Metabolism and Regulation of Glycerolipids in the Yeast *Saccharomyces cerevisiae*

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ABSTRACT Due to its genetic tractability and increasing wealth of accessible data, the yeast *Saccharomyces cerevisiae* is a model system of choice for the study of the genetics, biochemistry, and cell biology of eukaryotic lipid metabolism. Glycerolipids (*e.g.*, phospholipids and triacylglycerol) and their precursors are synthesized and metabolized by enzymes associated with the cytosol and membranous organelles, including endoplasmic reticulum, mitochondria, and lipid droplets. Genetic and biochemical analyses have revealed that glycerolipids play important roles in cell signaling, membrane trafficking, and anchoring of membrane proteins in addition to membrane structure. The expression of glycerolipid enzymes is controlled by a variety of conditions including growth stage and nutrient availability. Much of this regulation occurs at the transcriptional level and involves the Ino2–Ino4 activation complex and the Opi1 repressor, which interacts with Ino2 to attenuate transcriptional activation of UAS_{INO}-containing glycerolipid biosynthetic genes. Cellular levels of phosphatidic acid, precursor to all membrane phospholipids and the storage lipid triacylglycerol, regulates transcription of UAS_{INO}-containing genes by tethering Opi1 to the nuclear/endoplasmic reticulum membrane and controlling its translocation into the nucleus, a mechanism largely controlled by inositol availability. The transcriptional activator Zap1 controls the expression of some phospholipid synthesis genes in response to zinc availability. Regulatory mechanisms also include control of catalytic activity of glycerolipid enzymes by water-soluble precursors, products and lipids, and covalent modification of phosphorylation, while *in vivo* function of some enzymes is governed by their subcellular location. Genome-wide genetic analysis indicates coordinate regulation between glycerolipid metabolism and a broad spectrum of metabolic pathways.

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THE yeast, *Saccharomyces cerevisiae*, has emerged as a powerful model system for the elucidation of the metabolism, cell biology, and regulation of eukaryotic lipids. Due to the strong homology of yeast proteins, pathways, and regulatory networks with those in higher eukaryotes, yeast has provided numerous insights into the genetics and biochemistry of lipid-related diseases. As a system for the study of eukaryotic lipid metabolism, the advantages of yeast include its vast, well-curated, and electronically accessible archives of genetic data, including those detailing gene–enzyme relationships in the pathways for lipid synthesis and turnover. Another major advantage is the rapidly increasing understanding of the regulation and localization of enzymes and the movement of lipids within and among cellular membranes and compartments in yeast.

This article presents an overview of progress in elucidating gene–enzyme relationships, cellular localization, and regulatory mechanisms governing glycerolipid metabolism in yeast. The metabolism covered in this YeastBook chapter includes the regulation, synthesis, and turnover of phospholipids and triacylglycerol (TAG) and their precursors in the context of changing growth conditions and nutrient availability. All glycerophospholipids in yeast, including the major phospholipids, phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidylcholine (PC), are derived from the precursor lipid, phosphatidic acid (PA) (Figures 1 and 2). A major topic of this review article is the tremendous recent progress in elucidating the complex regulatory mechanisms that control the connected and coordinated pathways involved in the synthesis of glycerophospholipids and TAG, which is also derived from PA (Figures 2, 3, and 4). The regulation of glycerolipid metabolism occurs at many levels and is a major topic discussed in this article. Adding complexity to analysis of the regulatory networks connected to lipid metabolism is the fact that critical signals controlling this regulation arise during the ongoing biosynthesis and turnover of the lipids themselves and involve precursors and metabolites embedded in the metabolism.

For example, PA plays a number of signaling roles vital to the regulation of lipid metabolism in yeast (Figure 3), in addition to its function as precursor to all phospholipids and TAG (Figure 2). PI synthesis is regulated in response to its precursor, inositol, on several levels (Figures 3 and 4) and PI also serves as precursor to both phosphatidylinositol-phosphates and inositol-containing sphingolipids, both of which are implicated in a wide range of signaling and regulatory activities (Strahl and Thorner 2007; Dickson 2008, 2010), topics that will not be dealt with in detail in this YeastBook chapter. In addition, the enzymes controlling the metabolism of gycerolipids are localized to specific cellular compartments (Figure 2; Tables 1–3), while the lipids themselves are, for the most part, distributed to a much wider range of cellular compartments. Moreover, the regulation of TAG metabolism plays a major role in lipid droplet (LD) formation and depletion (Murphy and Vance 1999; Rajakumari *et al.* 2008; Kurat *et al.* 2009; Kohlwein 2010a), a topic that will also be addressed in detail in the chapter on *Lipid Droplets and Peroxisomes*. Thus, detailed knowledge of pathways, gene–enzyme relationships, and subcellular localization of enzymes and pools of lipids and metabolites involved in the synthesis and turnover of glycerolipids (Figures 2–4, Tables 1–3) is essential to the elucidation of the complex mechanisms responsible for their regulation.

Notably, in eukaryotes phospholipids play many vital roles in the biology of the cell that extend beyond lipid metabolism itself. These include roles in membrane trafficking and membrane identity (Vicinanza *et al.* 2008) and anchoring of membrane proteins (Roth *et al.* 2006; Pittet and Conzelmann 2007; Fujita and Jigami 2008), complex topics in their own right, which will be discussed only in brief in this YeastBook chapter. Phospholipids also serve as signaling molecules and as precursors of signaling molecules (Strahl

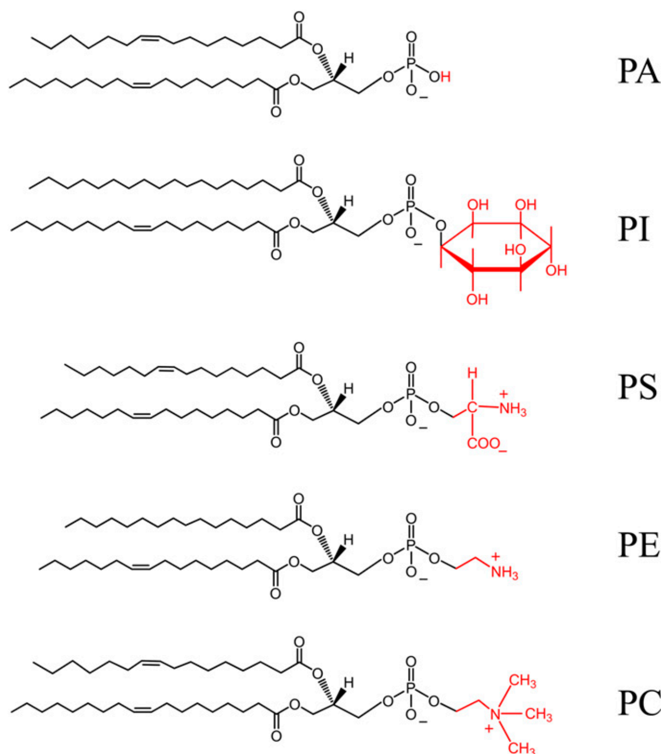

Figure 1 Phospholipid structures. The diagram shows the structures of the phospholipid PA and the major phospholipids PI, PS, PE, and PC that are derived from PA. The hydrophilic head groups (*H*, *inositol*, *serine*, *ethanolamine*, *and choline*) that are attached to the basic phospholipid structure are shown in red. The four most abundant fatty acids esterified to the glycerol-3-phosphate backbone of the phospholipids are palmitic acid, palmitoleic acid, steric acid, and oleic acid. The type and position of the fatty acyl moieties in the phospholipids are arbitrarily drawn. The relative amounts of the phospholipids as well as their fatty acyl compositions vary depending on strain (*e.g.*, mutation) and growth condition.

and Thorner 2007). Thus, the advancements in yeast glycerolipid metabolism discussed in this review article also have enormous potential to contribute critical insights into these vital roles of lipids and lipid-mediated signaling in eukaryotic cells.

Pathways of glycerolipid metabolism

Major glycerolipids of *S. cerevisiae* include the phospholipids PC, PE, PI, PS (Figure 1), phosphatidylglycerol (PG), and cardiolipin (CL) (Rattray *et al.* 1975; Henry 1982; Carman and Henry 1989; Paltauf *et al.* 1992; Guan and Wenk 2006; Ejsing *et al.* 2009). Minor phospholipids include intermediates such as PA, CDP-diacylglycerol (CDP-DAG), phosphatidylmonomethylethanolamine (PMME), phosphatidyldimethylethanolamine (PDME), the D-3, D-4, and D-5 polyphosphoinositides, and lysophospholipids (Rattray *et al.* 1975; Oshiro *et al.* 2003; Strahl and Thorner 2007). TAG and diacylglycerol (DAG) are the major neutral glycerolipids. The fatty acids that are commonly esterified to the glycerophosphate backbone of yeast glycerolipids include palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1}$), stearic acid ($C_{18:0}$), and oleic acid ($C_{18:1}$) (Rattray *et al.* 1975; Henry 1982; Bossie and Martin 1989; McDonough *et al.* 1992; Martin *et al.* 2007). The pathways for the synthesis of phospholipids and TAG are shown in Figure 2. The enzymes and transporters of glycerolipid metabolism and the genes that encode them are listed in Tables 1–3. The gene–protein relationships shown in the tables have been confirmed by the analysis of gene mutations and/or by the biochemical characterization of the enzymes and transporters (Carman and Henry 1989; Greenberg and Lopes 1996; Henry and Patton-Vogt 1998; Carman and Henry 1999; Black and Dirusso 2007; Tehlivets *et al.* 2007; Kohlwein 2010b; Carman and Han 2011).

Synthesis and turnover of phospholipids

In the *de novo* pathways (Figure 2, Table 1), all membrane phospholipids are synthesized from PA, which is derived from glycerol-3-P via lysoPA by two fatty acyl CoA-dependent reactions that are catalyzed in the endoplasmic reticulum (ER) by the *SCT1*- and *GPT2*-encoded glycerol-3-P acyltransferases and the *SLC1*- and *ALE1*-encoded lysoPA/lysophospholipid acyltransferases, respectively (Athenstaedt and Daum 1997; Athenstaedt *et al.* 1999b; Zheng and Zou 2001; Benghezal *et al.* 2007; Chen *et al.* 2007b; Jain *et al.* 2007; Riekhof *et al.* 2007b). The glycerol-3-P acyltransferase enzymes also utilize dihydroxyacetone-P as a substrate, and the product acyl dihydroxyacetone-P is converted to lysoPA by the lipid droplet (LD) and ER-associated *AYR1*-encoded reductase (Athenstaedt and Daum 2000). At this point in the pathway, PA is partitioned to CDP-DAG, catalyzed by *CDS1*-encoded CDP-DAG synthase (Carter and Kennedy 1966; Kelley and Carman 1987; Shen *et al.* 1996) or to DAG, catalyzed by *PAH1*-encoded PA phosphatase (Han *et al.* 2006) (Figure 1). CDP-DAG synthase activity has been detected in the ER and in mitochondria (Kuchler *et al.* 1986), whereas PA phosphatase is a cytosolic enzyme that must associate with membranes to catalyze the dephosphorylation of PA to produce DAG (Han *et al.* 2006; Carman and Han 2009a). CDP-DAG and DAG are used to synthesize PE and PC by two alternative routes, namely, the CDP-DAG and Kennedy pathways (Figure 2). In the CDP-DAG pathway, CDP-DAG is converted to PS by the ER localized *CHO1*-encoded PS synthase (Atkinson *et al.* 1980; Letts *et al.* 1983; Bae-Lee and Carman 1984; Kiyono *et al.* 1987; Nikawa *et al.* 1987b). Yeast has two PS decarboxylases encoded, by the *PSD1* and *PSD2* genes. Psd1, localized to the inner mitochondrial membrane, accounts for the majority of the enzymatic activity in yeast, while the minor activity, Psd2, associates with Golgi/vacuole (Clancey *et al.* 1993; Trotter *et al.* 1993, 1995; Voelker 2003). PE then undergoes three sequential methylation reactions in the ER (Gaynor and Carman 1990), the first of which is catalyzed by the *CHO2*-encoded PE methyltransferase, while the final two methylations are catalyzed by the *OPI3*-encoded phospholipid methyltransferase (Kodaki and Yamashita 1987; Summers *et al.* 1988; Kodaki and Yamashita 1989; McGraw and Henry 1989). The CDP-DAG pathway is the major route for synthesis of

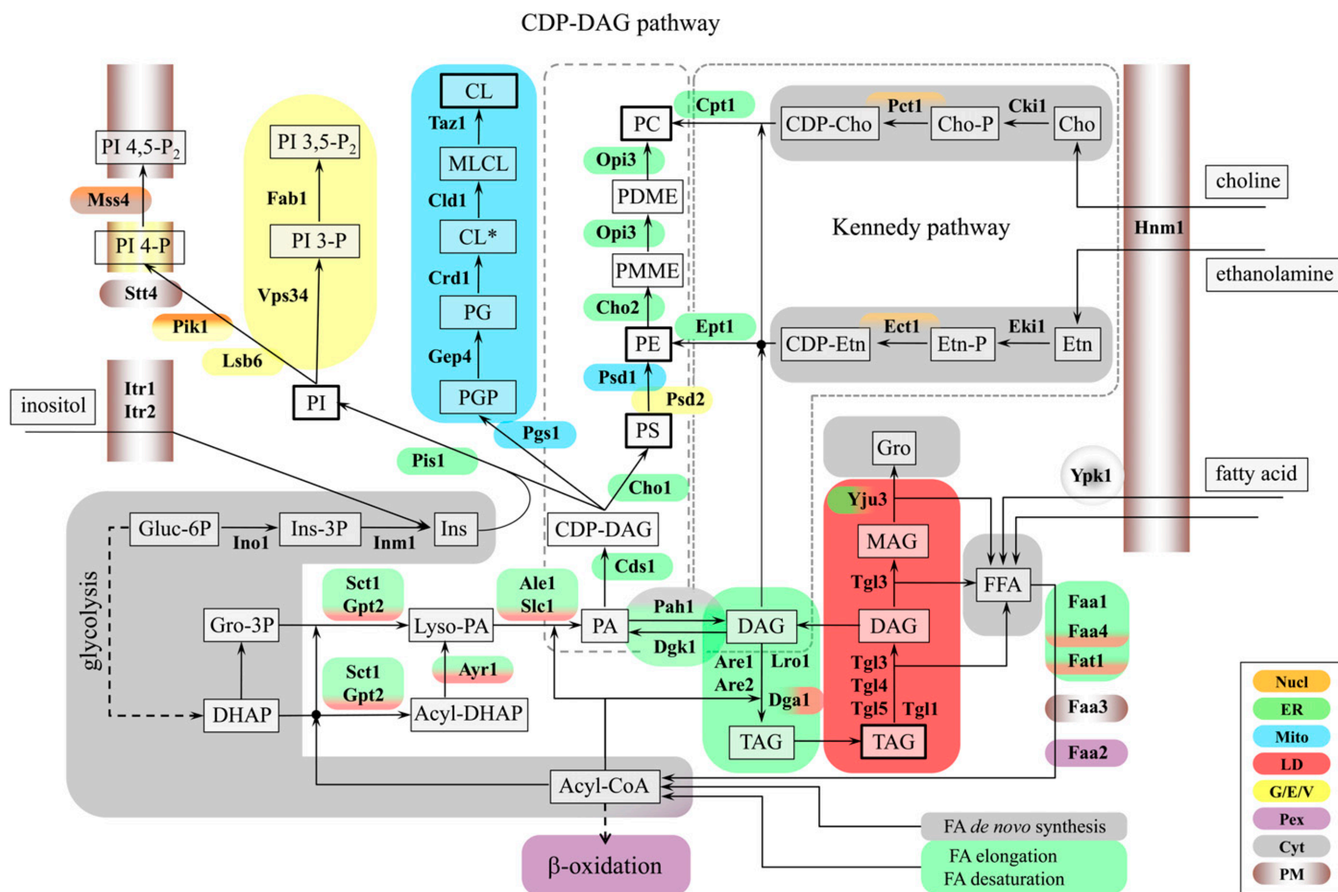

Figure 2 Pathways for the synthesis of glycerolipids and their subcellular localization. Phospholipids and TAG share DAG and PA as common precursors. In the *de novo* synthesis of phospholipids, PA serves as the immediate precursor of CDP-DAG, precursor to PI, PG, and PS. PS is decarboxylated to form PE, which undergoes three sequential methylations resulting in PC. PA also serves as a precursor for PGP, PG, and ultimately CL, which undergoes acyl-chain remodeling to the mature lipid. Alternatively, PA is dephosphorylated, producing DAG, which serves as the precursor of PE and PC in the Kennedy pathway. DAG also serves as the precursor for TAG and can be phosphorylated, regenerating PA. The names of the enzymes that are discussed in detail in this YeastBook chapter are shown adjacent to the arrows of the metabolic conversions in which they are involved and the gene–enzyme relationships are shown in Tables 1–3. Lipids and intermediates are boxed, with the most abundant lipid classes boxed in boldface type. Enzyme names are indicated in boldface type. The abbreviations used are: TAG, triacylglycerols; PI, phosphatidylinositol; PA, phosphatidic acid; CDP-DAG, CDP-diacylglycerol; DAG, diacylglycerol; MAG, monoacylglycerol; Gro, glycerol; DHAP, dihydroxyacetone phosphate, PS, phosphatidylserine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PGP phosphatidylglycerol phosphate; CL* precursor cardiolipin; MLCL, monolyso-cardiolipin; CL, mature cardiolipin; PMME, phosphatidylmonomethylethanolamine; PDME, phosphatidyl-dimethylethanolamine; PC, phosphatidylcholine; FFA, free fatty acids; Cho, choline, Etn, ethanolamine, Ins, inositol; Cho-P, choline phosphate; CDP-Cho, CDP-choline; Etn-P, ethanolamine phosphate; CDP-Etn, CDP-ethanolamine; PI 3-P, phosphatidylinositol 3-phosphate; PI 4-P, phosphatidylinositol 4-phosphate; PI 4,5-P_2, phosphatidylinositol 4,5-bisphosphate; PI 3,5-P_2, phosphatidylinositol 3,5-bisphosphate. Nucl, nucleus; ER, endoplasmic reticulum; Mito, mitochondria; LD, lipid droplets; G/E/V, Golgi, endosomes, vacuole; Pex, peroxisomes; Cyt, cytoplasma; PM, plasma membrane. CL* indicates a precursor of cardiolipin (CL) with saturated acyl-chain that undergoes deacylation/reacylation to mature CL. See text for details.

PE and PC when wild-type cells are grown in the absence of ethanolamine and choline, and *cho1*, *psd1 psd2*, and *cho2 opi3* mutants defective in this pathway have choline/ethanolamine auxotrophy phenotypes (Atkinson *et al.* 1980a Summers *et al.* 1988; McGraw and Henry 1989; Trotter and Voelker 1995; Trotter *et al.* 1995). PE and PC synthesis in mutants defective in the CDP-DAG pathway can also be supported by exogenously supplied lysoPE, lysoPC, or PC with short acyl chains, which are transported into the cell. LysoPE and lysoPC are acylated by the *ALE1*-encoded lysophospholipid acyltransferase (Jain *et al.* 2007; Tamaki *et al.* 2007; Riekhof *et al.* 2007a,b). Short chain PE and PC are remodeled with C16 and C18 acyl chains prior to incorporation into the membrane (Tanaka *et al.* 2008; Deng *et al.* 2010).

