD, Varela C, Pretorius IS (2012) Flavour-active wine yeasts. Appl Microbiol Biotechnol 96:601–618
Cowart LA, Obeid LM (2007) Yeast sphingolipids: recent developments in understanding biosynthesis, regulation, and function. Biochim Biophys Acta 1771:421–431
Crowley JH, Leak FW Jr, Shianna KV, Tove S, Parks LW (1998) A mutation in a purported regulatory gene affects control of sterol uptake in *Saccharomyces cerevisiae*. J Bacteriol 180:4177–4183
Cullis PR, Hope MJ, Tilcock CP (1986) Lipid polymorphism and the roles of lipids in membranes. Chem Phys Lipids 40:127–144
Czabany T, Athenstaedt K, Daum G (2007) Synthesis, storage and degradation of neutral lipids in yeast. Biochim Biophys Acta 1771:299–309
Czabany T, Wagner A, Zweytick D, Lohner K, Leitner E, Ingolic E, Daum G (2008) Structural and biochemical properties of lipid particles from the yeast *Saccharomyces cerevisiae*. J Biol Chem 283:17065–17074
Daum G (1985) Lipids of mitochondria. Biochim Biophys Acta 822:1–42
Daum G, Vance JE (1997) Import of lipids into mitochondria. Prog Lipid Res 36:103–130
Daum G, Lees ND, Bard M, Dickson R (1998) Biochemistry, cell biology and molecular biology of lipids of *Saccharomyces cerevisiae*. Yeast 14:1471–1510
De Jong B, Siewers V, Nielsen J (2012) Systems biology of yeast: enabling technology for development of cell factories for production of advanced biofuels. Curr Opin Biotechnol 23:624–630
Debelyy MO, Thoms S, Connerth M, Daum G, Erdmann R (2011) Involvement of the *Saccharomyces cerevisiae* hydrolase Ldh1p in lipid homeostasis. Eukaryot Cell 10:776–781
Dennis EA (2009) Lipidomics joins the omics evolution. Proc Natl Acad Sci USA 106:2089–2090
Dickson RC, Nagiec EE, Wells GB, Nagiec MM, Lester RL (1997) Synthesis of mannose-(inositol-P)2-ceramide, the major sphingolipid in *Saccharomyces cerevisiae*, requires the *IPT1* (*YDR072c*) gene. J Biol Chem 272:29620–29625
Dickson RC, Sumanasekera C, Lester RL (2006) Functions and metabolism of sphingolipids in *Saccharomyces cerevisiae*. Prog Lipid Res 45:447–465
Donova MV, Egorova OV (2012) Microbial steroid transformations: current state and prospects. Appl Microbiol Biotechnol 94:1423–1447
Dowhan W, Bogdanov M (2009) Lipid-dependent membrane protein topogenesis. Annu Rev Biochem 78:515–540
Dufour J-P, Malcorps P, Silcock P (2003) Control of ester synthesis during brewery fermentation. In: Smart K (ed) Brewing Yeast Fermentation Performance. Blackwell Publishing, Oxford, pp 213–233
Dunn TM, Haak D, Monaghan E, Beeler TJ (1998) Synthesis of monohydroxylated inositol-phosphorylceramide (IPC-C) in *Saccharomyces cerevisiae* requires Scs7p, a protein with both a cytochrome b5-like domain and a hydroxylase/desaturase domain. Yeast 14:311–321
Eggeling C, Ringemann C, Medda R, Schwarzmann G, Sandhoff K, Polyakova S, Belov VN, Hein B, von Middendorff C, Schönle A et al (2009) Direct observation of the nanoscale dynamics of membrane lipids in a living cell. Nature 457:1159–1162
Einerhand AW, Voorn-Brouwer TM, Erdmann R, Kunau WH, Tabak HF (1991) Regulation of transcription of the gene coding for peroxisomal 3-oxoacyl-CoA thiolase of *Saccharomyces cerevisiae*. Eur J Biochem 200:113–122
Ejsing CS, Sampaio JL, Surendranath V, Duchoslav E, Ekroos K, Klemm RW, Simons K, Shevchenko A (2009) Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. Proc Natl Acad Sci USA 106:2136–2141
Engels B, Dahm P, Jennewein S (2008) Metabolic engineering of taxadiene biosynthesis in yeast as a first step towards Taxol (Paclitaxel) production. Metab Eng 10:201–206

Fahy E, Subramaniam S, Murphy RC, Nishijima M, Raetz CRH, Shimizu T, Spener F, van Meer G, Wakelam MJO, Dennis EA (2009) Update of the LIPID MAPS comprehensive classification system for lipids. J Lipid Res 50(Suppl):S9–14

Fakas S, Qiu Y, Dixon JL, Han G-S, Ruggles KV, Garbarino J, Sturley SL, Carman GM (2011) Phosphatidate phosphatase activity plays key role in protection against fatty acid-induced toxicity in yeast. J Biol Chem 286:29074–29085

Farese RV, Walther TC (2009) Lipid Droplets Finally Get a Little R-E-S-P-E-C-T. Cell 139:855–860

Fei W, Wang H, Fu X, Bielby C, Yang H (2009) Conditions of endoplasmic reticulum stress stimulate lipid droplet formation in *Saccharomyces cerevisiae*. Biochem J 424:61–67

Fernández-Murray JP, Gaspard GJ, Jesch SA, McMaster CR (2009) *NTE1*-encoded phosphatidylcholine phospholipase b regulates transcription of phospholipid biosynthetic genes. J Biol Chem 284:36034–36046

Fickers P, Benetti PH, Waché Y, Marty A, Mauersberger S, Smit MS, Nicaud JM (2005) Hydrophobic substrate utilisation by the yeast *Yarrowia lipolytica*, and its potential applications. FEMS Yeast Res 5:527–543

Firestone GL, Sundar SN (2009) Anticancer activities of artemisinin and its bioactive derivatives. Expert Rev Mol Med 11:e32

Fischl AS, Carman GM (1983) Phosphatidylinositol biosynthesis in *Saccharomyces cerevisiae*: purification and properties of microsome-associated phosphatidylinositol synthase. J Bacteriol 154:304–311

Fraser PD, Bramley PM (2004) The biosynthesis and nutritional uses of carotenoids. Prog Lipid Res 43:228–265

Fujii T, Nagasawa N, Iwamatsu A, Bogaki T, Tamai Y, Hamachi M (1994) Molecular cloning, sequence analysis, and expression of the yeast alcohol acetyltransferase gene. Appl Environ Microbiol 60:2786–2792

Fujita M, Umemura M, Yoko-o T, Jigami Y (2006) *PER1* is required for GPI-phospholipase A_2 activity and involved in lipid remodeling of GPI-anchored proteins. Mol Biol Cell 17:5253–5264

Gable K, Slife H, Bacikova D, Monaghan E, Dunn TM (2000) Tsc3p is an 80-amino acid protein associated with serine palmitoyltransferase and required for optimal enzyme activity. J Biol Chem 275:7597–7603

Gaigg B, Timischl B, Corbino L, Schneiter R (2005) Synthesis of sphingolipids with very long chain fatty acids but not ergosterol is required for routing of newly synthesized plasma membrane ATPase to the cell surface of yeast. J Biol Chem 280:22515–22522

Gaigg B, Toulmay A, Schneiter R (2006) Very long-chain fatty acid-containing lipids rather than sphingolipids per se are required for raft association and stable surface transport of newly synthesized plasma membrane ATPase in yeast. J Biol Chem 281:34135–34145

García-López MC, Pelechano V, Mirón-García MC, Garrido-Godino AI, García A, Calvo O, Werner M, Pérez-Ortín JE, Navarro F (2011) The conserved foot domain of RNA pol II associates with proteins involved in transcriptional initiation and/or early elongation. Genetics 189:1235–1248

Georgiev AG, Sullivan DP, Kersting MC, Dittman JS, Beh CT, Menon AK (2011) Osh proteins regulate membrane sterol organization but are not required for sterol movement between the ER and PM. Traffic 12:1341–1355

Grilley MM, Stock SD, Dickson RC, Lester RL, Takemoto JY (1998) Syringomycin action gene *SYR2* is essential for sphingolipid 4-hydroxylation in *Saccharomyces cerevisiae*. J Biol Chem 273:11062–11068

Grillitsch K, Daum G (2011) Triacylglycerol lipases of the yeast. Front Biol 6:219–230

Grillitsch K, Connerth M, Köfeler H, Arrey TN, Rietschel B, Wagner B, Karas M, Daum G (2011) Lipid particles/droplets of the yeast *Saccharomyces cerevisiae* revisited: lipidome meets proteome. Biochim Biophys Acta 1811:1165–1176

Guan XL, Souza CM, Pichler H, Dewhurst G, Schaad O, Kajiwara K, Wakabayashi H, Ivanova T, Castillon GA, Piccolis M et al (2009) Functional Interactions between Sphingolipids and Sterols in Biological Membranes Regulating Cell Physiology. Mol Biol Cell 20:2083–2095

Haak D, Gable K, Beeler T, Dunn T (1997) Hydroxylation of *Saccharomyces cerevisiae* ceramides requires Sur2p and Scs7p. J Biol Chem 272:29704–29710

Han G-S, Wu W-I, Carman GM (2006) The *Saccharomyces cerevisiae* Lipin homolog is a Mg^{2+}-dependent phosphatidate phosphatase enzyme. J Biol Chem 281:9210–9218

Han G-S, O'Hara L, Carman GM, Siniossoglou S (2008) An unconventional diacylglycerol kinase that regulates phospholipid synthesis and nuclear membrane growth. J Biol Chem 283:20433–20442

Hearn JD, Lester RL, Dickson RC (2003) The uracil transporter Fur4p associates with lipid rafts. J Biol Chem 278:3679–3686

Heiderpriem RW, Livant PD, Parish EJ, Barbuch RJ, Broaddus MG, Bard M (1992) A simple method for the isolation of zymosterol from a sterol mutant of *Saccharomyces cerevisiae*. J Steroid Biochem Mol Biol 43:741–743

Henry SA, Kohlwein SD, Carman GM (2012) Metabolism and regulation of glycerolipids in the yeast *Saccharomyces cerevisiae*. Genetics 190:317–349

Herrero O, Ramón D, Orejas M (2008) Engineering the *Saccharomyces cerevisiae* isoprenoid pathway for de novo production of aromatic monoterpenes in wine. Metab Eng 10:78–86

Hiltunen JK, Wenzel B, Beyer A, Erdmann R, Fosså A, Kunau WH (1992) Peroxisomal multifunctional beta-oxidation protein of *Saccharomyces cerevisiae*. Molecular analysis of the *FOX2* gene and gene product. J Biol Chem 267:6646–6653

Hiltunen JK, Autio KJ, Schonauer MS, Kursu VAS, Dieckmann CL, Kastaniotis AJ (2010) Mitochondrial fatty acid synthesis and respiration. Biochim Biophys Acta 1797:1195–1202

Hjelmstad RH, Bell RM (1992) Choline- and ethanolaminephosphotransferases from *Saccharomyces cerevisiae*. Meth Enzymol 209:272–279

Hoppins S, Collins SR, Cassidy-Stone A, Hummel E, Devay RM, Lackner LL, Westermann B, Schuldiner M, Weissman JS, Nunnari J (2011) A mitochondrial-focused genetic interaction map reveals a scaffold-like complex required for inner membrane organization in mitochondria. J Cell Biol 195:323–340

Horvath A, Sütterlin C, Manning-Krieg U, Movva NR, Riezman H (1994) Ceramide synthesis enhances transport of GPI-anchored proteins to the Golgi apparatus in yeast. EMBO J 13:3687–3695

Horvath SE, Wagner A, Steyrer E, Daum G (2011) Metabolic link between phosphatidylethanolamine and triacylglycerol metabolism in the yeast *Saccharomyces cerevisiae*. Biochim Biophys Acta 1811:1030–1037

Horvath SE, Böttinger L, Vögtle F-N, Wiedemann N, Meisinger C, Becker T, Daum G (2012) Processing and topology of the yeast mitochondrial phosphatidylserine decarboxylase 1. J Biol Chem 287:36744–36755