In the Kennedy pathway (Hjelmstad and Bell 1990), PE and PC are synthesized, respectively, from ethanolamine and choline (Figure 2, Table1). Exogenous ethanolamine and choline are both transported into the cell by the *HNM1*-encoded choline/ethanolamine transporter (Nikawa *et al.* 1986). The *EKI1*-encoded ethanolamine kinase (Kim *et al.* 1999) and the *CKI1*-encoded choline kinase (Hosaka *et al.* 1989) are both cytosolic enzymes, which, respectively, phosphorylate ethanolamine and choline with ATP to form ethanolamine-P and

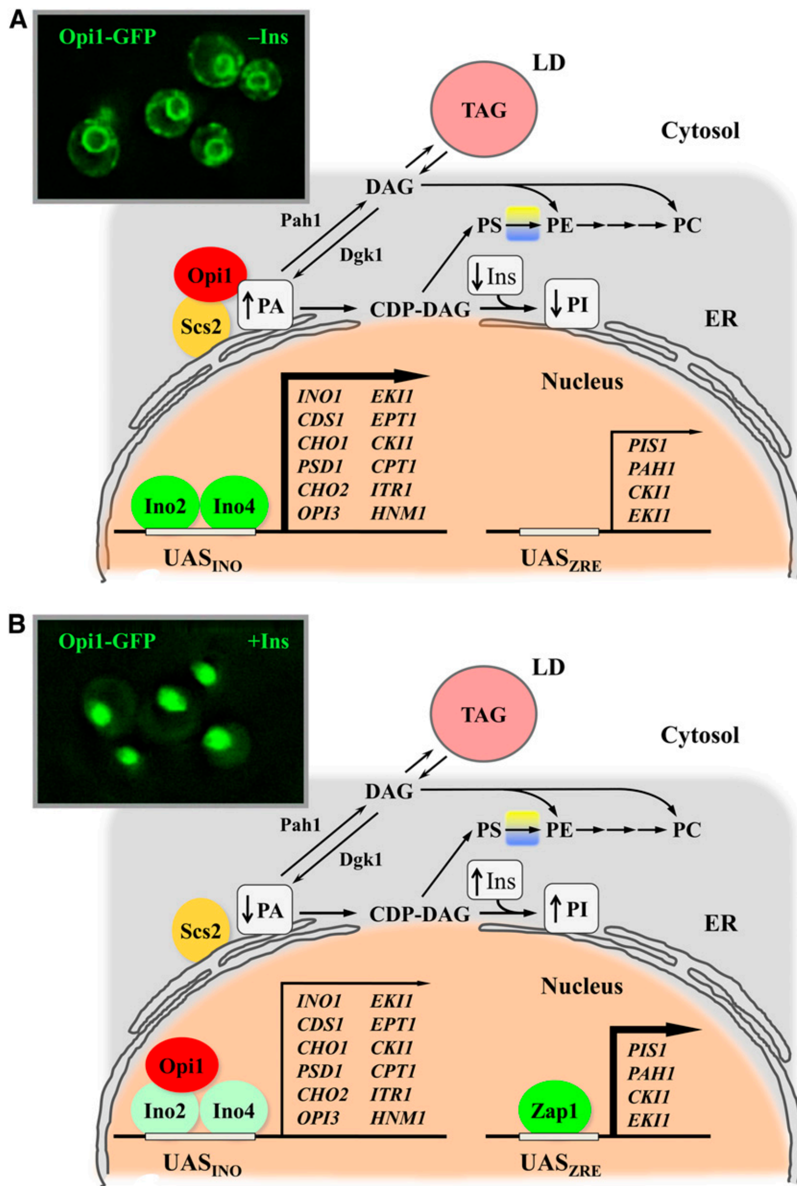

Figure 3 Model for PA-mediated regulation of phospholipid synthesis genes. (A) Growth conditions (*e.g.*, exponential phase, inositol depletion, or zinc supplementation) under which the levels of PA are relatively high, the Opi1 repressor is tethered to the nuclear/ER membrane, and UAS_{INO}-containing genes are maximally expressed (boldface arrow) by the Ino2-Ino4 activator complex. (Inset) Localization of Opi1, fused with GFP at its C-terminal end and integrated into the chromosome, being expressed under its own promoter in live cells growing logarithmically in synthetic complete medium lacking inositol (−Ins) and analyzed by fluorescence microscopy. (B) Growth conditions (*e.g.*, stationary phase, inositol supplementation, or zinc depletion) under which the levels of PA are reduced, Opi1 dissociates from the nuclear/ER membrane, and enters into the nucleus where it binds to Ino2 and attenuates (thin arrow) transcriptional activation by the Ino2–Ino4 complex. (Inset) Localization of Opi1, as described in A, except that the cells are growing logarithmically in medium containing 75 μM inositol. PA level decreases by the stimulation of PI synthesis in response to inositol (Ins) supplementation and by Zap1-mediated induction of *PIS1*, that results in an increase in PI synthesis in response to zinc depletion. The regulation in response to zinc depletion and stationary phase occurs without inositol supplementation. Pah1 and Dgk1 play important roles in controlling PA content and transcriptional regulation of UAS_{INO}-containing genes. The synthesis of TAG (which is stored in lipid droplets, LD) and phospholipids (with the exception of PE, which occurs in the mitochondria and Golgi) occurs in the ER. Fluorescence microscopy images of Opi1 localization courtesy of Yu-Fang Chang, Henry Laboratory, Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

choline-P. Ethanolamine-P may also be derived from sphingolipids by dihydrosphingosine-1-P lyase, encoded by the *DPL1* gene (Saba *et al.* 1997; Schuiki *et al.* 2010). These intermediates are then activated with CTP to form CDP-ethanolamine and CDP-choline, respectively, by the *ECT1*-encoded ethanolamine-P cytidylyltransferase (Min-Seok *et al.* 1996) and the *PCT1*-encoded choline-P cytidylyltransferase (Tsukagoshi *et al.* 1987), which are associated with the nuclear/ER membrane (Huh *et al.* 2003; Natter *et al.* 2005). Finally, the *EPT1*-encoded ethanolamine phosphotransferase (Hjelmstad and Bell 1988, 1991) and the *CPT1*-encoded choline phosphotransferase (Hjelmstad and Bell 1987, 1990), respectively, catalyze the reactions of CDP-ethanolamine and CDP-choline with DAG provided by the *PAH1*-encoded PA phosphatase to form PE and PC. Ept1 will also catalyze the CDP-choline–dependent reaction (Hjelmstad and Bell 1988). Cpt1 and Ept1 have somewhat ambiguous patterns of localization to vesicular structures, but have also been described to localize to mitochondria or ER (Huh *et al.* 2003; Natter *et al.* 2005).

The CDP-DAG and Kennedy pathways are both used by wild-type cells, regardless of whether or not ethanolamine and choline are present in the growth medium (Patton-Vogt *et al.* 1997; Henry and Patton-Vogt 1998; Kim *et al.* 1999). In the absence of exogenous choline, this Kennedy pathway precursor may be derived from hydrolysis of PC synthesized by the CDP-DAG pathway and subsequently hydrolyzed by phospholipase D (Patton-Vogt *et al.* 1997; Xie *et al.* 1998) encoded by the *SPO14* gene (Rose *et al.* 1995; Waksman *et al.* 1996). Choline for PC synthesis can also be derived from Nte1-catalyzed deacylation (Zaccheo *et al.* 2004) followed by hydrolysis by Gde1 to produce choline and glycerophosphate (Fernandez-Murray and McMaster 2005a; Fisher *et al.* 2005; Patton-Vogt 2007). The activity of a Ca^{++}-dependent phospholipase D with preference for PS

Figure 4 Regulation of phospholipid synthesis by soluble lipid precursors and metabolites. The diagram shows the major steps in the synthesis of phospholipids. The enzymes that are biochemically regulated by phospholipid precursors and products are shown. The green arrow designates the stimulation of enzyme activity, whereas the red line designates the inhibition of enzyme activity. Details on the biochemical regulation of these enzymes are discussed in the section *Water soluble precursors of phospholipids, metabolism, and regulatory roles* and in Carman and Han (2009a). See Figure 2 for abbreviations.

and PE has been detected (Mayr *et al.* 1996), but the corresponding gene encoding a PE-specific phospholipase D has yet to be identified. Thus, proof that ethanolamine derived from PE is recycled for PE synthesis via the Kennedy pathway is still lacking. Kennedy pathway mutants (*e.g.*, *cki1 eki1* and *cpt1 ept1*) defective in both the CDP-choline and CDP-ethanolamine branches can synthesize PC only by the CDP-DAG pathway (McMaster and Bell 1994; Morash *et al.* 1994; Kim *et al.* 1999). However, unlike the CDP-DAG pathway mutants, the Kennedy pathway mutants do not exhibit any auxotrophic requirements and have an essentially normal complement of phospholipids (Morash *et al.* 1994; Kim *et al.* 1999).

In the synthesis of the inositol-containing phospholipids (Figure 2, Table 1), CDP-DAG donates its phosphatidyl moiety to inositol to form PI (Paulus and Kennedy 1960; Fischl and Carman 1983) in a reaction that competes in the ER with PS synthase for their common substrate, CDP-DAG (Kelley *et al.* 1988). While *PIS1*-encoded PI synthase is essential, a strain expressing a mutant form of Pis1 with a lower affinity for inositol has been isolated as an inositol auxotroph (Nikawa and Yamashita 1984; Nikawa *et al.* 1987a). The inositol used in PI synthesis is either synthesized *de novo* (discussed below) or obtained from the growth medium via the *ITR1*- and *ITR2*-encoded inositol transporters (Table 2) (Nikawa *et al.* 1991). Once formed, PI may be converted to PI 3-P by the *VPS34*-encoded PI 3 kinase (Herman and Emr 1990; Schu *et al.* 1993) or to PI 4-P by the PI 4 kinases encoded by *LSB6* (Han G-S *et al.* 2002; Shelton *et al.* 2003), *STT4* (Yoshida *et al.* 1994a), and *PIK1* (Flanagan *et al.* 1993; Garcia-Bustos *et al.* 1994). PI 4-P may be further phosphorylated to PI 4,5-P_2 by the *MSS4*-encoded PI 4-P 5 kinase (Yoshida *et al.* 1994b), whereas PI 3-P may be phosphorylated to PI 3,5-P_2 by the *FAB1*-encoded PI 3-P 5 kinase (Yamamoto *et al.* 1995). The specific localization of these kinases and the lipids they produce play important roles in signaling, membrane identity, and membrane trafficking (Strahl and Thorner 2007). PI also serves as a precursor in the synthesis of the complex sphingolipids in yeast, which also play essential roles in signaling and membrane function (Dickson and Lester 2002; Dickson 2008), topics that are beyond the scope of this YeastBook chapter.

In the synthesis of mitochondrial-specific phospholipids (Figure 2, Table1), CDP-DAG donates its phosphatidyl moiety to glycerol-3-P to form phosphatidylglycerophosphate (PGP) in the reaction catalyzed by the *PGS1*-encoded PGP synthase (Janitor and Subik 1993; Chang *et al.* 1998a). PGP is then dephosphorylated to PG by the *GEP4*-encoded PGP phosphatase (Osman *et al.* 2010). The *CRD1*-encoded CL synthase (Jiang *et al.* 1997; Chang *et al.* 1998b; Tuller *et al.* 1998) catalyzes the reaction between PG and another molecule of CDP-DAG to generate CL. The *CLD1*-encoded cardiolipin-specific phospholipase and the *TAZ1*-encoded monolyso cardiolipin acyltransferase, involved in establishing specific unsaturated cardiolipin species, are also specifically associated with the mitochondria (Beranek *et al.* 2009; Brandner *et al.* 2005).

The enzymes that catalyze the turnover of phospholipids include both phospholipases and lipid phosphatases (Table 3). *NTE1*-encoded phospholipase B (Zaccheo *et al.* 2004; Fernandez-Murray and McMaster 2005b, 2007) is an integral ER membrane protein and removes both fatty acids from PC to produce glycerophosphocholine (GroPCho). GroPCho may be re-acylated by an uncharacterized acyltransferase to PC (Ståhlberg *et al.* 2008). The phospholipase B enzymes encoded by *PLB1*, *PLB2*, and *PLB3* catalyze the same type of reaction, but they are not specific and their localization is ambiguous. Plb1 localizes to the ER, vesicles, the plasma membrane, and the extracellular space and primarily utilizes PC and PE as substrates, whereas Plb3 is found in vesicles, vacuoles, as well as the cytosol and primarily uses PI as a substrate (Lee *et al.* 1994; Fyrst *et al.* 1999; Merkel *et al.* 1999, 2005). The GroPCho and glycerophosphoinositol (GroPIns) produced by the phospholipase B enzymes may be excreted into the growth medium and then transported back into the cell by the *GIT1*-encoded GroPCho/GroPIns transporter localized in the plasma membrane (Patton-Vogt and Henry 1998; Fisher *et al.* 2005). In turn, these molecules are hydrolyzed by a phosphodiesterase to produce the phospholipid precursor molecules choline

Table 1 Glycerolipid synthesis enzymes

Gene	Ino⁻ or Opi⁻ phenotype	Enzyme	Mol mass (kDa)	Isoelectric point	Molecules per cell[b]	Location[c]	Transmembrane domains	Phosphorylation sites[d]
SCT1 (*GAT2*)	Ino⁻	Glycerol-3-P /dihydroxyacetone-P acyltransferase	85.7	7.27	1050	ER	4	Few
GPT2 (*GAT1*)	—	Glycerol-3-P /dihydroxyacetone-P acyltransferase	83.6	10.3	3100	ER, lipid droplets	4	Several
AYR1	—	Acyl DHAP reductase	32.8	9.92	3670	ER, lipid droplets	None	None
SLC1	—	LysoPA/Acylglycerol-3-P acyltransferase	33.8	10.41	ND	ER, lipid droplets	1	None
ALE1 (*SLC4, LPT1, LCA1*)	—	LysoPA/Acylglycerol-3-P acyltransferase	72.2	10.3	ND	ER	7	Several
PSI1 (*CST26*)	—	LysoPI acyltransferase	45.5	10.15	2010	ER	4	None
TAZ1	—	LysoPC acyltransferase/ monolysoCL acyltransferase	44.2	9.38	1340	Mitochondria	None	None
***CDS1* (*CDG1*)**[a]	Opi⁻	CDP-DAG synthase	51.8	8.64	ND	ER, mitochondria	6	Few
***CHO1* (*PSS1*)**[a]	Opi⁻	PS synthase	30.8	6.23	ND	ER	2	Several
PSD1[a]	Opi⁻	PS decarboxylase	56.6	9.84	1080	Mitochondria	None	None
PSD2	—	PS decarboxylase	130	7.85	ND	Vacuole, endomembranes	None	Few
***CHO2* (*PEM1*)**[a]	Opi⁻	PE methyltransferase	101.2	8.56	1810	ER	8	Few
***OPI3* (*PEM2*)**[a]	Opi⁻	Phospholipid methyltransferase	23.1	9.6	5890	ER, mitochondria	None	None
PAH1 (*SMP2*)	—	PA phosphatase	95	4.68	3910	Cytoplasm, ER	None	Several
DGK1 (*HSD1*)	—	DAG kinase	32.8	9.48	784	ER	4	Few
EKI1[a]	—	Ethanolamine kinase	61.6	5.69	3420	Cytoplasm	None	Few
ECT1 (*MUQ1*)	—	Ethanolamine-P cytidylyltransferase	36.8	6.44	4700	Cytoplasm	None	None
EPT1[a]	—	Ethanolamine/choline phosphotransferase	44.5	6.5	ND	ER	7	None
CKI1[a]	—	Choline kinase	66.3	5.43	3930	Cytoplasm	None	Several
***PCT1* (*CCT1, BSR2*)**[a]	Opi⁻	Choline-P cytidylyltransferase	49.4	9.26	3050	Cytoplasm, nucleus	None	Several
CPT1[a]	—	Choline phosphotransferase	44.8	6.57	981	Membrane	8	None
PGS1 (*PEL1*)	—	PGP synthase	59.3	10.5	ND	Mitochondria	None	None
GEP4	—	PGP phosphatase	20.9	9.18	ND	Mitochondria	None	None
CRD1 (*CLS1*)	—	CL synthase	32	10.55	876	Mitochondria	3	None
PIS1	Ino⁻	PI synthase	24.8	8.92	3810	ER	3	None
LSB6	—	PI 4-kinase	70.2	6.68	57	Plasma membrane, vacuole membrane	None	None
STT4	Ino⁻	PI 4-kinase	214.6	7.44	846	Plasma membrane	None	Few
PIK1	—	PI 4-kinase	119.9	6.46	1600	Plasma membrane, nucleus, Golgi	None	Several
VPS34 (*END12, PEP15, VPL7, VPT29, STT8, VPS7*)	Opi⁻	PI 3-kinase	100.9	7.79	1080	Vacuole	None	None
MSS4	Ino⁻	PI 4-P 5-kinase	89.3	10.13	ND	Cytoplasm	None	Several
FAB1 (*SVL7*)	Ino⁻	PI 3-P 5-kinase	257.4	8.45	149	Vacuole	None	Several
DGA1[a]	—	Acyl-CoA diacylglycerol acyltransferase	47.7	10.39	907	ER, lipid droplets	1	Few
LRO1[a]	—	Phospholipid diacylglycerol acyltransferase	75.3	6.67	ND	ER	1	Few
***ARE1* (*SAT2*)**[a]	—	Acyl-CoA sterol acyltransferase	71.6	8.27	ND	ER	9	Several

(continued)

Table 1, *continued*

Gene	Ino⁻ or Opi⁻ phenotype	Enzyme	Mol mass (kDa)	Isoelectric point	Molecules per cell[b]	Location[c]	Transmembrane domains	Phosphorylation sites[d]
***ARE2* (*SAT1*)**[a]	—	Acyl-CoA sterol acyltransferase	74.0	7.71	279	ER	9	Several
Phospholipid synthesis regulatory proteins								
***INO2* (*DIE1, SCS1*)**[a]	Ino⁻	Transcriptional activator	34.2	6.23	784	Nucleus	None	None
INO4	Ino⁻	Transcriptional activator	17.4	10.21	521	Nucleus, cytoplasm	None	None
OPI1	Opi⁻	Transcriptional repressor	46	4.87	1280	Nuclear/ER membrane	None	Few

Much of the information in the table may be found in the *Saccharomyces* Genome Database. Ino⁻, inositol auxotrophy; Opi⁻, inositol excretion; ND, not determined.
[a] Genes containing the UAS_{INO} element and regulated by the Ino2-Ino4-Opi1 circuit. The names in parentheses are aliases.
[b] Ghaemmaghami *et al.* 2003.
[c] Habeler *et al.* 2002; Kumar *et al.* 2002; Huh *et al.* 2003; Natter *et al.* 2005.
[d] Li, X. *et al.* 2007; Bodenmiller *et al.* 2008.

and inositol, respectively (Patton-Vogt 2007). *PLC1*-encoded phospholipase C is cytosolic and specific for PI 4,5-P_2 and produces DAG and inositol 1,4,5-trisphosphate (Flick and Thorner 1993; Yoko-O *et al.* 1993), and the *PGC1*-encoded phospholipase C localizes to lipid droplets and mitochondria and is specific for PG and produces DAG and glycerol 3-P (Simockova *et al.* 2008). The *SPO14*-encoded cytosolic phospholipase D is specific for PC and produces PA and choline (Rose *et al.* 1995; Waksman *et al.* 1996). Most phospholipids undergo rapid turnover and acyl-chain remodeling, which yields the typical complex pattern of lipid molecular species found in yeast (Schneiter *et al.* 1999; Guan and Wenk 2006; Ejsing *et al.* 2009). This remodeling is governed by specific acyltransferases, such as the *PSI1*-encoded acyltransferase in the ER that is involved in stearoyl-acylation of PI (Le Guedard *et al.* 2009), or the *CLD1*-encoded cardiolipin-specific phospholipase A in mitochondria (Beranek *et al.* 2009) and the *TAZ1*-encoded acyltransferase (yeast tafazzin ortholog; Gu *et al.* 2004; Testet *et al.* 2005). Moreover, several enzymes of TAG synthesis and degradation have additional activities, suggesting that they also may play a role in phospholipid acyl-chain remodeling (Rajakumari *et al.* 2008; Kohlwein 2010b; Rajakumari and Daum 2010a,b).