Iwanyshyn WM, Han G-S, Carman GM (2004) Regulation of phospholipid synthesis in *Saccharomyces cerevisiae* by zinc. J Biol Chem 279:21976–21983

Jacquier N, Schneiter R (2012) Mechanisms of sterol uptake and transport in yeast. J Steroid Biochem Mol Biol 129:70–78

Jacquier N, Choudhary V, Mari M, Toulmay A, Reggiori F, Schneiter R (2011) Lipid droplets are functionally connected to the endoplasmic reticulum in *Saccharomyces cerevisiae*. J Cell Sci 124:2424–2437

Jahnke L, Klein HP (1983) Oxygen requirements for formation and activity of the squalene epoxidase in *Saccharomyces cerevisiae*. J Bacteriol 155:488–492

Jain S, Stanford N, Bhagwat N, Seiler B, Costanzo M, Boone C, Oelkers P (2007) Identification of a novel lysophospholipid acyltransferase in *Saccharomyces cerevisiae*. J Biol Chem 282:30562–30569

Janssen MJ, Koorengevel MC, de Kruijff B, de Kroon AI (2000) The phosphatidylcholine to phosphatidylethanolamine ratio of *Saccharomyces cerevisiae* varies with the growth phase. Yeast 16:641–650

Jiang F, Rizavi HS, Greenberg ML (1997) Cardiolipin is not essential for the growth of *Saccharomyces cerevisiae* on fermentable or non-fermentable carbon sources. Mol Microbiol 26:481–491

Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K (2006) Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 116:1784–1792

Karpichev IV, Cornivelli L, Small GM (2002) Multiple regulatory roles of a novel *Saccharomyces cerevisiae* protein, encoded by *YOL002c*, in lipid and phosphate metabolism. J Biol Chem 277:19609–19617

Kennedy EP, Weiss SB (1956) The function of cytidine coenzymes in the biosynthesis of phospholipides. J Biol Chem 222:193–214

Kim K, Kim KH, Storey MK, Voelker DR, Carman GM (1999) Isolation and characterization of the *Saccharomyces cerevisiae EKI1* gene encoding ethanolamine kinase. J Biol Chem 274:14857–14866

Klose C, Surma MA, Gerl MJ, Meyenhofer F, Shevchenko A, Simons K (2012) Flexibility of a eukaryotic lipidome–insights from yeast lipidomics. PLoS ONE 7:e35063

Kobayashi SD, Nagiec MM (2003) Ceramide/long-chain base phosphate rheostat in *Saccharomyces cerevisiae*: regulation of ceramide synthesis by Elo3p and Cka2p. Eukaryot Cell 2:284–294

Köffel R, Tiwari R, Falquet L, Schneiter R (2005) The *Saccharomyces cerevisiae YLL012/YEH1*, *YLR020/YEH2*, and *TGL1* genes encode a novel family of membrane-anchored lipases that are required for steryl ester hydrolysis. Mol Cell Biol 25:1655–1668

Kohlwein SD (2010) Triacylglycerol homeostasis: insights from yeast. J Biol Chem 285:15663–15667

Kohlwein SD, Veenhuis M, van der Klei IJ (2012) Lipid droplets and peroxisomes: key players in cellular lipid homeostasis or a matter of fat-store 'em up or burn 'em down. Genetics 193:1–50

Kolaczkowski M, Kolaczkowska A, Gaigg B, Schneiter R, Moye-Rowley WS (2004) Differential regulation of ceramide synthase components *LAC1* and *LAG1* in *Saccharomyces cerevisiae*. Eukaryot Cell 3:880–892

Kopec KO, Alva V, Lupas AN (2010) Homology of SMP domains to the TULIP superfamily of lipid-binding proteins provides a structural basis for lipid exchange between ER and mitochondria. Bioinformatics 26:1927–1931

Kornmann B, Currie E, Collins SR, Schuldiner M, Nunnari J, Weissman JS, Walter P (2009) An ER-mitochondria tethering complex revealed by a synthetic biology screen. Science 325:477–481

Kornmann B, Osman C, Walter P (2011) The conserved GTPase Gem1 regulates endoplasmic reticulum-mitochondria connections. Proc Natl Acad Sci USA 108:14151–14156

Kristan K, Rižner TL (2012) Steroid-transforming enzymes in fungi. J Steroid Biochem Mol Biol 129:79–91

Ktistakis NT (2010) Lipid signaling and homeostasis: PA- is better than PA-H, but what about those PIPs? Sci Signal 3:pe46

Kuchler K, Daum G, Paltauf F (1986) Subcellular and submitochondrial localization of phospholipid-synthesizing enzymes in *Saccharomyces cerevisiae*. J Bacteriol 165:901–910

Kupchak BR, Garitaonandia I, Villa NY, Mullen MB, Weaver MG, Regalla LM, Kendall EA, Lyons TJ (2007) Probing the mechanism of *FET3* repression by Izh2p overexpression. Biochim Biophys Acta 1773:1124–1132

Kuranda K, François J, Palamarczyk G (2010) The isoprenoid pathway and transcriptional response to its inhibitors in the yeast *Saccharomyces cerevisiae*. FEMS Yeast Res 10:14–27

Kurat CF, Natter K, Petschnigg J, Wolinski H, Scheuringer K, Scholz H, Zimmermann R, Leber R, Zechner R, Kohlwein SD (2006) Obese yeast: triglyceride lipolysis is functionally conserved from mammals to yeast. J Biol Chem 281:491–500

Kurat CF, Wolinski H, Petschnigg J, Kaluarachchi S, Andrews B, Natter K, Kohlwein SD (2009) Cdk1/Cdc28-dependent activation of the major triacylglycerol lipase Tgl4 in yeast links lipolysis to cell-cycle progression. Mol Cell 33:53–63

Kuroda T, Tani M, Moriguchi A, Tokunaga S, Higuchi T, Kitada S, Kuge O (2011) *FMP30* is required for the maintenance of a normal cardiolipin level and mitochondrial morphology in the absence of mitochondrial phosphatidylethanolamine synthesis. Mol Microbiol 80:248–265

Lauwers E, Grossmann G, André B (2007) Evidence for coupled biogenesis of yeast Gap1 permease and sphingolipids: essential role in transport activity and normal control by ubiquitination. Mol Biol Cell 18:3068–3080