There are several phosphatase enzymes that catalyze the dephosphorylation of the polyphosphoinositides. Some of these enzymes are specific and some have broad substrate specificity. The *YMR1*-encoded (Taylor *et al.* 2000) and *FIG4*-encoded (Gary *et al.* 2002) phosphatases are specific for PI 3-P and PI 3,5-P_2, respectively, whereas *INP51*-encoded (Stolz *et al.* 1998b) and *INP54*-encoded (Wiradjaja *et al.* 2001) phosphatases are specific for PI 4,5-P_2. The phosphatase encoded by *SAC1* will utilize PI 3-P, PI 4-P, and PI 3,5-P_2 (Guo *et al.* 1999), whereas the phosphatases encoded by *INP52* and *INP53* will utilize any polyphosphoinositide as a substrate (Stolz *et al.* 1998; Guo *et al.* 1999).

The *DPP1*- and *LPP1*-encoded lipid phosphate phosphatase enzymes dephosphorylate a broad spectrum of substrates that include DAG pyrophosphate (DGPP), PA, lysoPA, sphingoid-base phosphates, and isoprenoid phosphates (Toke *et al.* 1998, 1999; Faulkner *et al.* 1999). While these enzymes may utilize PA as a substrate, they are not involved in the *de novo* synthesis of phospholipids and TAG; the function of which is ascribed to the *PAH1*-encoded PA phosphatase (Han *et al.* 2006; Carman and Han 2009a). The *PHM8* gene encodes a lipid phosphatase that is specific for lysoPA and yields monoacylglycerol (MAG) and P_i (Reddy *et al.* 2008). *N*-acyl PE is a minor phospholipid species implicated in signaling processes (Merkel *et al.* 2005) and is degraded by the *FMP30*-encoded phospholipase D to *N*-acylethanolamide (NAE), which is related to endocannabinoids (Muccioli *et al.* 2009). NAE may be catabolized by Yju3, the major MAG lipase in yeast (see below).

Synthesis and turnover of TAG

The pathways for the synthesis of TAG and phospholipids share the same initial steps (Figure 2; Table 1; Kohlwein 2010b). Indeed, TAG is derived from the phospholipid, PA. The *PAH1*-encoded PA phosphatase provides DAG, which is acylated by the *DGA1*- and *LRO1*-encoded acyl-CoA–dependent and phospholipid-dependent diacylglycerol acyltransferases, respectively, to TAG (Oelkers *et al.* 2000; Oelkers *et al.* 2002; Sorger and Daum 2002; Kohlwein 2010b). The *ARE1*- and *ARE2*-encoded sterol acyltransferases utilize acyl-CoA but contribute only marginally to TAG synthesis from DAG (Yang, H. *et al.* 1996). Notably, deletion of both *DGA1* and *LRO1* genes does not result in a readily detectable growth phenotype in wild-type cells, indicating that TAG synthesis is not essential (Oelkers *et al.* 2002; Sorger and Daum 2002). Similarly, simultaneous deletion of the *ARE1* and *ARE2* genes does not impair cell growth (Yang *et al.* 1996). Even the *dga1*Δ *lro1*Δ *are1*Δ *are2*Δ quadruple mutant that lacks both TAG and steryl esters, and has no lipid droplets (LD), exhibits only a slight extension of the lag phase after recovery from quiescence (Petschnigg *et al.* 2009). The *dga1*Δ *lro1*Δ *are1*Δ *are2*Δ quadruple mutant, however, does exhibit a defect in sterol synthesis (Sorger *et al.* 2004) and is highly sensitive to unsaturated fatty acid (FA) supplementation (Garbarino

Table 2 Glycerolipid precursor enzymes and transporters

Gene	Ino⁻ or Opi⁻ phenotype	Enzyme	Mol mass (kDa)	Isoelectric point	Molecules per cell[b]	Location[c]	Transmembrane domains	Phosphorylation sites[d]
***ACC1* (*ABP2, FAS3, MTR7*)**[a]	—	Acetyl CoA carboxylase	250.4	6.22	20,200	Cytoplasm	None	Several
HFA1	—	Acetyl CoA carboxylase	241.8	8.05	396	Mitochondria	None	Few
FAS1[a]	—	Fatty acid synthase (β subunit)	228.7	5.79	91,800	Cytoplasm	None	Several
FAS2[a]	—	Fatty acid synthase (α subunit)	206.9	5.21	17,000	Cytoplasm	None	Several
ETR1(*MRF′*)	Ino⁻	2-Enoyl thioester reductase	42	9.78	1560	Mitochondria	None	None
HTD2 (*RMD12*)	—	3-Hydroxyacyl-thioester dehydratase	33	9	799	Mitochondria	None	None
MCT1	—	Malonyl-CoA:ACP transferase	40.7	6.9	1360	Mitochondria	None	None
OAR1	—	3-Oxoacyl-ACP reductase	31.2	9.3	1760	Mitochondria	None	None
ACP1	—	Acyl carrier protein	13.9	4.64	60,500	Mitochondria	None	Few
PPT2	—	Phosphopantetheine: protein transferase	19.9	8.46	486	Mitochondria	None	None
CEM1	—	β-keto-acyl synthase	47.6	8.26	1660	Mitochondria	None	None
FAA1	—	Fatty acyl CoA synthetase	77.8	7.58	7470	ER, lipid droplets	None	None
FAA2 (*FAM1*)	—	Fatty acyl CoA synthetase	83.4	7.7	358	Peroxisomes	None	None
FAA3	—	Fatty acyl CoA synthetase	77.9	9.7	6440	Plasma membrane	None	None
FAA4	—	Fatty acyl CoA synthetase	77.2	6.52	31,200	ER, lipid droplets	None	None
FAT1	—	Fatty acid transporter and fatty acyl CoA synthetase	77.1	8.47	16,900	ER, lipid droplets	3	None
OLE1 (*MDM2*)	—	Δ9 Fatty acid desaturase	58.4	9.71	ND	ER	4	None
ELO1	—	FA elongase, condensing enzyme	36.2	10.2	937	ER	5	None
FEN1 (*ELO2*)	—	FA elongase, condensing enzyme	40.0	10.35	3510	ER	7	Several
SUR4 (*ELO3*)	—	FA elongase, condensing enzyme	39.5	10.13	ND	ER	6	None
IFA38 (*YBR159w*)	—	β-keto acyl-CoA reductase	38.7	10.28	41900	ER	1	None
PHS1	—	3-Hydroxy acyl-CoA dehydratase	24.5	10.84	ND	ER	6	None
TSC13	—	Enoyl-CoA reductase	36.8	10.38	23600	ER	4	None
INO1[a] **(*APR1*)**	Ino⁻	Inositol 3-P synthase	59.6	5.77	ND	Cytoplasm	None	None
INM1	—	Inositol 3-P phosphatase	32.8	5	2440	Cytoplasm, nucleus	None	None
URA7	Ino⁻	CTP synthetase	64.7	5.93	57,600	Cytoplasm	None	Several
URA8	—	CTP synthetase	63	6.02	5370	Cytoplasm	None	None
ITR1[a]	Opi⁻	Inositol transporter	63.5	6.51	ND	Plasma membrane	12	Several
ITR2 (*HRB612*)	—	Inositol transporter	66.7	8.25	468	Plasma membrane	12	None
***HNM1* (*CTR1*)**[a]	—	Choline transporter	62	6.83	ND	Plasma membrane	12	Few
GIT1	—	GroPIns/GroPCho transporter	57.3	8.64	ND	Plasma membrane	11	None

Much of the information in the table may be found in the *Saccharomyces* Genome Database. Ino⁻, inositol auxotrophy; Opi⁻, inositol excretion; ND, not determined.
[a] Genes containing the UAS_{INO} element and are regulated by the Ino2-Ino4-Opi1 circuit, The names in parentheses are aliases.
[b] Ghaemmaghami *et al.* 2003.
[c] Habeler *et al.* 2002; Kumar *et al.* 2002; Huh *et al.* 2003; Natter *et al.* 2005.
[d] Li *et al.* 2007; Bodenmiller *et al.* 2008.

et al. 2009; Petschnigg *et al.* 2009). Strikingly, provision of exogenous unsaturated FA in the absence of TAG synthesis leads to respiration-dependent cell necrosis (Rockenfeller *et al.* 2010), challenging the dogma that lipid-induced cell death is exclusively apoptotic. The *dga1*Δ *lro1*Δ *are1*Δ *are2*Δ quadruple mutant also displays an Ino⁻ phenotype at elevated growth temperature, indicative of altered *INO1* expression and/or defective PI synthesis (Gaspar *et al.* 2011), ER stress induced by tunicamycin, which inhibits protein glycosylation and stimulates LD formation in wild-type cells. However, the *dga1*Δ *lro1*Δ *are1*Δ *are2*Δ quadruple mutant is no more sensitive to tunicamycin than wild type, indicating that LD formation is not protective against this form of stress (Fei *et al.* 2009). Dga1 and Lro1 acyltransferase-dependent TAG synthesis, on the other hand, is required for growth at semipermissive temperatures in the absence of inositol in the *sec13*^{ts}*-1* mutant, defective in COPII vesicle budding from the ER. When shifted to higher growth temperature, the *sec13*^{ts} mutant channels PA and DAG precursors from phospholipid into TAG, which apparently provides a degree of protection from the secretory stress caused by a block in membrane trafficking (Gaspar *et al.* 2008).

TAG hydrolysis (Figure 2, Table 3) clearly contributes lipid precursors that are essential to the resumption of

Table 3 Glycerolipid turnover enzymes

Gene	Ino⁻ or Opi⁻ phenotype	Enzyme	Mol mass (kDa)	Isoelectric point	Molecules per cell[a]	Location[b]	Transmembrane domains	Phosphorylation sites[c]
CLD1	—	CL specific phospholipase A2	52	10.3	ND	Mitochondria	None	None
NTE1	Ino⁻	PC specific phospholipase B	187.1	8.19	521	ER	3	Several
PLB1	—	PC/PE specific phospholipase B	71.6	4.36	ND	Plasma membrane, ER, vesicles, extracellular	None	None
PLB2	—	Nonspecific phospholipase B	75.4	4.35	623	Plasma membrane, ER, vesicles	None	None
PLB3	—	Nonspecific phospholipase B	75	4.7	ND	Cytoplasm, vacuole, vesicles	None	None
PLC1	—	PI 4,5-P_2 specific phospholipase C	110.5	9.84	ND	Cytoplasm	None	Few
PGC1	—	PG specific phospholipase C	37	8.93	3270	Mitochondria	None	None
SPO14 (*PLD1*)	—	PC specific phospholipase D	195.2	7.58	49	Cytoplasm	None	Several
SAC1 (*RSD1*)	Ino⁻	Nonspecific polyphosphoinositide phosphatase	71.1	7.75	48,000	ER, Golgi, vacuole	2	None
INP51 (*SJL1*)	—	PI 4,5-P_2 phosphatase	108.4	6.7	98	Cytoplasm	None	None
INP52 (*SJL2*)	—	Nonspecific polyphosphoinositide phosphatase	133.3	8.97	ND	Actin	None	Several
INP53 (*SJL3*, *SOP2*)	—	Nonspecific polyphosphoinositide phosphatase	124.6	7.18	1520	Actin	None	Several
INP54	—	PI 4,5-P_2 phosphatase	43.8	7.6	1200	ER	None	None
YRM1	—	PI 3-P phosphatase	91	7.19	125	Cytoplasm	None	Few
FIG4	—	PI 3,5-P_2 phosphatase	101.7	5.52	339	Vacuole	None	None
DPP1 (*ZRG1*)	—	DGPP phosphatase/nonspecific lipid phosphate phosphatase	33.5	6.42	3040	Vacuole	5	Few
LPP1	—	Nonspecific lipid phosphate phosphatase	31.5	8.25	300	Golgi	4	None
PHM8	—	LysoPA phosphatase	37.7	5.14	195	Cytoplasm, nucleus	None	None
TGL1	—	Triacylglycerol lipase, sterylester hydrolase	63.0	6.83	1470	ER, lipid droplets	None	Several
TGL2	—	Acylglycerol lipase	37.5	8.41	ND	Mitochondria	None	None
TGL3	—	Triacylglycerol lipase, lysoPA acyltransferase	73.6	8.50	3210	Lipid droplets	1	Few
TGL4	—	Triacylglycerol lipase, Ca^{++} dependent phospholipase A2, lysoPA acyltransferase	102.7	8.05	195	Lipid droplets	None	Several
TGL5	—	Triacylglycerol lipase, lysoPA acyltransferase	84.7	9.84	358	Lipid droplets	1	Several
YJU3	—	Monoacylglycerol lipase	35.6	8.5	2140	Lipid droplet, ER	None	None

Much of the information in the table may be found in the *Saccharomyces* Genome Database. The names in parentheses are aliases. Ino⁻, inositol auxotrophy; ND, not determined.
[a] Ghaemmaghami *et al.* 2003.
[b] Habeler *et al.* 2002; Kumar *et al.* 2002; Huh *et al.* 2003; Natter *et al.* 2005.
[c] Li *et al.* 2007; Bodenmiller *et al.* 2008

growth upon exit from stationary phase (Kurat *et al.* 2009; Kohlwein 2010b). TAG degradation provides substrates for the synthesis of phospholipids and sphingolipids (Rajakumari *et al.* 2010), which are required for efficient cell cycle progression upon exit from quiescence (Kurat *et al.* 2009). Degradation of TAG is catalyzed by *TGL1*-, *TGL3*-, *TGL4*-, and *TGL5*-encoded TAG lipases, all of which are localized to LD (Athenstaedt and Daum 2003, 2005; Jandrositz *et al.* 2005; Kurat *et al.* 2006; Rajakumari *et al.* 2008; Kurat *et al.* 2009; Kohlwein, 2010b). Tgl1 harbors the canonical lipase catalytic triad, consisting of serine201, aspartate369 and histidine396 (Jandrositz *et al.* 2005). In contrast, Tgl3,

Tgl4, and Tgl5 (and also Nte1, see above) are members of the patatin domain-containing family of (phospho) lipases, characterized by a catalytic diad of serine and aspartate (Kurat *et al.* 2006). The substrate specificities of the Tgl3, Tgl4, and Tgl5 TAG lipases differ. Tgl5 preferentially hydrolyses TAG molecular species harboring very long chain fatty acids (VLCFA) (Athenstaedt and Daum 2005), while Tgl3 also accepts DAG as a substrate in addition to TAG (Kurat *et al.* 2006). Tgl3, Tgl4, and Tgl5 also possess lysoPA acyltransferase activities, and Tgl4 additionally functions as a Ca^{++}-dependent phospholipase A2 and steryl ester hydrolase (Rajakumari and Daum 2010a,b). Although Tgl1 also contributes to TAG hydrolysis, its major activity is as a steryl ester hydrolase, in conjunction with the *YEH1*- and *YEH2*-encoded enzymes (Jandrositz *et al.* 2005; Koffel *et al.* 2005). *TGL2* encodes an acylglycerol lipase localized to mitochondria, but its role in TAG homeostasis has not been clarified yet (Ham *et al.* 2010). *YJU3* encodes the major monoacylglycerol (MAG) lipase in yeast (Heier *et al.* 2010), but *yju3*Δ mutants do not display any detectable growth phenotype when tested under multiple conditions (Heier *et al.* 2010).

Stationary phase cells shifted into fresh media rapidly initiate TAG and steryl ester breakdown, which leads to almost full depletion of cellular LD pools within 4–6 hr in lag phase. This initial phase of TAG breakdown is governed by the activity of *TGL3*- and *TGL4*-encoded lipases on LD and the *tgl3*Δ *tgl4*Δ mutant strain exhibits a delay in entering vegetative growth during exit from stationary phase (Kurat *et al.* 2006). Tgl4 is constitutively present on LD and activation of Tgl4 by Cdc28p-dependent phosphorylation is involved in TAG lipolysis that contributes to bud formation exiting from stasis (Kurat *et al.* 2009). Resumption of growth following stasis is also dependent on the *DGK1*-encoded DAG kinase. In comparison to wild-type cells, stationary phase *dgk1*Δ cells fail to initiate growth when *de novo* FA synthesis is impaired. The *dgk1*Δ mutant also fails to mobilize TAG under these conditions and accumulates TAG, phenotypes that are partially suppressed by the *pah1*Δ mutation or by channeling DAG into PC synthesis when choline is present (Fakas *et al.* 2011).

TAG synthesis and breakdown are also coordinated with phospholipid metabolism during logarithmic growth. Mutants with defects in TAG hydrolysis exhibit decreased synthesis of inositol containing sphingolipids and decreased PC and PI content during active growth (Rajakumari *et al.* 2010). Furthermore, mutants defective in synthesis or hydrolysis of TAG exhibit reduced capacity to restore cellular levels of PI when exogenous inositol is resupplied following an interval of inositol starvation during logarithmic growth. Under these conditions, alterations in the synthesis of inositol-containing sphingolipids are also observed in the *dga1*Δ *lro1*Δ *are1*Δ *are2*Δ strain (Gaspar *et al.* 2011).