Leber R, Zinser E, Zellnig G, Paltauf F, Daum G (1994) Characterization of lipid particles of the yeast, *Saccharomyces cerevisiae*. Yeast 10:1421–1428

Leber R, Landl K, Zinser E, Ahorn H, Spök A, Kohlwein SD, Turnowsky F, Daum G (1998) Dual localization of squalene epoxidase, Erg1p, in yeast reflects a relationship between the endoplasmic reticulum and lipid particles. Mol Biol Cell 9:375–386

Lee SY, Kim HU, Park JH, Park JM, Kim TY (2009) Metabolic engineering of microorganisms: general strategies and drug production. Drug Discov Today 14:78–88

Lees ND, Skaggs B, Kirsch DR, Bard M (1995) Cloning of the late genes in the ergosterol biosynthetic pathway of *Saccharomyces cerevisiae*–a review. Lipids 30:221–226

Linder ME, Deschenes RJ (2004) Model organisms lead the way to protein palmitoyltransferases. J Cell Sci 117:521–526

Lingwood D, Simons K (2010) Lipid rafts as a membrane-organizing principle. Science 327:46–50

Loewen CJR, Gaspar ML, Jesch SA, Delon C, Ktistakis NT, Henry SA, Levine TP (2004) Phospholipid metabolism regulated by a transcription factor sensing phosphatidic acid. Science 304:1644–1647

London E, Brown DA (2000) Insolubility of lipids in triton X-100: physical origin and relationship to sphingolipid/cholesterol membrane domains (rafts). Biochim Biophys Acta 1508:182–195

Lorenz RT, Casey WM, Parks LW (1989) Structural discrimination in the sparking function of sterols in the yeast *Saccharomyces cerevisiae*. J Bacteriol 171:6169–6173

Lyons TJ, Villa NY, Regalla LM, Kupchak BR, Vagstad A, Eide DJ (2004) Metalloregulation of yeast membrane steroid receptor homologs. Proc Natl Acad Sci USA 101:5506–5511

Madhavan A, Srivastava A, Kondo A, Bisaria VS (2012) Bioconversion of lignocellulose-derived sugars to ethanol by engineered *Saccharomyces cerevisiae*. Crit Rev Biotechnol 32:22–48

Mao C, Xu R, Bielawska A, Szulc ZM, Obeid LM (2000a) Cloning and characterization of a *Saccharomyces cerevisiae* alkaline ceramidase with specificity for dihydroceramide. J Biol Chem 275:31369–31378

Mao C, Xu R, Bielawska A, Obeid LM (2000b) Cloning of an alkaline ceramidase from *Saccharomyces cerevisiae*. An enzyme with reverse (CoA-independent) ceramide synthase activity. J Biol Chem 275:6876–6884

Matmati N, Hannun YA (2008) Thematic review series: sphingolipids. *ISC1* (inositol phosphosphingolipid-phospholipase C), the yeast homologue of neutral sphingomyelinases. J Lipid Res 49:922–928

Meisinger C, Pfannschmidt S, Rissler M, Milenkovic D, Becker T, Stojanovski D, Youngman MJ, Jensen RE, Chacinska A, Guiard B et al (2007) The morphology proteins Mdm12/Mmm1 function in the major beta-barrel assembly pathway of mitochondria. EMBO J 26:2229–2239

Merkel O, Schmid PC, Paltauf F, Schmid HHO (2005) Presence and potential signaling function of N-acylethanolamines and their phospholipid precursors in the yeast *Saccharomyces cerevisiae*. Biochim Biophys Acta 1734:215–219

Mesmin B, Maxfield FR (2009) Intracellular sterol dynamics. Biochimica et Biophysica Acta (BBA)-Molecular Cell Biol Lipids 1791:636–645

Mignery GA, Pikaard CS, Park WD (1988) Molecular characterization of the patatin multigene family of potato. Gene 62:27–44

Misawa N (2011) Pathway engineering for functional isoprenoids. Curr Opin Biotechnol 22:627–633

Moir RD, Gross DA, Silver DL, Willis IM (2012) *SCS3* and *YFT2* link transcription of phospholipid biosynthetic genes to ER stress and the UPR. PLoS Genet 8:e1002890

Mora G, Scharnewski M, Fulda M (2012) Neutral lipid metabolism influences phospholipid synthesis and deacylation in *Saccharomyces cerevisiae*. PLoS ONE 7:e49269

Müllner H, Zweytick D, Leber R, Turnowsky F, Daum G (2004) Targeting of proteins involved in sterol biosynthesis to lipid particles of the yeast *Saccharomyces cerevisiae*. Biochim Biophys Acta 1663:9–13

Müllner H, Deutsch G, Leitner E, Ingolic E, Daum G (2005) *YEH2/YLR020c* encodes a novel steryl ester hydrolase of the yeast *Saccharomyces cerevisiae*. J Biol Chem 280:13321–13328

Murphy DJ, Vance J (1999) Mechanisms of lipid-body formation. Trends Biochem Sci 24:109–115

Nadolski MJ, Linder ME (2007) Protein lipidation. FEBS J 274:5202–5210

Nagasawa N, Bogaki T, Iwamatsu A, Hamachi M, Kumagai C (1998) Cloning and nucleotide sequence of the alcohol acetyltransferase II gene (*ATF2*) from *Saccharomyces cerevisiae* Kyokai No. 7. Biosci Biotechnol Biochem 62:1852–1857

Nagiec MM, Baltisberger JA, Wells GB, Lester RL, Dickson RC (1994) The *LCB2* gene of Saccharomyces and the related LCB1 gene encode subunits of serine palmitoyltransferase, the initial enzyme in sphingolipid synthesis. Proc Natl Acad Sci USA 91:7899–7902

Nagiec MM, Nagiec EE, Baltisberger JA, Wells GB, Lester RL, Dickson RC (1997) Sphingolipid synthesis as a target for antifungal drugs. Complementation of the inositol phosphorylceramide synthase defect in a mutant strain of *Saccharomyces cerevisiae* by the *AUR1* gene. J Biol Chem 272:9809–9817