Glycerolipid precursors

Fatty acid synthesis and regulation: The FA that are esterified to phospholipids and TAG are derived from *de novo* synthesis, the growth medium, and from lipid turnover (Tehlivets *et al.* 2007). The spectrum of FA in yeast consists mainly of C_{16} and C_{18} FA that are either saturated or monounsaturated, harboring a single double bond between carbon atoms 9 and 10 (Δ9 desaturation). Whereas *de novo* FA synthesis mostly takes place in the cytosol, all the enzymes involved in FA desaturation and elongation are associated with the ER membrane (Table 2) (Tehlivets *et al.* 2007).

Minor FA species are C_{12}, C_{14}, and very long chain FA, up to C_{26}. However, FA compositions vary substantially between strains and are also dependent on cultivation conditions (Martin *et al.* 2007). Two different FA synthesis pathways exist in yeast, as in mammalian cells (Tehlivets *et al.* 2007): the major cytosolic pathway, which resembles the "eukaryotic" type I pathway and is responsible for the bulk synthesis of all major FA, and the mitochondrial (type II pathway), which is organized similarly to the bacterial FA biosynthetic pathway (Hiltunen *et al.* 2010). The latter is believed to synthesize FA up to C_8 carbons, which serve as precursors for the synthesis of lipoic acid. Cytosolic FA synthesis is initiated by the *ACC1*-encoded acetyl-CoA carboxylase, which synthesizes malonyl-CoA from acetyl-CoA (Al-Feel *et al.* 1992; Hasslacher *et al.* 1993). This reaction requires a covalently bound biotin prosthetic group, which is attached to lysine735 in the biotin-carrier domain of Acc1 by the *BPL1*-encoded biotin:apoprotein ligase. The FA synthase complex is composed of two subunits, encoded by *FAS1* (Fas1, β-subunit) and *FAS2* (Fas2, α-subunit) and is organized as an α6/β6 heterooligomeric complex (Kuziora *et al.* 1983; Schweizer *et al.* 1986; Chirala *et al.* 1987). Fas2 carries a pantetheine prostetic group on the acyl-carrier protein (ACP) domain, which is a central element in a cycling series of reactions. In a first condensation step, malonyl-CoA is condensed with pantetheine-bound acetyl-CoA to form 3-keto-acyl-ACP, which is reduced to 3-hydroxyacyl-ACP, dehydrated to 2,3-*trans*-enoyl-ACP, and further reduced to $acyl_{(C+2)}$-ACP. Both reduction steps require NADPH and, as a result, FA synthesis is a major consumer of this dinucleotide. This multistep process results in the addition of two carbon units to the growing FA chain and cycles up to seven times, resulting in acyl-chains typically of 16 carbon atoms. The newly formed FA is transferred to coenzyme A, to yield cytosolic long chain acyl-CoA (Tehlivets *et al.* 2007). Acyl-CoA molecules are precursors for all acylation reactions involved in the synthesis of phospholipids, TAG, long chain bases, ceramide, and steryl esters, and also serve as precursors in protein acylation. The acyl-CoAs, whether derived from FA *de novo* synthesis or lipid recycling, are subject to elongation and desaturation, yielding the typical pattern of saturated and unsaturated FA species (see below). Yeast also contains an *ACB1*-encoded acyl-CoA binding protein, which plays an important regulatory function in delivering acyl-CoA into various pathways (Schjerling *et al.* 1996; Gaigg *et al.* 2001; Faergeman *et al.* 2004; Rijken *et al.* 2009). Mitochondrial FA synthesis involves enzymatic steps similar to those catalyzed by the cytosolic FAS complex. However, in contrast

to cytosolic FA synthesis, but resembling archae- and eubacterial type II fatty acid synthase, the reactions of FA synthesis in mitochondria are catalyzed by individual polypeptides, encoded by separate genes (Hiltunen *et al.* 2010).

Defects in the *FAS1* or *FAS2* genes encoding cytosolic FA synthase lead to FA auxotrophy and can typically be rescued by the addition of exogenous C_{14} or C_{16} FAs. However, defects in *ACC1* (Hasslacher *et al.* 1993) and *BPL2* are lethal and cannot be suppressed by the addition of long chain FA. The essential role of these genes, when exogenous C_{14} and C_{16} FAs are present, is most likely the requirement for malonyl-CoA in the synthesis of essential VLCFA, which are components of glycerophosphoinositol (GPI) anchors and sphingolipids (Pittet and Conzelmann 2007; Dickson 2008; Dickson 2010). The synthesis of VLCFAs is accomplished by an ER membrane-localized elongase complex that acts on long chain acyl-CoA, consisting of the condensing enzymes encoded by *ELO1*, *ELO2*, and *ELO3* (Oh *et al.* 1997), the β-ketoacyl-CoA reductase encoded by gene *YBR159w* (Han *et al.* 2002), the dehydratase encoded by *PHS1* (Denic and Weissman 2007; Kihara *et al.* 2008), and the enoyl-CoA reductase, encoded by *TSC13* (Kohlwein *et al.* 2001). Tsc13 accumulates within a specialized region of the ER in juxtaposition to the vacuole, termed the nucleus-vacuole junction (Pan *et al.* 2000) through interaction with Osh1p and Nvj1p (Kvam *et al.* 2005). The physiological role for a localization of just one component of the FA elongation complex to this subregion of the ER is currently unclear, but indicates a role for VLCFA in the formation of microautophagic vesicles involved in piecemeal autophagy of the nucleus (Kvam *et al.* 2005). Triple mutations in all three condensing enzyme genes as well as mutations in *PHS1* or *TSC13* are lethal, further supporting the notion that VLCFAs are essential in yeast. Yeast also harbors a single FA Δ9-desaturase encoded by *OLE1*, which localizes to the ER (Stukey *et al.* 1989) and mutants defective in Ole1 require exogenous $C_{16:1}$ or $C_{18:1}$ FA for growth. Since FA desaturation is an oxygen-dependent process (see below), growth of yeast under anaerobic conditions also requires the supplementation of these unsaturated FA.

In the absence of *de novo* FA synthesis or desaturation, as in *fas1*, *fas2*, or *ole1* mutants, and in wild-type cells under anaerobic conditions, cellular growth is dependent on an exogenous supply of FA. Under these conditions, the activity of acyl-CoA synthetases (Table 2), encoded by *FAA1*, *FAA2*, *FAA3*, *FAA4*, and *FAT1* (Duronio *et al.* 1992; Harington *et al.* 1994; Johnson *et al.* 1994a,b; Watkins *et al.* 1998; Choi and Martin 1999; Black and Dirusso 2007) is also required. The acyl-CoA synthetases activate free FA with coenzyme A in an ATP-dependent reaction, and are also believed to be required for FA uptake into yeast cells (Faergeman *et al.* 2001; Black and Dirusso 2007; Obermeyer *et al.* 2007). Acyl-CoA synthetases differ in their substrate specificity and subcellular localization and are present in the ER membrane, plasma membrane, peroxisome, and LD (Natter *et al.* 2005; Black and Dirusso 2007). The acyl-CoA synthetase Faa2 and the enzymes for FA β-oxidation, a cyclic series of reactions breaking down FA into acetyl-CoA and generating $FADH_2$ and NADH, are localized to the peroxisomes (planned YeastBook chapter "Lipid particles and peroxisomes" by Kohlwein and van der Klei). FA uptake into yeast cells is also mediated by an endocytotic mechanism that requires the activity of Ypk1, the yeast ortholog of the human serum- and glucocorticoid-induced kinase (Jacquier and Schneiter 2010). In the absence of acyl-CoA synthetases, FA released by lipid turnover (see above) cannot be activated and utilized for lipid synthesis. For this reason, mutants lacking these activities excrete FA (Scharnewski *et al.* 2008).

FA synthesis is regulated at multiple levels (Tehlivets *et al.* 2007). *ACC1*, *FAS1*, and *FAS2* expression is under control of an UAS_{INO} element and coregulated with genes involved in phospholipid synthesis (Chirala 1992; Schuller *et al.* 1992) (see below). *ACC1* expression is also regulated by the SAGA protein complex and TFIID (Huisinga and Pugh 2004) and may therefore depend on histone acetylation. *ACC1* expression was also found to fluctuate during the cell division cycle, with a peak of expression in early G1 phase (Cho *et al.* 1998). Compensatory changes in *ACC1* expression in mutants defective in Acc1 activity indicate an autoregulatory loop (Shirra *et al.* 2001). Expression of the *FAS1* and *FAS2* genes is regulated by the transcription factors Gcr1, Reb1, Rap1, and Abf1 (Schuller *et al.* 1994; Greenberg and Lopes 1996) and repressed by long chain FA (Chirala 1992). *FAS1* is also regulated by the SAGA complex and TFIID, and both genes show cell cycle-dependent expression, with a peak level at M/G1 (Spellman *et al.* 1998; Huisinga and Pugh 2004).

Acc1 enzyme activity is greatly elevated in the *snf1*Δ mutant and the data are consistent with Acc1 being a substrate of AMP-activated protein kinase, encoded by the *SNF1* (Woods *et al.* 1994; Shirra *et al.* 2001). Snf1 also regulates chromatin by phosphorylating histones among other proteins and, therefore, has multiple effects on expression of genes, including *ACC1*, *FAS1*, and *FAS2*. Notably, *FAS1* and *FAS2* promoter sequences differ, and the stoichiometry of the FAS complex is established both by the level of *FAS1* gene expression and Fas1 protein abundance (Schuller *et al.* 1992; Wenz *et al.* 2001; Tehlivets *et al.* 2007). Excess of either protein is rapidly eliminated by degradation via vacuolar proteases (Fas1) or ubiquitination and proteasomal digestion (Fas2), respectively (Peng *et al.* 2003).

Knowledge about the regulation of FA elongation leading to VLCFA synthesis is limited. Microarray experiments have shown that *ELO1* expression is upregulated in the presence of myristic acid ($C_{14:0}$), consistent with the preference of the Elo1 protein for this FA (Toke and Martin 1996). Expression of *ELO1*, *ELO2*, and *ELO3* genes also responds to stationary phase, nitrogen limitation, glycosylation defects, or α-factor treatment, indicating a regulatory cross-talk between nutritional state and cell proliferation to VLCFA synthesis (Gasch *et al.* 2000).

Ole1 is a nonheme iron-containing integral ER membrane protein (Table 2), which harbors an intrinsic cytochrome b_5

domain as an electron carrier (Martin *et al.* 2007). To subtract two electrons and two protons from the saturated acyl-chain and transfer them to oxygen, Ole1 activity is complemented by the activity of an NADH cytochrome b_5 reductase (dehydrogenase) that is also localized to the ER. Desaturation of FA by Ole1 is a highly regulated, oxygen-requiring process (Martin *et al.* 2007), which is discussed in detail in the section below on cell biology of lipids.

Water soluble precursors of phospholipids, metabolism, and regulatory roles: A number of water-soluble molecules used in phospholipid synthesis, including inositol, choline, ethanolamine, serine, CTP, and S-adenosyl methionine (AdoMet) and the enzymes involved in these reactions are largely cytosolic (Table 2). However, choline and ethanolamine are produced in yeast in the context of ongoing synthesis and turnover of PE and PC in the membranes as discussed above. Considerable attention has been paid to inositol and CTP, which have major regulatory roles in phospholipid metabolism. The effects of inositol on transcriptional regulation UAS_{INO}-containing phospholipid biosynthetic genes controlled by the Opi1 repressor are shown in Figure 3 and will be discussed in a subsequent section. The regulatory effects of the soluble metabolites inositol, CTP, and S-adenosyl homocysteine (AdoHcy) on enzyme activity and metabolic flux in the CDP-DAG pathway (Figure 4) are discussed here.

Inositol is the precursor to PI (Figure 2), which is essential to the synthesis of polyphosphoinositides (Strahl and Thorner 2007), sphingolipids (Cowart and Obeid 2007; Dickson 2008, 2010), and GPI anchors (Pittet and Conzelmann 2007). Inositol is derived from glucose-6-P via the reactions catalyzed by the cytosolic *INO1*-encoded inositol-3-P synthase (Donahue and Henry 1981; Klig and Henry 1984; Dean-Johnson and Henry 1989) and the *INM1*-encoded inositol-3-P phosphatase (Murray and Greenberg 2000). Exogenously supplied inositol stimulates PI synthase and inhibits PS synthase activity, alleviating the competition with PI synthase for their common substrate, CDP-DAG (Figure 4). The presence of exogenous inositol leads to increased PI synthesis and reduced levels of both CDP-DAG and its precursor PA in wild-type cells (Kelley *et al.* 1988; Loewen *et al.* 2004). TAG, which is derived from PA by the action of Pah1 (Figure 2), is decreased in the presence of inositol and increases in its absence (Gaspar *et al.* 2006, 2011). In addition, the levels of the complex sphingolipids, which are derived from PI, are reduced when cells are deprived of inositol and increase when inositol is supplied (Becker and Lester 1977; Hanson and Lester 1980; Jesch *et al.* 2010). The changes that occur in phospholipid synthesis and composition in response to exogenous serine (Kelley *et al.* 1988) and choline (Gaspar *et al.* 2006) are much less dramatic than the effects of exogenous inositol. However, when inositol is present in the growth medium, serine (Homann *et al.*1987), ethanolamine, and choline (Poole *et al.* 1986) result in the reduction of the activities of PS synthase and CDP-DAG synthase at the transcriptional level (see below). Exogenous choline also results in a dramatic change in the rate and mechanism of PC turnover, leading to a switch from a phospholipase D to a phospholipase B-mediated route (Dowd *et al.* 2001). The phospholipase responsible for turnover of PC under these conditions is Nte1 (Zaccheo *et al.* 2004).

CTP is derived from UTP by the cytosolic *URA7*- and *URA8*-encoded CTP synthetase enzymes (Ozier-Kalogeropoulos *et al.* 1991, 1994).

The nucleotide CTP plays a critical role in phospholipid synthesis as the direct precursor of the high-energy intermediates CDP-DAG, CDP-choline, and CDP-ethanolamine that are used in the CDP-DAG and Kennedy pathways (Figure 2) (Chang and Carman 2008). It is also used as the phosphate donor for the synthesis of PA by DAG kinase (Han *et al.* 2008b). CTP synthetase (Ozier-Kalogeropoulos *et al.* 1991, 1994) that produces CTP is allosterically inhibited by the product (Yang *et al.* 1994; Nadkarni *et al.* 1995), and this regulation ultimately determines the intracellular concentration of CTP (Yang *et al.* 1994; McDonough *et al.* 1995). CTP inhibits the CTP synthetase activity by increasing the positive cooperativity of the enzyme for UTP, and at the same time by decreasing the affinity for UTP (Yang *et al.* 1994; Nadkarni *et al.* 1995). However, CTP synthetases containing an E161K mutation are less sensitive to CTP product inhibition (Ostrander *et al.* 1998), and cells expressing the mutant enzymes exhibit a 6- to 15-fold increase in the CTP level (Ostrander *et al.* 1998). They also show alterations in the synthesis of membrane phospholipids, which include a decrease in the amounts of PS and increases in the amounts of PC, PE, and PA (Ostrander *et al.* 1998). The decrease in the amount of PS results from direct inhibition of PS synthase activity by CTP (McDonough *et al.* 1995), and this inhibition favors the synthesis of phospholipids by the Kennedy pathway (Figure 4). The increase in PC synthesis is ascribed to a higher utilization of the CDP-choline branch of the Kennedy pathway due to the stimulation of choline P cytidylyltransferase activity (McDonough *et al.* 1995; Ostrander *et al.* 1998) by increased substrate availability of CTP (McDonough *et al.* 1995; Kent and Carman 1999). Likewise, the increase in PE synthesis could be attributed to stimulation of ethanolamine-P cytidylyltransferase activity. The increase in PA content may result from the stimulation of DAG kinase activity by increased availability of its substrate CTP (Han *et al.* 2008b). CTP also inhibits the activity of Pah1 (Wu and Carman 1994), another factor that contributes to an elevation of PA content. The cells expressing the E161K mutant CTP synthase excrete inositol (Ostrander *et al.* 1998), a characteristic phenotype that typifies the misregulation of UAS_{INO}-containing phospholipid synthesis genes (see below) in cells that accumulate an excess of PA (Carman and Henry 2007). It is unclear whether UAS_{INO}-containing genes in the CDP-DAG and Kennedy pathways are derepressed in CTP overproducing cells, but the overriding regulation that governs the utilization of the two pathways appears to be biochemical in nature.

AdoHcy is a product of the AdoMet-dependent methylation reactions that are catalyzed by Cho2 and Opi3 in the CDP-DAG pathway (Figure 2). *SAH1*-encoded AdoHcy hydrolase (Malanovic *et al.* 2008) requires NADH for the hydrolytic breakdown of AdoHcy to adenosine and homocysteine (Takata *et al.* 2002). AdoHcy (Figure 4) is a competitive inhibitor of the phospholipid methyltransferase enzymes (Gaynor and Carman 1990). Thus, down-regulation of the AdoHcy hydrolase causes the accumulation of AdoHcy and the inhibition of PC synthesis, which leads to an increase in PA content and consequently, as described below, to the derepression of UAS_{INO}-containing genes (Malanovic *et al.* 2008). Although the effects of AdoHcy on phospholipid composition have not been addressed, its accumulation causes an increase in TAG synthesis and LD content (Malanovic *et al.* 2008), further underscoring the metabolic interconnection between phospholipid and TAG homeostasis discussed above.

Cell Biology of Lipids

In yeast, most subcellular membranes are composed of similar glycerophospholipid classes but the quantitative phospholipid composition of subcellular organelles differs substantially (Schneiter *et al.* 1999). Moreover, as described above, multiple cellular organelles and compartments contribute to glycerolipid metabolism (Figure 2, Tables 1–3). While multiple compartments contribute to lipid synthesis in yeast (Zinser *et al.* 1991; Natter *et al.* 2005), most reactions are confined to single membrane compartments. Thus, extensive regulated flux of lipids, across and among individual membranes and organelles, is required to enable the cell to adjust lipid composition in specific compartments under changing growth conditions. A number of mechanisms involved in these complex interactions have been identified.

For example, a set of membrane-bound flippases are known to govern transmembrane movement of phospholipids (Pomorski *et al.* 2004; Holthuis and Levine 2005). Intermembrane transfer of lipids is facilitated by a family of proteins, termed oxysterol-binding protein (OSBP) related proteins (ORP), of which seven members, Osh1–7, with overlapping functions, exist in yeast (Beh and Rine 2004). Some of the yeast Osh proteins have been localized to membrane contacts sites (Levine and Loewen 2006; Raychaudhuri and Prinz 2010). While none of these genes individually are essential, simultaneous deletion of all seven *OSH* genes is lethal and conditional alleles, following a shift to restrictive conditions, result in pleiotropic effects on vesicular trafficking and phospholipid and sterol composition of membranes, as do combinations of individual deletions (Beh and Rine 2004; Fei *et al.* 2008). The six members of the Sec14 superfamily constitute another major class of proteins involved in sensing and regulating membrane lipid composition (Griac 2007; Bankaitis *et al.* 2010). The five Sec14 homologs (Sfh1–5) share 28–76% similarity to Sec14 (Griac 2007) and localize to multiple subcellular organelles (Schnabl *et al.* 2003). Sec14, which performs an essential function at the Golgi, was originally identified as a PI/PC transfer protein, but its role in establishing phospholipid homeostasis is complex (Bankaitis *et al.* 2010).