Nagiec MM, Skrzypek M, Nagiec EE, Lester RL, Dickson RC (1998) The *LCB4* (*YOR171c*) and *LCB5* (*YLR260w*) genes of *Saccharomyces* encode sphingoid long chain base kinases. J Biol Chem 273:19437–19442

Natter K, Kohlwein SD (2012) Yeast and cancer cells—common principles in lipid metabolism. Biochim Biophys Acta 1831:314–326

Natter K, Leitner P, Faschinger A, Wolinski H, McCraith S, Fields S, Kohlwein SD (2005) The spatial organization of lipid synthesis in the yeast *Saccharomyces cerevisiae* derived from large scale green fluorescent protein tagging and high resolution microscopy. Mol Cell Proteomics 4:662–672

Nes WD, Janssen GG, Crumley FG, Kalinowska M, Akihisa T (1993) The structural requirements of sterols for membrane function in *Saccharomyces cerevisiae*. Arch Biochem Biophys 300:724–733

Nguyen TT, Lewandowska A, Choi J-Y, Markgraf DF, Junker M, Bilgin M, Ejsing CS, Voelker DR, Rapoport TA, Shaw JM (2012) Gem1 and ERMES do not directly affect phosphatidylserine transport from ER to mitochondria or mitochondrial inheritance. Traffic 13:880–890

Nikawa J, Tsukagoshi Y, Yamashita S (1991) Isolation and characterization of two distinct myo-inositol transporter genes of *Saccharomyces cerevisiae*. J Biol Chem 266:11184–11191

Niles BJ, Powers T (2012) Plasma membrane proteins Slm1 and Slm2 mediate activation of the AGC kinase Ypk1 by TORC2 and sphingolipids in *S. cerevisiae*. Cell Cycle 11:3745–3749

O'Hara L, Han G-S, Peak-Chew S, Grimsey N, Carman GM, Siniossoglou S (2006) Control of phospholipid synthesis by phosphorylation of the yeast lipin Pah1p/Smp2p Mg^{2+}-dependent phosphatidate phosphatase. J Biol Chem 281:34537–34548

Opekarová M, Tanner W (2003) Specific lipid requirements of membrane proteins–a putative bottleneck in heterologous expression. Biochim Biophys Acta 1610:11–22

Osman C, Haag M, Wieland FT, Brügger B, Langer T (2010) A mitochondrial phosphatase required for cardiolipin biosynthesis: the PGP phosphatase Gep4. EMBO J 29:1976–1987

Ostergaard S, Olsson L, Nielsen J (2000) Metabolic engineering of *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 64:34–50

Panwar SL, Moye-Rowley WS (2006) Long chain base tolerance in *Saccharomyces cerevisiae* is induced by retrograde signals from the mitochondria. J Biol Chem 281:6376–6384

Pascual F, Carman GM (2013) Phosphatidate phosphatase, a key regulator of lipid homeostasis. Biochim Biophys Acta 1831:514–522

Pichler H (2005) Modulating membrane properties-sterol cell biology in yeast. In: Daum G (ed) Cell biology and dynamics of yeast lipids, Research Signpost, Trivandrum (Kerala), pp. 179–199

Ploegh HL (2007) A lipid-based model for the creation of an escape hatch from the endoplasmic reticulum. Nature 448:435–438

Ploier B, Scharwey M, Koch B, Schmidt C, Schatte J, Rechberger G et al (2013) Screening for hydrolytic enzymes reveals Ayr1p as a novel triacylglycerol lipase in *Saccharomyces cerevisiae*. J Biol Chem 288:36061–36072

Poirier Y, Antonenkov VD, Glumoff T, Hiltunen JK (2006) Peroxisomal β-oxidation–a metabolic pathway with multiple functions. Biochim Biophys Acta 1763:1413–1426

Polakowski T, Stahl U, Lang C (1998) Overexpression of a cytosolic hydroxymethylglutaryl-CoA reductase leads to squalene accumulation in yeast. Appl Microbiol Biotechnol 49:66–71

Potting C, Wilmes C, Engmann T, Osman C, Langer T (2010) Regulation of mitochondrial phospholipids by Ups1/PRELI-like proteins depends on proteolysis and Mdm35. EMBO J 29:2888–2898

Pu J, Ha CW, Zhang S, Jung JP, Huh W-K, Liu P (2011) Interactomic study on interaction between lipid droplets and mitochondria. Protein Cell 2:487–496

Rajakumari S, Daum G (2010a) Janus-faced enzymes yeast Tgl3p and Tgl5p catalyze lipase and acyltransferase reactions. Mol Biol Cell 21:501–510

Rajakumari S, Daum G (2010b) Multiple functions as lipase, steryl ester hydrolase, phospholipase, and acyltransferase of Tgl4p from the yeast *Saccharomyces cerevisiae*. J Biol Chem 285:15769–15776

Rajakumari S, Grillitsch K, Daum G (2008) Synthesis and turnover of non-polar lipids in yeast. Prog Lipid Res 47:157–171

Rajakumari S, Rajasekharan R, Daum G (2010) Triacylglycerol lipolysis is linked to sphingolipid and phospholipid metabolism of the yeast *Saccharomyces cerevisiae*. Biochim Biophys Acta 1801:1314–1322

Raychaudhuri S, Young BP, Espenshade PJ, Loewen C Jr (2012) Regulation of lipid metabolism: a tale of two yeasts. Curr Opin Cell Biol 24:502–508

Rebecchi MJ, Pentyala SN (2000) Structure, function, and control of phosphoinositide-specific phospholipase C. Physiol Rev 80:1291–1335

Reue K, Brindley DN (2008) Thematic Review Series: Glycerolipids. Multiple roles for lipins/phosphatidate phosphatase enzymes in lipid metabolism. J Lipid Res 49:2493–2503

Reue K, Dwyer JR (2009) Lipin proteins and metabolic homeostasis. J Lipid Res 50(Suppl):S109–S114

Riad M, Mogos M, Thangathurai D, Lumb PD (2002) Steroids. Curr Opin Crit Care 8:281–284

Riekhof WR, Voelker DR (2006) Uptake and utilization of lyso-phosphatidylethanolamine by *Saccharomyces cerevisiae*. J Biol Chem 281:36588–36596