In some instances, movement of lipids between membrane compartments is required to carry out a sequence of reactions. For example, PS is synthesized in the ER, but the major PS decarboxylase, Psd1, is located in the mitochondria (Figure 2, Table 1). Subsequently, the PE produced by Psd1 must be transported back to the ER to undergo methylation leading to PC. PS is transported into the mitochondria by an ubiquitin-regulated process that is insensitive to disruption of vesicular trafficking and involves specialized regions of apposition of mitochondria and ER known as the mitochondria-associated ER or MAM (Clancey *et al.* 1993; Trotter *et al.* 1993; Trotter and Voelker 1995; Achleitner *et al.* 1999; Voelker 2003; Osman *et al.* 2011). An ER–mitochondria tethering complex potentially involved in movement of phospholipids between the ER and mitochondria has been described (Kornmann *et al.* 2009).

The complex relationship between phospholipid metabolism in the ER and the synthesis and breakdown of cytosolic LD represents another example of the intricate interaction among cellular compartments that occurs in the course of lipid metabolism. LD are not merely storage depots for TAG and steryl esters. They are metabolically active; harboring multiple enzymes involved in lipid metabolism (Athenstaedt *et al.* 1999a; Rajakumari *et al.* 2008; Goodmann 2009; Kohlwein 2010b) (See also chapter: *Lipid Droplets and Peroxisomes*). Interaction of LD with the ER, mitochondria, and peroxisomes has been reported (Goodmann 2009). Pah1 clearly plays a critical role in TAG synthesis, LD formation, and the balance between nuclear/ER membrane proliferation and LD formation (Carman and Han 2009a; Adeyo *et al.* 2011). Several enzymes involved in TAG synthesis show a dual localization to the ER and LD (Figure 2, Table 1), including acyl-DHAP reductase (Ayr1), glycerol-3-P acyltransferase (Gpt2), and acyl-CoA–dependent DAG acyltransferase (Dga1). Formation of LD is believed to occur between the leaflets of the ER membrane, but alternative models such as vesicular trafficking have also been suggested (Thiele and Spandl 2008; Farese and Walther 2009; Guo *et al.* 2009). The close interaction of LD with mitochondria and peroxisomes also underscores the important role of LD in metabolism (Goodmann 2009). TAG levels fluctuate drastically during cellular growth and division, and multiple conditions contribute to the level of TAG storage in LD in stationary phase cells (Kurat *et al.* 2006; Czabany *et al.* 2007; Daum *et al.* 2007; Rajakumari *et al.* 2008; Zanghellini *et al.* 2008). As cells exit stationary phase, TAG hydrolysis supplies precursors for membrane lipid synthesis. As active cellular growth progresses, *de novo* FA synthesis increases, satisfying cellular requirements for lipid synthesis but even while TAG turnover is still in progress, *de novo* formation of TAG and LD is initiated (Kurat *et al.* 2006; Zanghellini *et al.* 2008). Methods for imaging-based screening of mutant libraries have been designed to identify factors involved in LD content, morphology, and mobilization (Wolinski

and Kohlwein 2008; Wolinski *et al.* 2009) and screens using such methods have identified >200 genes that potentially influence these processes (Szymanski *et al.* 2007; Fei *et al.* 2008). However, there is surprisingly little overlap between the mutants identified in the published screens, indicating that current screens are far from being saturated.

Roles of lipids in organelle function and morphology

Due to the ubiquitous presence of most phospholipids in all subcellular membranes it can be difficult to assess specific phospholipid functions, in particular organelles. However, quite specific functions have been attributed to several lipids in the mitochondrion. For example, PE synthesized in the mitochondria by Psd1 plays an important role in stabilizing mitochondrial protein complexes (Birner *et al.* 2003; Osman *et al.* 2009a). Consequently, the *psd1*Δ mutant is nonviable in conjunction with defects in components of the prohibitin complex, which functions as a protein and lipid scaffold and ensures the integrity and functionality of the mitochondrial inner membrane (Osman *et al.* 2009b; Potting *et al.* 2010; van Gestel *et al.* 2010; Osman *et al.* 2011). In another example, CL, which is present only in the mitochondrion (Li, G. *et al.* 2007), plays an important role in mitochondrial genome stabilization and higher order organization of components of the mitochondrial respiratory chain (Koshkin and Greenberg 2002; Zhang *et al.* 2003; Zhong *et al.* 2004; Mileykovskaya *et al.* 2005; Joshi *et al.* 2009). However, CL function reaches beyond the mitochondria, indirectly regulating morphology and acidification of the vacuole through the retrograde (*RTG2*) signaling pathway and the *NHX1*-encoded vacuolar Na^+/H^+ exchanger (Chen *et al.* 2008). Lack of CL in *crd1*Δ mutants is in part compensated by an upregulation of the PE content, which is dependent on the mitochondrial synthesis through Psd1 (Gohil *et al.* 2005). Defects in Crd1 are also, in part, compensated by an increase in the precursor lipid PGP. Accordingly, mutation of the *PGS1* gene encoding PGP synthase leads to more pronounced mitochondrial defects and a petite negative phenotype (Janitor *et al.* 1996; Chang *et al.* 1998a).

Alterations in organelle morphology and/or function observed in response to changes in lipid composition due to mutation or pharmacological inhibition of enzymes (Schneiter and Kohlwein 1997) provide tantalizing clues as to the roles of specific lipids. Notably, the *pah1*Δ mutant exhibits the expansion of the nuclear/ER membrane and upregulation of phospholipid biosynthesis genes (Santos-Rosa *et al.* 2005). As discussed previously, dephosphorylation of PA by Pah1 provides DAG for the synthesis of TAG, as well as PE and PC in the Kennedy pathway. Consequently, the *pah1*Δ mutant has elevated PA levels, which result in upregulation of phospholipid biosynthesis (see below) and membrane proliferation (Han *et al.* 2005; Carman and Henry 2007; Carman and Han 2009b). A number of mutants defective in FA metabolism also exhibit morphological abnormalities. For example, conditional mutants defective in FA desaturation (*ole1*/*mdm2*) show defective mitochondrial morphology and distribution upon cell division (Stewart and Yaffe 1991; Tatzer *et al.* 2002). The finding that several dozen proteins species are required to sustain cell viability in the presence of unsaturated FA suggests an enormously complex network of processes and interactions to maintain membrane homeostasis and function (Lockshon *et al.* 2007). In another example, a conditional *acc1* mutant defective in FA *de novo* synthesis has impaired morphology of the vacuole due to reduced acylation of Vac8 that is involved in stabilizing the vacuolar membrane and vacuole inheritance (Schneiter *et al.* 2000). Mutants defective in FA elongation show similar fragmentation of the vacuole due to impaired synthesis of sphingolipids, which are important for the stability of the vacuolar ATPase complex (Chung *et al.* 2003).

Due to their important roles in protein modification, myristic and palmitic acid affect the membrane association of numerous proteins and consequently influence signaling, membrane function and organelle morphology (Dietrich and Ungermann 2004). *NMT1* encodes the essential N-myristoyl transferase that attaches coenzyme A-activated myristic acid (C_{14}-CoA) to a glycine residue close to the N-terminus of target proteins, resulting in cleavage of the peptide bond and *N*-terminal glycine acylation (Towler *et al.* 1987). Although the myristoyl residue is shorter than the typical C_{16} or C_{18} chain length found in membrane phospholipids and, therefore may not fully interdigitate into a membrane leaflet, it serves an essential function in promoting membrane association of proteins (Duronio *et al.* 1989). Palmitoylation of internal cysteine residues within the peptide chain plays an important role in post-translational modification of some 50 proteins in yeast (Roth *et al.* 2006), modulating membrane association, folding and activity. Protein palmitoylation is catalyzed by seven members of the "Asp-His-His-Cys-cystein-rich" (DHHC-CRD) domain family of proteins, and includes the Erf2/Shr5 complex, Akr1, Akr2, Pfa3, Pfa4, Pfa5, and Swf1. Among the substrates for palmitoylation are Vac8, SNAREs, and Ras2, indicating important regulatory functions for this FA modification of proteins in multiple pathways involved in membrane trafficking and signaling.

Mechanisms of compartmentalization and localization of enzymes of lipid metabolism

Many enzymes involved in glycerolipid metabolism have transmembrane domains (Tables 1–3), which anchor the proteins in their specific membrane environment and promote access to hydrophobic lipid substrates. However, several membrane-associated enzymes lack defined transmembrane spanning domains and membrane interaction is mediated, perhaps in a regulated manner, with specific membrane-resident anchor proteins. For example, *PIK1*-encoded PI 4-kinase binds to the membrane through Frq1 and the *VPS34*-encoded PI 3-kinase binds through Vps15 (Strahl and Thorner 2007). In addition, enzymes may bind to the membrane through specific interaction with lipids in the membrane, such as PI 3-P, PI 4-P, PI 3,5-P_2 and PI 4,5-P_2 through

PH, PX, FYVE or ENTH domains (Strahl and Thorner 2007). A number of enzymes involved in lipid biosynthesis utilize water soluble lipid precursors, including the choline and ethanolamine kinases and the enzymes of the FAS complex, involved in long chain FA synthesis, and are localized to the cytosol. Acyl-CoA, generated by the FAS complex, has a highly amphiphilic structure and easily associates with membrane surfaces, facilitating the subsequent incorporation of the acyl chains into lipids by membrane-bound enzymes. However, Pah1 is an example of an enzyme that is associated with both cytosolic and membrane compartments. PA, the substrate for Pah1, is present in the membrane, but the largest pool of Pah1 is cytosolic, highly phosphorylated and inactive. Pah1 must be dephosphorylated by the Nem1-Spo7 complex in order to be functional *in vivo* (Santos-Rosa *et al.* 2005; Han *et al.* 2006; Carman and Han 2009a; Choi *et al.* 2011). Dephosphorylation of Pah1 by the Nem1-Spo7 complex leads to its membrane association and its activation (Choi *et al.* 2011), leading to the production of DAG and TAG and the lowering of PA levels, which in turn affects regulation of phospholipid biosynthetic genes (see below) (Carman and Henry 2007; Carman and Han 2009b).

Regulation of lipid metabolism in response to membrane function

Several regulatory circuits have been uncovered that govern, by different mechanisms, the cross-talk between membrane function and lipid synthesis. Proteins involved in this regulation may sense specific changes in lipid composition, membrane charge, fluidity, or curvature. One such mechanism controls the expression of the fatty acid desaturase, Ole1, by the ER membrane-bound transcription factors Mga2 and Spt23, which are cleaved and released from the membrane in response to changes in membrane fluidity or permeability (Hoppe *et al.* 2000). Both proteins are synthesized as larger, ER membrane-bound proteins that are ubiquitinated and cleaved by the activity of the 26S proteasome under low oxygen conditions, in conjunction with the ubiquitin-selective chaperone *CDC48*$^{UFD1/NPL4}$. This cleavage detaches the soluble fragments from their membrane anchors, and allows them to enter the nucleus to control transcription of *OLE1* and several other genes through the low oxygen response (LORE) promoter element. Proteasomal cleavage of Mga2 and Spt23 requires the essential E3 ubiquitin ligase, Rsp5, and lack of this activity results in a cellular requirement for unsaturated FA. Cleavage of Spt23 and Mga2 in the ER is believed to be regulated by the membrane environment, adjusting membrane lipid composition through the control of FA desaturation. Under normoxic conditions, *OLE1* expression is activated by the oxygen-responsive transcription factor Hap1. Addition of unsaturated FA, including linoleic acid ($C_{18:2}$) or arachidonic acid ($C_{20:4}$), which are normally not present in yeast, results in a drastic and rapid reduction of *OLE1* mRNA expression and stability. However, low oxygen tension leads to a massive induction of *OLE1* expression through the LORE element present in its promoter. This induction is believed to increase efficiency of FA desaturation to support cellular growth under oxygen limiting (hypoxic) conditions (Martin *et al.* 2007; see also the chapter on Lipid Droplets and Peroxisomes for more discussion of fatty acid metabolism and regulation). Mga2 (but not Spt23) was also shown to be responsible for the rapid, but transient, up-regulation of *OLE1* and other LORE-containing genes observed when inositol is added to cultures of wild-type yeast growing logarithmically in the absence of inositol (Jesch *et al.* 2006). Regulation of the phospholipid biosynthetic genes containing the UAS_{INO} promoter element also occurs in response to changes in the lipid composition in the ER membrane, through the binding of the Opi1 repressor to PA (see below). The ER is also the locus of complex regulatory cross-talk involving membrane expansion, the secretory pathway, the unfolded protein response (UPR) pathway, and phospholipid metabolism (Cox *et al.* 1997; Travers *et al.* 2000; Block-Alper *et al.* 2002; Chang *et al.* 2002, 2004; Hyde *et al.* 2002; Brickner and Walter 2004; Jesch *et al.* 2006; Gaspar *et al.* 2008; Schuck *et al.* 2009).

Another example of regulation of lipid metabolism in yeast in response to changing membrane conditions was discovered in the course of analysis of a *cho2*Δ *opi3*Δ mutant strain completely defective in PC formation via the PE methylation pathway (Boumann *et al.* 2006). When this mutant is deprived of choline, PC levels decline and as this process progresses, concomitant changes in acyl-chain distribution occur in PC and other phospholipids, especially PE. However, similar changes were not observed in the neutral lipid fraction. The shortening and increased saturation of PE acyl chains decreases the bilayer forming potential of PE and Boumann *et al.* (2006) suggest that phospholipid remodeling under these conditions may provide a mechanism for maintaining intrinsic membrane curvature. The nature of such a mechanism has not yet been defined, but yeast has a number of proteins harboring a BAR domain, including Rvs161 and Rvs167, which bind to liposomes in a curvature-dependent manner and promote tubule formation *in vitro*. *In vivo* studies in yeast indicate inappropriate regulation of phosphoinositide and sphingolipid metabolism impinges on Rsv161 and Rsv167 function (Ren *et al.* 2006; Youn *et al.* 2010). The Rim101 pathway, which is involved in regulating cellular pH in response to alkaline conditions and cell wall biogenesis, also appears to be involved in sensing membrane curvature (Mattiazzi *et al.* 2010).

Genetic Regulation of Glycerolipid Synthesis

In wild-type cells, both glycerolipid composition and the expression of lipid biosynthetic genes are influenced by a wide variety of growth conditions, including growth phase, temperature, pH, and the availability of nutrients such as carbon, nitrogen, phosphate, zinc, and lipid precursors. Transcript levels of certain lipid biosynthetic genes

in yeast are also regulated by message stability. A number of viable mutants defective in genes encoding lipid metabolic enzymes, transporters, and regulatory factors exhibit changes in lipid composition. These complex and interrelated topics have been the subjects of numerous reports and reviews (Henry 1982; Carman and Henry 1989; Paltauf *et al.* 1992; Henry and Patton-Vogt 1998; Carman and Henry 1999; Gardocki *et al.* 2005; Jesch and Henry 2005b; Santos-Rosa *et al.* 2005; Carman and Henry 2007; Chen *et al.* 2007a; Daum *et al.* 2007; Gaspar *et al.* 2007; Li, G. *et al.* 2007; Patton-Vogt 2007; Gaspar *et al.* 2008; Schuiki *et al.* 2010; Young *et al.* 2010; Carman and Han 2011), as discussed below with a focus on the transcriptional regulation of lipid biosynthetic genes in response to PA.

Regulation of transcript abundance by mRNA degradation

The transcript abundance of some glycerolipid metabolic genes is regulated at the level of mRNA degradation. Genes that exhibit this level of regulation include *CHO1* (Choi and Carman 2007) and *OLE1* (Gonzalez and Martin 1996; Vemula *et al.* 2003; Martin *et al.* 2007). In wild-type cells, *CHO1* mRNA is moderately stable with a half-life of 12 min when compared with other *S. cerevisiae* mRNAs that have half-lives ranging from 1 to 60 min (Herrick *et al.* 1990). However, *CHO1* mRNA is greatly stabilized with a half-life >45 min in respiratory mutants (Choi and Carman 2007). This results in increased levels of the PS synthase protein and its associated activity (Choi and Carman 2007). Given that *CHO1* mRNA decays by the primary 5′–3′ decay pathway when cells are respiratory sufficient (Parker and Song 2004), it is reasonable to predict that the rate of deadenylation and/or decapping may be reduced when respiration is blocked. The *OLE1* transcript is destabilized when cells are supplemented with unsaturated FA (Gonzalez and Martin 1996; Vemula *et al.* 2003; Martin *et al.* 2007). This FA-regulated decay of *OLE1* mRNA occurs through both the 5′–3′ general pathway and via exosomal 3′–5′ degradation activities (Martin *et al.* 2007).

Transcriptional regulation by inositol and choline

Expression of the *INO1* gene (Figure 3, Table 2) is repressed by ≥100-fold in the presence of inositol. Exogenous choline results in an additional severalfold reduction in *INO1* transcript level but only if inositol is also present. However, in the absence of exogenous inositol, choline has little or no effect on *INO1* expression (Hirsch and Henry 1986; Griac *et al.* 1996; Jesch *et al.* 2005a). Other genes encoding enzymes of lipid metabolism, including many involved in PC biosynthesis, show similar patterns of regulation in response to inositol and choline (Carman and Henry 1989, 1999, 2007; Paltauf *et al.* 1992; Henry and Patton-Vogt 1998; Jesch and Henry 2005; Jesch *et al.* 2005; Chen *et al.* 2007a; Carman and Han 2011). However, no other gene in the yeast genome shows as great a repression ratio in the presence of inositol and choline as *INO1* (Santiago and Mamoun 2003; Jesch *et al.* 2005, 2006).

The regulation (Figure 3) controlling the expression of *INO1* and coregulated genes of lipid metabolism has been the subject of a number of recent comprehensive reviews (Carman and Henry 2007; Chen *et al.* 2007a; Carman and Han 2011). Expression of these genes is controlled by the *cis*-acting element, consensus 5′CATGTGAAT3′ (Bachhawat *et al.* 1995; Greenberg and Lopes 1996), known as the inositol sensitive upstream activating element (UAS_{INO}), or alternatively as the inositol/choline responsive element (ICRE) (Schuller *et al.* 1992). The Ino2-Ino4 heterodimer, which is required for activation of transcription of UAS_{INO}-containing genes, binds to this site (Ambroziak and Henry 1994). Genome-wide location analysis confirmed the sequence of the UAS_{INO} element and the binding of the Ino2-Ino4 heterodimer to the element across the genome (Lee *et al.* 2002; Harbison *et al.* 2004). Since both Ino2 and Ino4 are required for activation of expression of *INO1*, *ino2*, and *ino4* mutants exhibit inositol auxotrophy (Ino^- phenotype). However, since many genes involved in lipid metabolism in addition to *INO1* contain the UAS_{INO} promoter element (Greenberg and Lopes 1996), the *ino2* and *ino4* mutants exhibit altered phospholipid compositions including reduced PC content, even when growing in the presence of inositol (Loewy and Henry 1984). The fact that the *INO2* regulatory gene also contains a UAS_{INO} element in its promoter, and is auto-regulated in response to inositol, adds an additional layer of complexity to this regulatory mechanism (Ashburner and Lopes 1995, Chen *et al.* 2007a). The *INO1* locus has also recently been reported to localize at the nuclear periphery when it is being actively transcribed (Brickner and Walter 2004), through a chromatin-mediated mechanism that persists for several generations following addition of inositol (Brickner *et al.* 2007; Brickner 2009).

Repression of UAS_{INO}-containing genes in response to the presence of exogenous inositol involves the Opi1 repressor (Hirsch and Henry 1986; White *et al.* 1991) (Figure 3), which interacts with transcriptional activation domains in Ino2 (Wagner *et al.* 2001; Dietz *et al.* 2003). Opi1 has also been reported to interact with a number of other regulatory proteins (Chen *et al.* 2007a), including the pleiotropic corepressors, Sin3 and Ssn6 (Jaschke *et al.* 2011). Deletion of the *OPI1* gene renders the cell incapable of repressing UAS_{INO} genes in response to inositol, and leads to expression of UAS_{INO}-containing genes at levels that exceed their normal derepressed levels by severalfold, whether inositol or choline are present or not (White *et al.* 1991; Santiago and Mamoun 2003; Jesch *et al.* 2005). Such high levels of *INO1* expression lead to **o**ver-**p**roduction of **i**nositol (the Opi^- phenotype for which the *opi1* mutants are named) and the excess inositol excreted from Opi^- mutants can be detected in a plate assay (Greenberg *et al.* 1982a,b). In addition, even when growing in the absence of inositol, *opi1*Δ cells have elevated levels of PI and levels of other lipids that resemble those of wild-type cells growing in the presence of inositol (Klig *et al.* 1985).

Role of PA in regulation of UAS_{INO}-containing genes

Opi⁻ phenotypes are also associated with mutants (*cho1*, *cho2*, *and opi3*) defective in the synthesis of PC via the CDP-DAG pathway (Greenberg *et al.* 1983; Letts *et al.* 1983; Summers *et al.* 1988; McGraw and Henry 1989) (Figure 2). Similar to the *opi1* mutant, *cho2* and *opi3* mutants overexpress *INO1* and fail to repress *INO1* and other UAS_{INO}-containing genes in response to inositol. However, in contrast to *opi1* mutants, the constitutive overexpression of *INO1* and Opi⁻ phenotypes of *opi3* and *cho2* mutants are suppressed when they are grown in the presence of exogenous soluble precursors that restore PC biosynthesis by entering the Kennedy pathway downstream of their respective genetic lesions in PE methylation (Figure 2). Thus, the Opi⁻ phenotype is eliminated and PC biosynthesis is restored in *cho2* mutants grown in the presence of monomethylethanolamine (MME), dimethylethanolamine (DME), or choline (Summers *et al.* 1988), while these phenotypes are corrected only by DME and choline in *opi3* mutants (McGraw and Henry 1989). Such observations led to the hypothesis that the mechanism by which the yeast cell senses the presence of exogenous inositol, choline, and other phospholipid precursors, such as serine, ethanolamine, MME, and DME, involves their effect on phospholipid synthesis (Carman and Henry 1989).

Further support for this hypothesis came from studies involving strains carrying temperature-sensitive mutations of the essential PI/PC transport protein, Sec14. While a full discussion of the biology of Sec14 is beyond the scope of this review article, the role it has played in discovery that PA is the signaling molecule controlling *INO1* expression will be covered in brief here. The growth and secretory phenotypes of *sec14*^{ts} mutants are suppressed by mutations in any one of the three genes encoding enzymes in the Kennedy pathway for PC biosynthesis (*i.e.*, *cki1*, *pct1*, and *cpt1*) (Cleves *et al.* 1991). Such strains (for example: *sec14*^{ts} *cki1*Δ) have Opi⁻ phenotypes and excrete choline (Opc⁻ phenotype) (Patton-Vogt *et al.* 1997). These phenotypes are due to increased PA levels resulting from elevated turnover of PC, catalyzed by the *SPO14*-encoded phospholipase D (Sreenivas *et al.* 1998; Xie *et al.* 1998). These observations led to the identification of PA as the probable signaling lipid responsible for derepression of *INO1* and coregulated genes in the absence of exogenous inositol (Griac *et al.* 1986; Summers *et al.* 1988; McGraw and Henry 1989; Henry and Patton-Vogt 1998). This model is consistent with the Opi⁻ phenotypes of mutants defective in reactions in the CDP-DAG pathway for PC synthesis and the fact that these phenotypes are suppressed by supplying these mutants with precursor that enters the Kennedy pathway downstream of the defect in the CDP-DAG pathway (Henry and Patton-Vogt 1998). Interruption of any of the reactions downstream of PA via the CDP-DAG pathway leads to a buildup of PA and overexpression of *INO1*, which raises endogenous inositol production. This leads to excretion of inositol and renders the cell insensitive to exogenous inositol (Henry and Patton-Vogt 1998). Metabolites that enter the Kennedy pathway downstream of the specific metabolic lesion counteract PA build up by increasing the use of DAG in the Kennedy pathway (Figure 2). Also consistent with this model is the Opi⁻ phenotype observed in cells engineered to have reduced expression of *PIS1*, thereby slowing PI synthesis and raising PA levels (Jani and Lopes 2009).

The mechanism by which PA regulates *INO1* expression was elucidated when Loewen *et al.* (2004) showed that Opi1 contains PA binding domains that facilitate its interaction with PA in the perinuclear ER in cells growing in the absence of inositol (Figure 3). Opi1 also contains a motif known as FFAT (two phenylalanines in an acidic tract) that binds to Scs2, an integral ER membrane protein (Loewen *et al.* 2003). To remain localized to the ER membrane in the absence of inositol, Opi1 must interact with both Scs2 and PA. In wild-type yeast cells, addition of inositol to the growth medium leads to a dramatic increase in the rate of synthesis of PI, resulting in consumption of both PA and CDP-DAG. As PA levels drop, Opi1 is released from the ER and enters the nucleus where it represses expression of *INO1* and other UAS_{INO}-containing genes (Loewen *et al.* 2004). Consistant with this model, mutations in Opi1 that interfere with its interaction with either PA or Scs2 result in Ino⁻ phenotypes (Loewen *et al.* 2004) and *scs2* mutants also exhibit an Ino⁻ phenotype (Nikawa *et al.* 1995). Increased PC synthesis via the Kennedy pathway is also characteristic of *scs2* mutants, a phenotype that has not been fully explored, and mutations blocking PC synthesis by this route suppress both the Ino⁻ phenotype and increased synthesis of PC in the *scs2* mutant (Kagiwada and Zen 2003). Since, as described above, blocking the reuse of choline via the Kennedy pathway should reduce the draw on PA and thus enhance *INO1* expression, these phenotypes are consistent with the model of Loewen *et al.* (2004) concerning Opi1 function (Figure 3).

Regulation of gene expression by zinc

The expression of several phospholipid synthesis genes is also regulated in coordination with mechanisms that control zinc homeostasis (Figure 3) (Carman and Han 2007; Eide 2009). Zinc is an essential nutrient required for the growth and metabolism of *S. cerevisiae* (Eide 2009). It is a cofactor for key metabolic enzymes and a structural component of a diverse set of proteins that include chaperons, lipid binding proteins, and transcription factors (Vallee and Falchuk 1993; Schwabe and Klug 1994; Ellis *et al.* 2004; Eide 2009). Cells grown in medium lacking zinc exhibit the induced expression of plasma membrane and vacuolar membrane zinc transporters (encoded by *ZRT1*, *ZRT2*, *ZRT3*, and *FET4*) to maintain the cytosolic levels of zinc (Guerinot and Eide 1999; Eide 2003, 2009). At the same time, a reduction in zinc causes changes in membrane phospholipid composition that are brought about by changes in the expression of phospholipid synthesis enzyme activities (Han

et al. 2004, 2005; Iwanyshyn *et al.* 2004; Kersting and Carman 2006; Carman and Han 2007). It is postulated that changes in phospholipid composition may govern the insertion, topology, and/or function of the membrane-associated zinc transporters (Carman and Han 2007).

The regulation of phospholipid synthesis genes by zinc availability (Figure 3) involves the control of PA content through the activation of PI synthase function. This regulation occurs in the absence of inositol supplementation and is mediated by the zinc-sensing and zinc-inducible transcriptional activator Zap1 and the zinc-responsive *cis*-acting element (UAS_{ZRE}) (Carman and Han 2007). Zinc depletion causes an increase in the synthesis of PI through increased expression of PI synthase (Iwanyshyn *et al.* 2004; Han *et al.* 2005). This regulation is controlled by the interaction of Zap1 with a UAS_{ZRE} in the *PIS1* promoter (Iwanyshyn *et al.* 2004; Han *et al.* 2005). As discussed above, increased PI synthesis causes a decrease in PA content, which results in the Opi1-mediated repression of UAS_{INO}-containing genes and a decrease in the activities of the CDP-DAG pathway enzymes (Iwanyshyn *et al.* 2004). The major effects of zinc depletion on phospholipid composition include an increase in PI and a decrease in PE (Iwanyshyn *et al.* 2004). Although levels of enzymes in the CDP-DAG pathway are reduced by zinc depletion, the amount of PC is not significantly affected (Iwanyshyn *et al.* 2004). This is attributed to the Zap1-mediated inductions of choline kinase and ethanolamine kinase for PC synthesis via the Kennedy pathway (Kersting and Carman 2006; Soto and Carman 2008). Like *PIS1*, the *CKI1* and *EKI1* genes contain a UAS_{ZRE} in their promoters that interact with Zap1 for gene activation (Kersting and Carman 2006; Soto and Carman 2008). Opi1-mediated regulation of *CKI1* and *EKI1* are overcome by their derepression via Zap1 (Kersting and Carman 2006; Soto and Carman 2008).

Among the genes in lipid metabolism that contain the UAS_{ZRE}, *DPP1* is the most highly regulated by zinc availability (Lyons *et al.* 2000; Carman and Han 2007; Eide 2009). The *DPP1* gene encodes DGPP phosphatase, an enzyme associated with the vacuole membrane (Wu *et al.* 1996; Toke *et al.* 1998; Han *et al.* 2001). This enzyme catalyzes the removal of the β-phosphate from DGPP, a minor phospholipid in yeast, to form PA, followed by the dephosphorylation of PA to form DAG (Wu *et al.* 1996). The zinc-mediated regulation of *DPP1* expression correlates with the metabolism of DGPP and PA in the vacuole membrane (Han *et al.* 2004). In zinc-replete medium, DGPP and PA account for 0.6 mol% and 1.4 mol% of the total phospholipids in vacuole membranes, but in zinc-depleted medium, the amounts of DGPP and PA are decreased to an undetectable level and 0.3 mol%, respectively (Han *et al.* 2004). The function of *DPP1*-encoded DGPP phosphatase is still unclear. However, it is speculated that the enzyme controls the levels of DGPP and PA in vacuolar membranes, which in turn mediates the cellular functions occurring in response to zinc depletion (Carman and Han 2007).

Regulation by phosphorylation

Phosphorylation is a major covalent post-translational modification by which the activity of an enzyme or a transcription factor is regulated (Karin and Hunter 1995; Calkhoven and Ab 1996; Hung *et al.* 1997; Kaffman *et al.* 1998; Komeili and O'Shea 1999; Liu *et al.* 2000). Global analyses of protein phosphorylation indicate that several enzymes and transporters of glycerolipid metabolism are subject to phosphorylation (Tables 1–3). Some proteins have many sites of phosphorylation, whereas others have only a few. It is also telling that many enzymes and transporters have no sites of phosphorylation, and thus the function of these proteins might be regulated by other mechanisms (*e.g.*, substrate availability). The identity of the protein kinases involved and the physiological consequences of their phosphorylations have only been determined for a few proteins in glycerolipid metabolism (Carman and Han 2011). The protein kinases known to regulate the function of catalytic and regulatory proteins in glycerolipid metabolism include AMP-activated protein kinase, protein kinases A and C, casein kinase II, and cyclin-dependent kinase. Glycerolipid enzymes known to be *bona fide* substrates of protein kinases and regulated by phosphorylation include CTP synthetase, choline kinase, PS synthase, PA phosphatase, and TAG lipase. The repressor Opi1 is also regulated by phosphorylation. A discussion of these phosphorylations may be found in a recent review by Carman and Han (2011).

Regulation of PA phosphatase

Pah1 is one of the most highly regulated enzymes in lipid metabolism. Its activity is governed by several of the biochemical mechanisms discussed above including phosphorylation, enzyme location, and modulation by components of lipid metabolism. As discussed above, the DAG generated in the Pah1 reaction is used for the synthesis of TAG (Han *et al.* 2006) and for the synthesis of PE and PC via the Kennedy pathway (Carman and Han 2006, 2008). The enzyme also plays a major role in controlling the cellular concentration of its substrate PA (Figure 3) (Han *et al.* 2006), the precursor of phospholipids that are synthesized via the CDP-DAG pathway (Carman and Zeimetz 1996; Carman and Henry 1999; Carman and Han 2008). In addition, the substrate PA plays a signaling role (see above) in the transcriptional regulation of phospholipid synthesis genes (Carman and Henry 2007). *pah1*Δ mutants exhibit a >90% reduction in TAG content, as well as derepression of phospholipid synthesis genes and massive expansion of the nuclear/ER membrane (Santos-Rosa *et al.* 2005; Han *et al.* 2007). Thus, the regulation of Pah1 activity governs the synthesis of TAG, the pathways by which phospholipids are synthesized, PA signaling, and the growth of the nuclear/ER membrane (Carman and Han 2008). Pah1 is associated with the cytosolic and membrane fractions of the cell, and its association with the membrane is peripheral in nature (Han *et al.* 2006). Chromatin immunoprecipitation analysis indicates that Pah1 may also be localized in the nucleus (Santos-Rosa *et al.* 2005).

The association of Pah1 with the membrane where its substrate PA resides is essential to its function *in vivo*, and membrane association is largely governed by the phosphorylation state of the enzyme (Karanasios *et al.* 2010; Choi *et al.* 2011). Phosphorylation favors a cytosolic association, whereas dephosphorylation favors a membrane association (Choi *et al.* 2011). The important sites of phosphorylation that govern this regulation include the seven Ser/Thr-Pro targets for *CDC28*-encoded and *PHO85*-encoded cyclin-dependent kinases. Pah1 is dephosphorylated by the Nem1-Spo7 phosphatase complex that is associated with the nuclear/ER membrane (Siniossoglou *et al.* 1998; Santos-Rosa *et al.* 2005). In the absence of Nem1-Spo7, wild-type Pah1 is enriched in the cytosol where it is physiologically inactive, whereas a nonphosphorylatable mutant of Pah1 with alanine substitutions of Cdc28 and Pho85 target sites is enriched in the membrane and is physiologically active (Choi *et al.* 2011). The requirement of Nem1-Spo7 indicates that Pah1 is recruited to the membrane for its physiological function. Indeed, the Nem1-Spo7–dependent membrane localization of Pah1 is enhanced by elevated levels of PA (Karanasios *et al.* 2010). Once the enzyme is dephosphorylated, it anchors onto the nuclear/ER membrane via a short N-terminal amphipathic helix allowing for the production of DAG for TAG synthesis (Karanasios *et al.* 2010).

Under normal physiological conditions (*i.e.*, presence of the Nem1-Spo7 complex), the level of wild-type Pah1 detected on the membrane is very low. In fact, microscopic analysis of live *S. cerevisiae* cells expressing Pah1-GFP show a cytosolic localization without a detectable fluorescence signal associated with the nuclear/ER membrane unless PA levels are elevated (Karanasios *et al.* 2010). Yet we know that Pah1 is physiologically active with respect to lipid metabolism throughout cell growth (Han *et al.* 2006). Purified Pah1 has a relatively high catalytic efficiency when compared with other enzymes of lipid metabolism (Lin and Carman 1989), and the lethal phenotype of cells that overexpress Nem1-Spo7 (Santos-Rosa *et al.* 2005) indicates that an excess of Pah1 function is detrimental to cell physiology. Indeed, suppression of this phenotype by overexpression of Pah1 (Santos-Rosa *et al.* 2005) led to the discovery of Pah1 function (Han *et al.* 2007). Thus, under normal physiological conditions, the amount of Pah1 associated with membranes must be small for its physiological function to be controlled, and this regulation must be mediated by the amount of the Nem1-Spo7 on the membrane. In support of this hypothesis, the expression level of Nem1 is 10-fold lower when compared with Pah1 (Ghaemmaghami *et al.* 2003).

Pah1 activity is also modulated by cytosolic- and membrane-associated factors. The nucleotides ATP and CTP, which are precursors for the synthesis of phospholipids (Carman and Henry 1999), are inhibitors of Pah1 activity (Wu and Carman 1994). Indeed high levels of ATP favor elevated PA content and phospholipid synthesis, whereas low levels of ATP favor reduced PA content and an increase in the synthesis of TAG (Wu and Carman 1994). As discussed elsewhere, elevated CTP content favors an increase in PA content and derepression of UAS_{INO}-containing phospholipid synthesis genes (Ostrander *et al.* 1998). The patterns of regulation of PA phosphatase activity by ATP and by CTP are consistent with the regulation of lipid synthesis observed in cells that have fluctuations in ATP and CTP (Wu and Carman 1994; Ostrander *et al.* 1998).

Membrane lipids modulate Pah1 activity. For example, CDP-DAG, PI, and CL enhance activity (Wu and Carman 1996), whereas the sphingoid bases phytosphingosine and sphinganine inhibit activity (Wu *et al.* 1993). The major effect of the lipid activators is to decrease the K_m of Pah1 for PA. Sphinganine antagonizes the activation of PA phosphatase activity by CL and PI, whereas it causes an increase in the cooperativity of CL activation (Wu and Carman 1996). Conversely, sphinganine has little effect on the cooperativity of PI activation, but causes an increase in the activation constant for PI (Wu and Carman 1996). On the basis of the activation/inhibitor constants and cellular concentrations for these lipid effector molecules, their regulatory roles on Pah1 should be physiologically relevant (Wu *et al.* 1993; Wu and Carman 1996).