Riekhof WR, Wu J, Jones JL, Voelker DR (2007) Identification and characterization of the major lysophosphatidylethanolamine acyltransferase in *Saccharomyces cerevisiae*. J Biol Chem 282:28344–28352

Roelants FM, Breslow DK, Muir A, Weissman JS, Thorner J (2011) Protein kinase Ypk1 phosphorylates regulatory proteins Orm1 and Orm2 to control sphingolipid homeostasis in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 108:19222–19227

Rudge SA, Morris AJ, Engebrecht J (1998) Relocalization of phospholipase D activity mediates membrane formation during meiosis. J Cell Biol 140:81–90

Ruenwai R, Neiss A, Laoteng K, Vongsangnak W, Dalfard AB, Cheevadhanarak S, Petranovic D, Nielsen J (2011) Heterologous production of polyunsaturated fatty acids in *Saccharomyces cerevisiae* causes a global transcriptional response resulting in reduced proteasomal activity and increased oxidative stress. Biotechnol J 6:343–356

Saba JD, Nara F, Bielawska A, Garrett S, Hannun YA (1997) The *BST1* gene of *Saccharomyces cerevisiae* is the sphingosine-1-phosphate lyase. J Biol Chem 272:26087–26090

Saerens SMG, Verstrepen KJ, Van Laere SDM, Voet ARD, Van Dijck P, Delvaux FR, Thevelein JM (2006) The *Saccharomyces cerevisiae EHT1* and *EEB1* genes encode novel enzymes with medium-chain fatty acid ethyl ester synthesis and hydrolysis capacity. J Biol Chem 281:4446–4456

Saerens SMG, Delvaux FR, Verstrepen KJ, Thevelein JM (2010) Production and biological function of volatile esters in *Saccharomyces cerevisiae*. Microb Biotechnol 3:165–177

Sajbidor J, Ciesarová Z, Smogrovicová D (1995) Influence of ethanol on the lipid content and fatty acid composition of *Saccharomyces cerevisiae*. Folia Microbiol (Praha) 40:508–510

Santos AXS, Riezman H (2012) Yeast as a model system for studying lipid homeostasis and function. FEBS Lett 586:2858–2867

Sasser T, Qiu Q-S, Karunakaran S, Padolina M, Reyes A, Flood B, Smith S, Gonzales C, Fratti RA (2012) Yeast lipin 1 orthologue pah1p regulates vacuole homeostasis and membrane fusion. J Biol Chem 287:2221–2236

Sawai H, Okamoto Y, Luberto C, Mao C, Bielawska A, Domae N, Hannun YA (2000) Identification of *ISC1* (*YER019w*) as inositol phosphosphingolipid phospholipase C in *Saccharomyces cerevisiae*. J Biol Chem 275:39793–39798

Schneiter R, Brügger B, Sandhoff R, Zellnig G, Leber A, Lampl M, Athenstaedt K, Hrastnik C, Eder S, Daum G et al (1999) Electrospray ionization tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals acyl chain-based sorting/remodeling of distinct molecular species en route to the plasma membrane. J Cell Biol 146:741–754

Schorling S, Vallée B, Barz WP, Riezman H, Oesterhelt D (2001) Lag1p and Lac1p are essential for the Acyl-CoA-dependent ceramide synthase reaction in *Saccharomyces cerevisae*. Mol Biol Cell 12:3417–3427

Schrag JD, Cygler M (1997) Lipases and alpha/beta hydrolase fold. Meth Enzymol 284:85–107

Schulz TA, Prinz WA (2007) Sterol transport in yeast and the oxysterol binding protein homologue (OSH) family. Biochimica et Biophysica Acta (BBA)-Molecular Cell Biol Lipids 1771:769–780

Shang F, Wen S, Wang X, Tan T (2006) Effect of nitrogen limitation on the ergosterol production by fed-batch culture of *Saccharomyces cerevisiae*. J Biotechnol 122:285–292

Shen H, Heacock PN, Clancey CJ, Dowhan W (1996) The *CDS1* gene encoding CDP-diacylglycerol synthase in *Saccharomyces cerevisiae* is essential for cell growth. J Biol Chem 271:789–795

Simocková M, Holic R, Tahotná D, Patton-Vogt J, Griac P (2008) Yeast Pgc1p (Ypl206c) controls the amount of phosphatidylglycerol via a phospholipase C-type degradation mechanism. J Biol Chem 283:17107–17115

Simons K, Sampaio JL (2011) Membrane organization and lipid rafts. Cold Spring Harb Perspect Biol 3:a004697

Siniossoglou S, Santos-Rosa H, Rappsilber J, Mann M, Hurt E (1998) A novel complex of membrane proteins required for formation of a spherical nucleus. EMBO J 17:6449–6464

Skrzypek M, Lester RL, Dickson RC (1997) Suppressor gene analysis reveals an essential role for sphingolipids in transport of glycosylphosphatidylinositol-anchored proteins in *Saccharomyces cerevisiae*. J Bacteriol 179:1513–1520

Souza CM, Pichler H (2007) Lipid requirements for endocytosis in yeast. Biochim Biophys Acta 1771:442–454

Spanova M, Daum G (2011) Squalene—biochemistry, molecular biology, process biotechnology, and applications. Eur J Lipid Sci Technol 113:1299–1320

Spanova M, Czabany T, Zellnig G, Leitner E, Hapala I, Daum G (2010) Effect of lipid particle biogenesis on the subcellular distribution of squalene in the yeast *Saccharomyces cerevisiae*. J Biol Chem 285:6127–6133

Spanova M, Zweytick D, Lohner K, Klug L, Leitner E, Hermetter A, Daum G (2012) Influence of squalene on lipid particle/droplet and membrane organization in the yeast *Saccharomyces cerevisiae*. Biochim Biophys Acta 1821:647–653

Sreenivas A, Patton-Vogt JL, Bruno V, Griac P, Henry SA (1998) A role for phospholipase D (Pld1p) in growth, secretion, and regulation of membrane lipid synthesis in yeast. J Biol Chem 273:16635–16638