DAG kinase counteracts PA phosphatase in regulating PA levels

Dgk1 is a unique CTP-dependent nuclear/ER membrane-associated enzyme that catalyzes the formation of PA from DAG (Han *et al.* 2008a,b). Dgk1 counteracts Pah1 in controlling PA content and consequently, transcriptional regulation of UAS_{INO}-containing genes (Han *et al.* 2008a,b). *DGK1* overexpression causes an increase in PA content, derepression of UAS_{INO}-containing genes, and abnormal nuclear/ER membrane expansion (Han *et al.* 2008a) like those that occur in the *pah1*Δ mutant (Santos-Rosa *et al.* 2005; Han *et al.* 2007). Also consistent with the regulatory role of PA, *DGK1* overexpression suppresses the inositol auxotrophy caused by *PAH1* overexpression (Han *et al.* 2008a), whereas the *dgk1*Δ mutation suppresses the phenotypes caused by the *pah1*Δ mutation (Han *et al.* 2008a,b).

Ino$^-$ and Opi$^-$ phenotypes are associated with mutations affecting many functions

To date, the Ino$^-$ and Opi$^-$ phenotypes of mutants affecting lipid metabolism, which have been examined in detail for correlation of changes in lipid metabolism with changes in *INO1* expression, have been consistent with predictions based on the role of PA as discussed above (Carman and Henry 2007). However, mutants defective in a wide variety of other cellular functions have also been reported to have these phenotypes (Henry and Patton-Vogt 1998) and several genome-wide screens for mutants with Opi$^-$ or Ino$^-$ phenotypes have been conducted (Hancock *et al.* 2006; Young *et al.* 2010; Villa-Garciá *et al.* 2011).

The screen of the *Mat*α viable yeast deletion collection conducted by Hancock *et al.* (2006), using the Opi$^-$ plate

test, as well as an *INO1*-LacZ reporter construct, identified 89 Opi⁻ mutants. Among these mutants were *opi1*, *cho2*, *opi3*, *ume6*, *rpd3*, *sin3*, and *reg1*, which had previously been shown to affect *INO1* expression (Henry and Patton-Vogt 1998; Shirra and Arndt 1999; Ouyang *et al.* 1999; Elkhaimi *et al.* 2000). Hancock *et al.* (2006) also reported Opi⁻ phenotypes for the first time in mutants affecting functions such as the NuA4 histone acetyl transferase, vacuolar protein sorting, and other aspects of membrane trafficking and the UPR.

Young *et al.* (2010) identified 231 mutants from the yeast haploid deletion collection that showed measurable growth reduction at 37°, when grown in medium lacking inositol. The major focus of this study was on the discovery of pH sensitive Ino⁻ phenotypes associated with mutants defective in all of the subunits of the vacuolar adenosine triphosphatase (V-ATPase) complex, as well as factors in the ER responsible for the assembly of this complex. The *pma1-007* mutant, defective in the essential P-type ATPase of the plasma membrane, which regulates cellular pH, was also found to have an Ino⁻ phenotype, as was *trk1*Δ, defective in a K^+ transporter that activates Pma1. The Ino⁻ phenotypes of these mutants were observed at pH 3 but were suppressed at pH 4 and 5 and this pH sensitivity was attributed to pH dependence of binding of Opi1 to PA. The binding of PA to Spo20, a yeast SNARE (Nakanishi *et al.* 2004), was also shown to be pH sensitive, suggesting that binding of proteins to PA is affected by cellular pH. Changes in the ionization state of PA affect binding of Opi1 to PA *in vitro*, supporting the electrostatic/hydrogen bond switch model of Kooijman *et al.* (2007) for interaction of proteins with PA. Repression of *INO1* in response to glucose starvation also correlates to cellular pH, but not to PA levels. Young *et al.* (2010) have concluded that the Reg1-mediated mechanism that regulates *INO1* expression via the glucose response pathway (Ouyang *et al.* 1999; Shirra and Arndt 1999) is most likely not dependent on the absolute level of PA, but rather on the effect of pH on the ionization state of PA.

The screen for Ino⁻ mutants of the diploid viable yeast deletion collection conducted by Villa-Garciá *et al.* (2011) has resulted in the identification of 419 genes, which when deleted confer the Ino⁻ phenotype under one or more growth conditions. This screen involves comparing growth in the presence and absence of inositol at two growth temperatures, 30° and 37°, in the presence and absence of choline. Choline sensitive Ino⁻ phenotypes and the strengthening of weak Ino⁻ phenotypes by choline are potentially attributable to reduction in PA levels due to the consumption of DAG produced from PA when PC is synthesized via the Kennedy pathway (see above) using exogenous choline (Carman and Henry 2007). Growth temperature has also been shown to influence lipid metabolism in wild-type and mutant strains. Growth at 37° results in an increased rate of synthesis of PI in wild-type cells growing in the presence of inositol (Gaspar *et al.* 2008), as well as an increase in PC synthesis and turnover (Dowd *et al.* 2001). Among the gene ontology (GO) categories enriched among the Ino⁻ mutants identified by Villa-Garciá *et al.* (2011) are: response to stress, protein modification, chromosome organization, response to chemical stimulus, cellular carbohydrate metabolism, psuedohyphal growth, and transcription. Mutants in the category response to stress include a number with defects in stress response pathways that had previously been reported to be associated with Ino⁻ phenotypes, including the protein kinase C-cell wall integrity (PKC-CWI) (Nunez *et al.* 2008; Jesch *et al.* 2010), the UPR (Cox *et al.* 1997; Chang *et al.* 2002) and the glucose response pathway (Shirra *et al.* 2001, 2005). However, Ino⁻ phenotypes have also been observed in mutants affecting signaling pathways not previously reported to be associated with such phenotypes including: target of rapamycin (TOR), high osmolarity glycerol (HOG), cAMP-dependent protein kinase, calcineurin and filamentous growth and cell cycle regulation (Villa-Garciá *et al.* 2011).

Causes of Ino⁻ phenotypes

It is often assumed that an Ino⁻ phenotype provides evidence that a mutant has impaired *INO1* expression or, alternatively, that a signaling pathway and/or a transcription factor defective in the mutant in question, is directly involved in regulating *INO1* transcription. For example, the Ino⁻ phenotypes of the *ire1*Δ and *hac1*Δ mutants, defective in UPR activation, have been attributed to a role for the Hac1 transcription factor in activating expression of *INO1* (Cox *et al.* 1997). The evidence for this interpretation includes the observations that *ire1*Δ and *hac1*Δ mutants fail to sustain expression of *INO1* when shifted to medium lacking inositol and that the *opi1*Δ mutation supresseses their Ino⁻ phenotypes (Cox *et al.* 1997). Cox *et al.* (1997) also reported that growth in the absence of inositol activates the UPR in wild-type cells and that *INO1* expression is activated in wild-type cells treated with tunicamycin in the presence of normally repressing levels of inositol. However, activation of *INO1* expression in response to tunicamycin in the presence of inositol was not observed by Chang *et al.* (2002) and *INO1* is repressed with normal kinetics in response to addition of inositol in cells in which the UPR was constitutively induced by constitutive expression of activated Hac1 (Jesch *et al.* 2006). The reasons for these divergent results with respect to the role of Hac1 in *INO1* transcription are not clear.

A number of mutants in the PKC-CWI pathway have Ino⁻ phenotypes that are intensified at 37° and in the presence of choline (Nunez *et al.* 2008; Fernandez-Murray *et al.* 2009; Jesch *et al.* 2010; Villa-Garciá *et al.* 2011). Futhermore, the Ino⁻ phenotypes of these mutants are suppressed by *opi1*Δ. It is clear, however, that the PKC pathway has no direct role in regulating *INO1* expression. The PKC pathway mutant, *slt2*Δ/*mpk1*Δ, exhibits normal *INO1* derepression in response to a shift to inositol medium even when choline is present at 37°, a condition under which growth ceases and the mutant begins to lose viability within 4–5 hr (Nunez *et al.* 2008). The *stt4*^{ts} and *mss4*^{ts} mutants, defective in the

PI kinases that are responsible for producing the PI 4-P and PI 4,5-P_2 pools on the plasma membrane and are essential for PKC-CWI signaling during heat stress (Audhya and Emr 2002; Audhya and Emr 2003), also exhibit Ino^- phenotypes at semipermissive growth temperatures (Jesch *et al.* 2010). However, *INO1* undergoes normal derepression immediately following a shift to medium lacking inositol in the *stt4*^{ts} and *mss4*^{ts} mutants, even at the temperature at which they exhibit an Ino^- phenotype (Jesch *et al.* 2010). Significantly, the PKC-CWI pathway is activated in wild-type cells grown in the absence of inositol (Nunez *et al.* 2008) in response to reduction in synthesis of inositol-containing sphingolipids (Jesch *et al.* 2010). Thus, it is clear that the Ino^- phenotypes of PKC-CWI mutants are not attributable to a defect in *INO1* expression, but rather to failure to mount a stress response that is essential for survival of stress caused by growth in the absence of inositol (Nunez *et al.* 2008; Jesch *et al.* 2010; Villa *et al.* 2011).

Supression of an Ino^- phenotype by *opi1Δ* is also often cited as evidence that the mutant in question is defective in a functon that regulates *INO1*. However, the *opi1Δ* mutation also suppresses the Ino^- phenotypes of *slt2Δ* and other PKC pathway mutants (Nunez *et al.* 2008), which, as discussed above, are not attributable to failure to express *INO1*. The explanation for this observation lies in the fact that the *opi1Δ* mutation does not simply restore *INO1* expression to the level seen in wild-type cells growing in the absence of inositol. Instead, the deletion of the *OPI1* gene completely eliminates the ability of the cell sense inositol, whether endogenously produced or exogenously supplied, leading expression of *INO1* at levels as high as 5- to 6-fold higher than normal derepressed levels (Bachhwat *et al.* 1995). As a consequence of the resulting overproduction of inositol, *opi1* cells have levels of PI and other lipids resembling the lipid composition of wild-type cells grown in the presence of high levels of exogenous inositol (Klig *et al.* 1985). Indeed, *opi1Δ* mutants excrete so much inositol that they support growth of any Ino^- mutant in their vicinity, the characteristic used in the original bioassay used in the isolation of Opi^- mutants (Greenberg *et al.* 1982a). Microarray experiments showed that, in contrast to wild-type cells, *opi1Δ* cells do not exhibit activation of UPR target genes when grown in the absence of inositol (Jesch *et al.* 2005). This is presumably due to the fact that overexpression of the *INO1* gene in *opi1Δ* cells produces sufficient inositol to repress expression of all genes that are normally activated in absence of inositol, except of course the UAS_{INO}-containing genes, which are directly dependent on Opi1p for repression in the presence of inositol (Jesch and Henry 2005). Thus, suppression by *opi1Δ* of an Ino^- phenotype conferred by mutation of a given gene does not consitute sufficient evidence on its own that the gene product in question is involved in regulation of *INO1*.

Microarray studies have revealed that the transcript levels of hundreds of genes in yeast are affected by the availability of inositol and/or inositol plus choline in the medium (Santiago and Mamoun 2003; Jesch *et al.* 2005, 2006; Nunez *et al.* 2008). Most of these "inositol-regulated" genes are not under the control of Opi1 and/or involved in lipid metabolism and a number of these genes are known targets of the stress response pathways that are associated with Ino^- phenotypes, including the UPR and PKC pathways (Jesch *et al.* 2005, 2006; Nunez *et al.* 2008; Jesch *et al.* 2010; Villa *et al.* 2011). As discussed above, expression of *INO1* at the level observed in wild-type cells growing in the absence of inositol does not suffice to support the rate of PI synthesis observed in the same cells growing in the presence of exogenous inositol (Kelley *et al.* 1988; Gaspar *et al.* 2006). Moreover, in wild-type cells, the changes in lipid metabolism that occur in response to removal of inositol and/or its resupply have been shown to affect signaling associated with PA (Loewen *et al.* 2004; Young *et al.* 2010), and with inositol containing sphingolipids and the pools of PI 4-P and PI 4,5-P_2 in the plasma membrane (Jesch *et al.* 2010).

As the exploration of the regulation and cell biology of lipid metabolism in yeast accelerates in response to genomic approaches, additional examples of lipid-mediated signaling influenced by the availability of lipid precursors are likely to be discovered. A major advantage of yeast in such studies is the fact, as described in this YeastBook chapter, that its lipid metabolism can readily be manipulated by changing the supply of exogenous precursors and/or by introducing mutations affecting specific steps in lipid metabolism.

Perspectives

Monumental advancements have been made in determining gene enzyme relationships and elucidating the fundamental biochemistry, regulatory mechanisms, and cellular roles of lipids in the 20 years since the publication of the last edition of the *Molecular Biology of the Yeast Saccharomyces: Gene Expression* in 1992. Yet while the identities of many of the genes encoding enzymes, transporters, and regulatory factors controlling lipid metabolism in yeast have been discovered, described, and analyzed, there remain many gaps in our knowledge. The functions of many genes, genome-wide, have yet to be clarified and among them are certainly a considerable number of "missing links" in yeast lipid metabolism and its regulation. For example, the molecular organization of lipid biosynthetic pathways that ensures metabolic channeling of critical compounds, enzyme topology, and the integration of soluble and membrane-bound intermediates in these pathways is only beginning to be addressed. Furthermore, we do not have complete knowledge of the mechanisms by which lipids synthesized in one compartment are trafficked to other locations within the cell or the mechanisms by which the precise lipid compositions of specific membrane compartments are established and maintained. High-resolution lipidomic analyses that have uncovered some 100 different molecular species of glycero- and sphingolipids have added another level of complexity to this question. Moreover, our understanding of the regulatory

networks that link lipid metabolism to other aspects of cell biology and metabolism, including energy metabolism, membrane biogenesis, and trafficking, signaling, and cell division, remains limited. However, the increasing availability of archived genetic and biochemical data on a wide variety of eukaryotic organisms and the extensive homologies that exist among eukaryotes are opening new avenues for pursuing these important challenges. Due to its genetic tractability and comparably "simple" lipid complement, yeast continues to be among the most attractive and important model systems for such research.

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Literature Cited

Achleitner, G., D. Zweytick, P. J. Trotter, D. R. Voelker, and G. Daum, 1995 Synthesis and intracellular transport of aminoglycerophospholipids in permeabilized cells of the yeast, Saccharomyces cerevisiae. J. Biol. Chem. 270: 29836–29842.

Achleitner, G., B. Gaigg, A. Krasser, E. Kainersdorfer, S. D. Kohlwein *et al.*, 1999 Association between the endoplasmic reticulum and mitochondria of yeast facilitates interorganelle transport of phospholipids through membrane contact. Eur. J. Biochem. 264: 545–553.

Adeyo O., P. J. Horn, S. Lee, D. D. Binns, A. Chandrahas *et al.*, 2011 The yeast lipin orthologue Pah1p is important for biogenesis of lipid droplets. J. Cell Biol. 192: 1043–1055.

Al-Feel, W., S. S. Chirala, and S. J. Wakil, 1992 Cloning of the yeast *FAS3* gene and primary structure of yeast acetyl-CoA carboxylase. Proc. Natl. Acad. Sci. USA 89: 4534–4538.

Ambroziak, J., and S. A. Henry, 1994 The *INO2* and *INO4* gene products, positive regulators of phospholipid biosynthesis in *Saccharomyces cerevisiae*, form a complex that binds to the *INO1* promoter. J. Biol. Chem. 269: 15344–15349.

Ashburner, B. P., and J. M. Lopes, 1995 Autoregulated expression of the yeast *INO2* and *INO4* helix-loop-helix activator genes effects cooperative regulation on their target genes. Mol. Cell. Biol. 15: 1709–1715.

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

Athenstaedt, K., and G. Daum, 2000 1-Acyldihydroxyacetone-phosphate reductase (Ayr1p) of the yeast *Saccharomyces cerevisiae* encoded by the open reading frame YIL124w is a major component of lipid particles. 2000. J. Biol. Chem. 275: 235–240.

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

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

Athenstaedt, K., S. Weys, F. Paltauf, and G. Daum, 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.

Atkinson, K. D., B. Jensen, A. I. Kolat, E. M. Storm, S. A. Henry *et al.*, 1980a Yeast mutants auxotropic for choline or ethanolamine. J. Bacteriol. 141: 558–564.

Atkinson, K., S. Fogel, and S. A. Henry, 1980b Yeast mutant defective in phosphatidylserine synthesis. J. Biol. Chem. 255: 6653–6661.

Audhya, A., and S. D. Emr, 2002 Stt4 PI 4-kinase localizes to the plasma membrane and functions in the Pkc1-mediated MAP kinase cascade. Dev. Cell 2: 593–605.

Audhya, A., and S. D. Emr, 2003 Regulation of PI4,5P2 synthesis by nuclear-cytosolic shuttling of the Mss4 lipid kinase. EMBO J. 22: 4223–4236.

Bachhawat, N., Q. Ouyang, and S. A. Henry, 1995 Functional Characterization of an Inositol-sensitive Upstream Activation Sequence in Yeast. A *cis*-Regulatory Element Responsible for Inositol-Choline Mediated Regulation of Phospholipid Biosynthesis. J. Biol. Chem. 270: 25087–25095.

Bae-Lee, M. S., and G. M. Carman, 1984 Phosphatidylserine synthesis in Saccharomyces cerevisiae. Purification and characterization of membrane-associated phosphatidylserine synthase. J. Biol. Chem. 259: 10857–10862.

Bankaitis, V. A., C. J. Mousley, and G. Schaaf, 2010 The Sec14 superfamily and mechanisms for crosstalk between lipid metabolism and lipid signaling. Trends Biochem. Sci. 35: 150–160.

Beh, C. T., and J. Rine, 2004 A role for yeast oxysterol-binding protein homologs in endocytosis and in the maintenance of intracellular sterol-lipid distribution. J. Cell Sci. 117: 2983–2996.

Benghezal, M., C. Roubaty, V. Veepuri, J. Knudsen, and A. Conzelmann, 2007 *SLC1* and *SLC4* encode partially redundant Acyl-Coenzyme A 1-Acylglycerol-3-phosphate O-Acyltransferases of budding yeast. J. Biol. Chem. 282: 30845–30855.

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

Birner, R., R. Nebauer, R. Schneiter, and G. Daum, 2003 Synthetic lethal interaction of the mitochondrial phosphatidylethanolamine biosynthetic machinery with the prohibitin complex of Saccharomyces cerevisiae. Mol. Biol. Cell 14: 370–383.

Black, P. N., and C. C. Dirusso, 2007 Yeast acyl-CoA synthetases at the crossroads of fatty acid metabolism and regulation. Biochim. Biophys. Acta 1771: 286–298.

Block-Alper, L., P. Webster, X. Zhou, L. Supekova, W. H. Wong *et al.*, 2002 *INO2*, a positive regulator of lipid biosynthesis, is essential for formation of inducible membranes in yeast. Mol. Biol. Cell 13: 40–51.

Bodenmiller, B., D. Campbell, B. Gerrits, H. Lam, M. Jovanovic *et al.*, 2008 PhosphoPep–a database of protein phosphorylation sites in model organisms. Nat. Biotechnol. 26: 1339–1340.