Strahl T, Thorner J (2007) Synthesis and function of membrane phosphoinositides in budding yeast, *Saccharomyces cerevisiae*. Biochim Biophys Acta 1771:353–404

Stroud DA, Oeljeklaus S, Wiese S, Bohnert M, Lewandrowski U, Sickmann A, Guiard B, van der Laan M, Warscheid B, Wiedemann N (2011) Composition and topology of the endoplasmic reticulum-mitochondria encounter structure. J Mol Biol 413:743–750

Stukey JE, McDonough VM, Martin CE (1990) The *OLE1* gene of *Saccharomyces cerevisiae* encodes the Δ9–fatty acid desaturase and can be functionally replaced by the rat stearoyl-CoA desaturase gene. J Biol Chem 265:20144–20149

Sumby KM, Grbin PR, Jiranek V (2010) Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. Food Chem 121:1–16

Sun Y, Taniguchi R, Tanoue D, Yamaji T, Takematsu H, Mori K, Fujita T, Kawasaki T, Kozutsumi Y (2000) Sli2 (Ypk1), a homologue of mammalian protein kinase SGK, is a downstream kinase in the sphingolipid-mediated signaling pathway of yeast. Mol Cell Biol 20:4411–4419

Tamura Y, Iijima M, Sesaki H (2010) Mdm35p imports Ups proteins into the mitochondrial intermembrane space by functional complex formation. EMBO J 29:2875–2887

Tamura Y, Onguka O, Hobbs AEA, Jensen RE, Iijima M, Claypool SM, Sesaki H (2012) Role for two conserved intermembrane space proteins, Ups1p and Ups2p, in intra-mitochondrial phospholipid trafficking. J Biol Chem 287:15205–15218

Tanaka S, Maeda Y, Tashima Y, Kinoshita T (2004) Inositol deacylation of glycosylphosphatidylinositol-anchored proteins is mediated by mammalian PGAP1 and yeast Bst1p. J Biol Chem 279:14256–14263

Tehlivets O, Scheuringer K, Kohlwein SD (2007) Fatty acid synthesis and elongation in yeast. Biochim Biophys Acta 1771:255–270

Tevzadze GG, Pierce JV, Esposito RE (2007) Genetic evidence for a *SPO1*-dependent signaling pathway controlling meiotic progression in yeast. Genetics 175:1213–1227

Thoms S, Debelyy MO, Nau K, Meyer HE, Erdmann R (2008) Lpx1p is a peroxisomal lipase required for normal peroxisome morphology. FEBS J 275:504–514

Thoms S, Debelyy MO, Connerth M, Daum G, Erdmann R (2011) The putative *Saccharomyces cerevisiae* hydrolase Ldh1p is localized to lipid droplets. Eukaryot Cell 10:770–775

Tinkelenberg AH, Liu Y, Alcantara F, Khan S, Guo Z, Bard M, Sturley SL (2000) Mutations in yeast *ARV1* alter intracellular sterol distribution and are complemented by human *ARV1*. J Biol Chem 275:40667–40670

Tiwari R, Köffel R, Schneiter R (2007) An acetylation/deacetylation cycle controls the export of sterols and steroids from *S. cerevisiae*. EMBO J 26:5109–5119

Toth MJ, Huwyler L (1996) Molecular cloning and expression of the cDNAs encoding human and yeast mevalonate pyrophosphate decarboxylase. J Biol Chem 271:7895–7898

Trotter PJ, Voelker DR (1995) Identification of a non-mitochondrial phosphatidylserine decarboxylase activity (*PSD2*) in the yeast *Saccharomyces cerevisiae*. J Biol Chem 270:6062–6070

Tuller G, Hrastnik C, Achleitner G, Schiefthaler U, Klein F, Daum G (1998) *YDL142c* encodes cardiolipin synthase (Cls1p) and is non-essential for aerobic growth of *Saccharomyces cerevisiae*. FEBS Lett 421:15–18

Uemura H (2012) Synthesis and production of unsaturated and polyunsaturated fatty acids in yeast: current state and perspectives. Appl Microbiol Biotechnol 95:1–12

Uemura S, Kihara A, Inokuchi J-I, Igarashi Y (2003) Csg1p and newly identified Csh1p function in mannosylinositol phosphorylceramide synthesis by interacting with Csg2p. J Biol Chem 278:45049–45055

Vallée B, Riezman H (2005) Lip1p: a novel subunit of acyl-CoA ceramide synthase. EMBO J 24:730–741

Van der Laan M, Bohnert M, Wiedemann N, Pfanner N (2012) Role of MINOS in mitochondrial membrane architecture and biogenesis. Trends Cell Biol 22:185–192

Van Meer G, Voelker DR, Feigenson GW (2008) Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol 9:112–124

Van Roermund CW, Elgersma Y, Singh N, Wanders RJ, Tabak HF (1995) The membrane of peroxisomes in *Saccharomyces cerevisiae* is impermeable to NAD(H) and acetyl-CoA under in vivo conditions. EMBO J 14:3480–3486

Van Roermund CW, Drissen R, van Den Berg M, Ijlst L, Hettema EH, Tabak HF, Waterham HR, Wanders RJ (2001) Identification of a peroxisomal ATP carrier required for medium-chain fatty acid beta-oxidation and normal peroxisome proliferation in *Saccharomyces cerevisiae*. Mol Cell Biol 21:4321–4329

Van Roermund CWT, Ijlst L, Majczak W, Waterham HR, Folkerts H, Wanders RJA, Hellingwerf KJ (2012) Peroxisomal fatty acid uptake mechanism in *Saccharomyces cerevisiae*. J Biol Chem 287:20144–20153

Veen M, Lang C (2004) Production of lipid compounds in the yeast *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 63:635–646

Veen M, Stahl U, Lang C (2003) Combined overexpression of genes of the ergosterol biosynthetic pathway leads to accumulation of sterols in *Saccharomyces cerevisiae*. FEMS Yeast Res 4:87–95

Vehring S and Pomorski T (2005) Dynamics and regulation of phospholipid distribution in the yeast. In: Daum G (ed) Cell biology and dynamics of yeast lipids, Research Signpost, Trivandrum (Kerala), pp. 85–104

Verger R (1997) "Interfacial activation" of lipases: facts and artifacts. Trends Biotechnol 15:32–38

Verstrepen KJ, Van Laere SDM, Vercammen J, Derdelinckx G, Dufour J-P, Pretorius IS, Winderickx J, Thevelein JM, Delvaux FR (2004) The *Saccharomyces cerevisiae* alcohol acetyl transferase Atf1p is localized in lipid particles. Yeast 21:367–377

Verwaal R, Wang J, Meijnen JP, Visser H, Sandmann G, van den Berg JA, van Ooyen AJJ (2007) High-level production of beta-carotene in*Saccharomyces cerevisiae* by successive transformation with carotenogenic genes from Xanthophyllomyces dendrorhous. Appl Environ Microbiol 73:4342–4350

Voelker DR (2003) New perspectives on the regulation of intermembrane glycerophospholipid traffic. J Lipid Res 44:441–449

Wagner A, Grillitsch K, Leitner E, Daum G (2009) Mobilization of steryl esters from lipid particles of the yeast *Saccharomyces cerevisiae*. Biochim Biophys Acta 1791:118–124

Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT (1971) Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. J Am Chem Soc 93:2325–2327

Wilcox LJ, Balderes DA, Wharton B, Tinkelenberg AH, Rao G, Sturley SL (2002) Transcriptional profiling identifies two members of the ATP-binding cassette transporter superfamily required for sterol uptake in yeast. J Biol Chem 277:32466–32472

Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussey H et al (1999) Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. Science 285:901–906

Wolinski H, Kolb D, Hermann S, Koning RI, Kohlwein SD (2011) A role for seipin in lipid droplet dynamics and inheritance in yeast. J Cell Sci 124:3894–3904

Wriessnegger T, Pichler H (2013) Yeast metabolic engineering—Targeting sterol metabolism and terpenoid formation. Prog Lipid Res 52:277–293

Wu WI, Carman GM (1994) Regulation of phosphatidate phosphatase activity from the yeast *Saccharomyces cerevisiae* by nucleotides. J Biol Chem 269:29495–29501

Wu WI, Carman GM (1996) Regulation of phosphatidate phosphatase activity from the yeast *Saccharomyces cerevisiae* by phospholipids. Biochemistry 35:3790–3796

Wu WI, Lin YP, Wang E, Merrill AH Jr, Carman GM (1993) Regulation of phosphatidate phosphatase activity from the yeast *Saccharomyces cerevisiae* by sphingoid bases. J Biol Chem 268:13830–13837

Xue Z, Sharpe PL, Hong SP, Yadav NS, Xie D, Short DR, Damude HG, Rupert RA, Seip JE, Wang J, Pollak DW, Bostick MW, Bosak MD, Macool DJ, Hollerbach DH, Zhang H, Arcilla DM, Bledsoe SA, Croker K, McCord EF, Tyreus BD, Jackson EN, Zhu Q (2013) Production of omega-3 eicosapentaenoic acid by metabolic engineering of *Yarrowia lipolytica*. Nat Biotechnol 31:734–740

Yamano S, Ishii T, Nakagawa M, Ikenaga H, Misawa N (1994) Metabolic engineering for production of beta-carotene and lycopene in *Saccharomyces cerevisiae*. Biosci Biotechnol Biochem 58:1112–1114

Yang H, Bard M, Bruner DA, Gleeson A, Deckelbaum RJ, Aljinovic G, Pohl TM, Rothstein R, Sturley SL (1996) Sterol esterification in yeast: a two-gene process. Science 272:1353–1356

Yazawa H, Iwahashi H, Kamisaka Y, Kimura K, Aki T, Ono K, Uemura H (2007) Heterologous production of dihomo-gamma-linolenic acid in *Saccharomyces cerevisiae*. Appl Environ Microbiol 73:6965–6971

York JD (2006) Regulation of nuclear processes by inositol polyphosphates. Biochim Biophys Acta 1761:552–559

Yu C, Kennedy NJ, Chang CC, Rothblatt JA (1996) Molecular cloning and characterization of two isoforms of *Saccharomyces cerevisiae* acyl-CoA:sterol acyltransferase. J Biol Chem 271:24157–24163

Yu X, Zheng Y, Dorgan KM, Chen S (2011) Oil production by oleaginous yeasts using the hydrolysate from pretreatment of wheat straw with dilute sulfuric acid. Bioresour Technol 102:6134–6140

Zanghellini J, Natter K, Jungreuthmayer C, Thalhammer A, Kurat CF, Gogg-Fassolter G, Kohlwein SD, von Grünberg H-H (2008) Quantitative modeling of triacylglycerol homeostasis in yeast–metabolic requirement for lipolysis to promote membrane lipid synthesis and cellular growth. FEBS J 275:5552–5563

Zerbes RM, Bohnert M, Stroud DA, von der Malsburg K, Kram A, Oeljeklaus S, Warscheid B, Becker T, Wiedemann N, Veenhuis M et al (2012) Role of MINOS in mitochondrial membrane architecture: cristae morphology and outer membrane interactions differentially depend on mitofilin domains. J Mol Biol 422:183–191

Zhao X, Hu C, Wu S, Shen H, Zhao ZK (2011) Lipid production by Rhodosporidium toruloides Y4 using different substrate feeding strategies. J Ind Microbiol Biotechnol 38:627–632

Zinser E, Daum G (1995) Isolation and biochemical characterization of organelles from the yeast, *Saccharomyces cerevisiae*. Yeast 11:493–536

Zinser E, Sperka-Gottlieb CD, Fasch EV, Kohlwein SD, Paltauf F, Daum G (1991) Phospholipid synthesis and lipid composition of subcellular membranes in the unicellular eukaryote *Saccharomyces cerevisiae*. J Bacteriol 173:2026–2034

Zinser E, Paltauf F, Daum G (1993) Sterol composition of yeast organelle membranes and subcellular distribution of enzymes involved in sterol metabolism. J Bacteriol 175:2853–2858

Zweytick D, Leitner E, Kohlwein SD, Yu C, Rothblatt J, Daum G (2000) Contribution of Are1p and Are2p to steryl ester synthesis in the yeast *Saccharomyces cerevisiae*. Eur J Biochem 267:1075–1082