Bossie, M. A., and C. E. Martin, 1989 Nutritional regulation of yeast delta-9 fatty acid desaturase activity. J. Bacteriol. 171: 6409–6413.
Boumann, H. A., J. Gubbens, M. C. Koorengevel, C. S. Oh, C. E. Martin *et al.*, 2006 Depletion of phosphatidylcholine in yeast induces shortening and increased saturation of the lipid acyl chains: evidence for regulation of intrinsic membrane curvature in a eukaryote. Mol. Biol. Cell 17: 1006–1017.
Brandner, K., D. U. Mick, A. E. Frazier, R. D. Taylor, C. Meisinger *et al.*, 2005 Taz1, an outer mitochondrial membrane protein, affects stability and assembly of inner membrane protein complexes: implications for Barth syndrome. Mol. Biol. Cell 16: 5202–5214.
Brickner, D.G., I. Cajigas, Y. Fondufe-Mittendorf, S. Ahmed, P. Lee, 2007 H2A.Z-mediated localization of genes at the nuclear periphery confers epigenetic memory of previous transcriptional state. PloS Biol. 5: 0704–0716.
Brickner, J. H., and P. Walter, 2004 Gene recruitment of the activated *INO1* locus to the nuclear membrane. PLoS Biol. 2: 1843–1853.
Brickner, J. H., 2009 Transcriptional memory at the nuclear periphery. Curr. Opin. Cell Biol. 21: 127–133.
Calkhoven, C. F., and G. Ab, 1996 Multiple steps in the regulation of transcription-factor level and activity. Biochem. J. 317: 329–342.
Carman, G. M., and G. S. Han, 2006 Roles of phosphatidate phosphatase enzymes in lipid metabolism. Trends Biochem. Sci. 31: 694–699.
Carman, G. M., and G. S. Han, 2007 Regulation of phospholipid synthesis in *Saccharomyces cerevisiae* by zinc depletion. Biochim. Biophys. Acta 1771: 322–330.
Carman, G. M., and G. S. Han, 2009a Phosphatidic acid phosphatase, a key enzyme in the regulation of lipid synthesis. J. Biol. Chem. 284: 2593–2597.
Carman, G. M., and G.-S. Han, 2009b Regulation of phospholipid synthesis in yeast. J. Lipid Res. 50: S69–S73.
Carman, G. M., and G.-S. Han, 2011 Regulation of phospholipid synthesis in the yeast *Saccharomyces cerevisiae*. Annu. Rev. Biochem. 80: 859–883.
Carman, G. M., and S. A. Henry, 1989 Phospholipid biosynthesis in yeast. Annu. Rev. Biochem. 58: 635–669.
Carman, G. M., and S. A. Henry, 1999 Phospholipid biosynthesis in the yeast *Saccharomyces cerevisiae* and interrelationship with other metabolic processes. Prog. Lipid Res. 38: 361–399.
Carman, G. M., and S. A. Henry, 2007 Phosphatidic acid plays a central role in the transcriptional regulation of glycerophospholipid synthesis in *Saccharomyces cerevisiae*. J. Biol. Chem. 282: 37293–37297.
Carman, G. M., and G. M. Zeimetz, 1996 Regulation of phospholipid biosynthesis in the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. 271: 13293–13296.
Carter, J. R., and E. P. Kennedy, 1966 Enzymatic synthesis of cytidine diphosphate diglyceride. J. Lipid Res. 7: 678–683.
Chang, H. J., E. W. Jones, and S. A. Henry, 2002 Role of the unfolded protein response pathway in regulation of *INO1* and in the *sec14* bypass mechanism in *Saccharomyces cerevisiae*. Genetics 162: 29–43.
Chang, H. J., S. A. Jesch, M. L. Gaspar and S. A. Henry, 2004 Role of the unfolded protein response pathway in secretory stress and regulation of *INO1* expression in *Saccharomyces cerevisiae*. Genetics 168: 1899–1913.
Chang, S. C., P. N. Heacock, C. J. Clancey, and W. Dowhan, 1998a The *PEL1* gene (renamed *PGS1*) encodes the phosphatidylglycerophosphate synthase of *Saccharomyces cerevisiae*. J. Biol. Chem. 273: 9829–9836.
Chang, S. C., P. N. Heacock, E. Mileykovskaya, D. R. Voelker, and W. Dowhan, 1998b Isolation and characterization of the gene (*CLS1*) encoding cardiolipin synthase in *Saccharomyces cerevisiae*. J. Biol. Chem. 273: 14933–14941.
Chang, Y.-F., and G. M. Carman, 2008 CTP synthetase and its role in phospholipid synthesis in the yeast *Saccharomyces cerevisiae*. Prog. Lipid Res. 47: 333–339.
Chen, M., L. C. Hancock, and J. M. Lopes, 2007a Transcriptional regulation of yeast phospholipid biosynthetic genes. Biochim. Biophys. Acta 1771: 310–321.
Chen, Q., M. Kazachkov, Z. Zheng, and J. Zou, 2007b The yeast acylglycerol acyltransferase LCA1 is a key component of Lands cycle for phosphatidylcholine turnover. FEBS Lett. 581: 5511–5516.
Chen, S., M. Tarsio, P. M. Kane, and M. L. Greenberg, 2008 Cardiolipin mediates cross-talk between mitochondria and the vacuole. Mol. Biol. Cell 19: 5047–5058.
Chen-Wu, J. L., R. Padmanabha, and C. V. Glover, 1988 Isolation, sequencing, and disruption of the CKA1 gene encoding the alpha subunit of yeast casein kinase II. Mol. Cell. Biol. 8: 4981–4990.
Chirala, S. S., 1992 Coordinated regulation and inositol-mediated and fatty acid-mediated repression of fatty acid synthase genes in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 89: 10232–10236.
Chirala, S. S., M. A. Kuziora, D. M. Spector, and S. J. Wakil, 1987 Complementation of mutations and nucleotide sequence of FAS1 gene encoding beta subunit of yeast fatty acid synthase. J. Biol. Chem. 262: 4231–4240.
Cho, R. J., M. J. Campbell, E. A. Winzeler, L. Steinmetz, A. Conway *et al.*, 1998 A genome-wide transcriptional analysis of the mitotic cell cycle. Mol. Cell 2: 65–73.
Choi, H.-S., and G. M. Carman, 2007 Respiratory deficiency mediates the regulation of *CHO1*-encoded phosphatidylserine synthase by mRNA stability in *Saccharomyces cerevisiae*. J. Biol. Chem. 282: 31217–31227.
Choi, H.-S., W.-M. Su, J. M. Morgan, G.-S. Han, Z. Xu *et al.*, 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.
Choi, J. Y., and C. E. Martin, 1999 The *Saccharomyces cerevisiae FAT1* gene encodes an acyl-CoA synthetase that is required for maintenance of very long chain fatty acid levels. J. Biol. Chem. 274: 4671–4683.
Chung, J. H., R. L. Lester, and R. C. Dickson, 2003 Sphingolipid requirement for generation of a functional v1 component of the vacuolar ATPase. J. Biol. Chem. 278: 28872–28881.
Clancey, C. J., S. C. Chang, and W. Dowhan, 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.
Cleves, A. E., T. P. McGee, E. A. Whitters, K. M. Champion, J. R. Aitken *et al.*, 1991 Mutations in the CDP-choline pathway for phospholipid biosynthesis bypass the requirement for an essential phospholipid transfer protein. Cell 64: 789–800.
Cowart, L. A., and L. M. Obeid, 2007 Yeast sphingolipids: recent developments in understanding biosynthesis, regulation, and function. Biochim. Biophys. Acta 1771: 421–431.
Cox, J. S., R. E. Chapman, and P. Walter, 1997 The unfolded protein response coordinates the production of endoplasmic reticulum protein and endoplasmic reticulum membrane. Mol. Biol. Cell 8: 1805–1814.
Czabany, T., K. Athenstaedt, and G. Daum, 2007 Synthesis, storage and degradation of neutral lipids in yeast. Biochim. Biophys. Acta 1771: 299–309.
Daum, G., A. Wagner, T. Czabany, and K. Athenstaedt, 2007 Dynamics of neutral lipid storage and mobilization in yeast. Biochimie 89: 243–248.
Dean-Johnson, M., and S. A. Henry, 1989 Biosynthesis of inositol in yeast. Primary structure of *myo-* inositol-1-phosphate syn-

thase (EC 5.5.1.4) and functional analysis of its structural gene, the *INO1* locus. J. Biol. Chem. 264: 1274–1283.

Deng, L., R. Fukuda, T. Kakihara, K. Narita, and A. Ohta, 2010 Incorporation and remodeling of phosphatidylethanolamine containing short acyl residues in yeast. Biochim. Biophys. Acta 1801: 635–645.

Denic, V., and J. S. Weissman, 2007 A molecular caliper mechanism for determining very long-chain fatty acid length. Cell 130: 663–677.

Dickson, R. C., 2008 New insights into sphingolipid metabolism and function in budding yeast. J. Lipid Res. 49: 909–921.

Dickson, R. C., 2010 Roles for sphingolipids in *Saccharomyces cerevisiae*. Adv. Exp. Med. Biol. 688: 217–231.

Dickson, R. C., and R. L. Lester, 2002 Sphingolipid functions in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta 1583: 13–25.

Dietrich, L. E., and C. Ungermann, 2004 On the mechanism of protein palmitoylation. EMBO Rep. 5: 1053–1057.

Dietz, M., W. T. Heyken, J. Hoppen, S. Geburtig, and H. J. Schuller, 2003 TFIIB and subunits of the SAGA complex are involved in transcriptional activation of phospholipid biosynthetic genes by the regulatory protein Ino2 in the yeast *Saccharomyces cerevisiae*. Mol. Microbiol. 48: 1119–1130.

Donahue, T. F., and S. A. Henry, 1981 Myoinositol-1-phosphate synthetase: Characteristics of the enzyme and identification of its structural gene in yeast. J. Biol. Chem. 256: 7077–7085.

Dowd, S. R., M. E. Bier, and J. L. Patton-Vogt, 2001 Turnover of phosphatidylcholine in *Saccharomyces cerevisiae*. The role of the CDP-choline pathway. J. Biol. Chem. 276: 3756–3763.

Duronio, R. J., D. A. Towler, R. O. Heuckeroth, and J. I. Gordon, 1989 Disruption of the yeast N-myristoyl transferase gene causes recessive lethality. Science 243: 796–800.

Duronio, R. J., L. J. Knoll, and J. I. Gordon, 1992 Isolation of a *Saccharomyces cerevisiae* long chain fatty acyl:CoA synthetase gene (*FAA1*) and assessment of its role in protein N-myristoylation. J. Cell Biol. 117: 515–529.

Eide, D. J., 2003 Multiple regulatory mechanisms maintain zinc homeostasis in *Saccharomyces cerevisiae*. J. Nutr. 133: 1532S–1535S.

Eide, D. J., 2009 Homeostatic and adaptive responses to zinc deficiency in Saccharomyces cerevisiae. J. Biol. Chem. 284: 18565–18569.

Ejsing, C. S., J. L. Sampaio, V. Surendranath, E. Duchoslav, K. Ekroos *et al.*, 2009 Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. Proc. Natl. Acad. Sci. USA 106: 2136–2141.

Elkhaimi, M., M. R. Kaadige, D. Kamath, J. C. Jackson, H. Biliran Jr. *et al.* 2000 Combinatorial regulation of phospholipid gene expression by the *UME6*, *SIN3* and *RPD6* genes. Nucleic Acids Res. 28: 3160–3167.

Ellis, C. D., F. Wang, C. W. MacDiarmid, S. Clark, T. Lyons *et al.*, 2004 Zinc and the Msc2 zinc transporter protein are required for endoplasmic reticulum function. J. Cell Biol. 166: 325–335.

Faergeman, N. J., P. N. Black, X. D. Zhao, J. Knudsen, and C. C. DiRusso, 2001 The Acyl-CoA synthetases encoded within *FAA1* and *FAA4* in *Saccharomyces cerevisiae* function as components of the fatty acid transport system linking import, activation, and intracellular Utilization. J. Biol. Chem. 276: 37051–37059.

Faergeman, N. J., S. Feddersen, J. K. Christiansen, M. K. Larsen, R. Schneiter *et al.*, 2004 Acyl-CoA-binding protein, Acb1p, is required for normal vacuole function and ceramide synthesis in *Saccharomyces cerevisiae*. Biochem. J. 380: 907–918.

Fakas, S., C. Konstantinou, and G. M. Carman, 2011 *DGK1*-encoded diacylglycerol kinase activity is required for phospholipid synthesis during growth resumption from stationary phase in *Saccharomyces cerevisiae*. J. Biol. Chem. 286: 1464–1474.

Farese, R. V. Jr. and T. C. Walther, 2009 Lipid droplets finally get a little R-E-S-P-E-C-T. Cell 139: 855–860.

Faulkner, A. J., X. Chen, J. Rush, B. Horazdovsky, C. J. Waechter *et al.*, 1999 The *LPP1* and *DPP1* gene products account for most of the isoprenoid phosphatase activities in *Saccharomyces cerevisiae*. J. Biol. Chem. 274: 14831–14837.

Fei, W., G. Alfaro, B. P. Muthusamy, Z. Klaassen, T. R. Graham *et al.*, 2008 Genome-wide analysis of sterol-lipid storage and trafficking in *Saccharomyces cerevisiae*. Eukaryot. Cell 7: 401–414.

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

Fernandez-Murray, J. P., and C. R. McMaster, 2005a Glycerophophocholine catabolism as a new route for choline formation for phosphatidylcholine synthesis by the Kennedy pathway. J. Biol. Chem. 280: 38290–38296.

Fernandez-Murray, J. P., and C. R. McMaster, 2005b Nte1p-mediated deacylation of phosphatidylcholine functionally interacts with Sec14p. J. Biol. Chem. 280: 8544–8552.

Fernandez-Murray, J. P., and C. R. McMaster, 2007 Phosphatidylcholine synthesis and its catabolism by yeast neuropathy target esterase 1. Biochim. Biophys. Acta 1771: 331–336.

Fernandez-Murray, J. P., G. J. Gaspard, S. A. Jesch, and C. R. McMaster, 2009 *NTE1*-encoded phosphatidylcholine phospholipase b regulates transcription of phospholipid biosynthetic genes. J. Biol. Chem. 284: 36034–36046.

Fischl, A. S., and G. M. Carman, 1983 Phosphatidylinositol biosynthesis in *Saccharomyces cerevisiae*: purification and properties of microsome-associated phosphatidylinositol synthase. J. Bacteriol. 154: 304–311.

Fisher, E., C. Almaguer, R. Holic, P. Griac, and J. Patton-Vogt, 2005 Glycerophosphocholine-dependent growth requires Gde1p (YPL110c) and Git1p in *Saccharomyces cerevisiae*. J. Biol. Chem. 280: 36110–36117.

Flanagan, C. A., E. S. Schnieders, A. W. Emerick, R. Kunisawa, A. Admon *et al.*, 1993 Phosphatidylinositol 4-kinase: Gene structure and requirement for yeast cell viability. Science 262: 1444–1448.

Flick, J. S., and J. Thorner, 1993 Genetic and biochemical characterization of a phosphatidylinositol-specific phospholipase C in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 13: 5861–5876.

Fujita, M., and Y. Jigami, 2008 Lipid remodeling of GPI-anchored proteins and its function. Biochim. Biophys. Acta. 1780: 410–420.

Fyrst, H., B. Oskouian, F. A. Kuypers, and J. D. Saba, 1999 The *PLB2* gene of *Saccharomyces cerevisiae* confers resistance to lysophosphatidylcholine and encodes a phospholipase B/lysophospholipase. Biochemistry 38: 5864–5871.

Gaigg, B., T. B. Neergaard, R. Schneiter, J. K. Hansen, N. J. Faergeman *et al.*, 2001 Depletion of acyl-coenzyme A-binding protein affects sphingolipid synthesis and causes vesicle accumulation and membrane defects in *Saccharomyces cerevisiae*. Mol. Biol. Cell 12: 1147–1160.

Garbarino, J., M. Padamsee, L. Wilcox, and P. M. Oelkers, D. D'Ambrosio *et al*., 2009 Sterol and diacylglycerol acyltransferase deficiency triggers fatty acid mediated cell death. J. Biol. Chem. 184: 30994–31005.

Garcia-Bustos, J. F., F. Marini, I. Stevenson, C. Frei, and M. N. Hall, 1994 *PIK1*, an essential phosphatidylinositol 4-kinase associated with the yeast nucleus. EMBO J. 13: 2352–2361.

Gardocki, M. E., N. Jani, and J. M. Lopes, 2005 Phosphatidylinositol biosynthesis: biochemistry and regulation. Biochim. Biophys. Acta 1735(2): 89–100.

Gary, J. D., T. K. Sato, C. J. Stefan, C. J. Bonangelino, L. S. Weisman *et al.*, 2002 Regulation of Fab1 phosphatidylinositol 3-phosphate 5-kinase pathway by Vac7 protein and Fig4, a polyphosphoinositide phosphatase family member. Mol. Biol. Cell 13: 1238–1251.

Gasch, A. P., P. T. Spellman, C. M. Kao, O. Carmel-Harel, M. B. Eisen *et al.*, 2000 Genomic expression programs in the re-

sponse of yeast cells to environmental changes. Mol. Biol. Cell 11: 4241–4257.
Gaspar, M. L., M. A. Aregullin, S. A. Jesch, and S. A. Henry, 2006 Inositol induces a profound alteration in the pattern and rate of synthesis and turnover of membrane lipids in *Saccharomyces cerevisiae*. J. Biol. Chem. 281: 22773–22785.
Gaspar, M. L., M. A. Aregullin, S. A. Jesch, L. R. Nunez, M. Villa-Garcia *et al.*, 2007 The emergence of yeast lipidomics. Biochim. Biophys. Acta 1771: 241–254.
Gaspar, M. L., S. A. Jesch, R. Viswanatha, A. L. Antosh, W. J. Brown *et al.*, 2008 A block in endoplasmic reticulum-to-Golgi trafficking inhibits phospholipid synthesis and induces neutral lipid accumulation. J. Biol. Chem. 283: 25735–25751.
Gaspar, M. L., H. F. Hofbauer, S. D. Kohlwein, and S. A. Henry, 2011 Coordination of storage lipid synthesis and membrane biogenesis: evidence for cross-talk between triacylglycerol metabolism and phosphatidylinositol synthesis. J. Biol. Chem. 286: 1696–1708.
Gaynor, P. M., and G. M. Carman, 1990 Phosphatidylethanolamine methyltransferase and phospholipid methyltransferase activities from *Saccharomyces cerevisiae*. Enzymological and kinetic properties. Biochim. Biophys. Acta 1045: 156–163.
Ghaemmaghami, S., W. K. Huh, K. Bower, R. W. Howson, A. Belle *et al.*, 2003 Global analysis of protein expression in yeast. Nature 425: 737–741.
Gohil, V. M., M. N. Thompson, and M. L. Greenberg, 2005 Synthetic lethal interaction of the mitochondrial phosphatidylethanolamine and cardiolipin biosynthetic pathways in *Saccharomyces cerevisiae*. J. Biol. Chem. 280: 35410–35416.
Gonzalez, C. I., and C. E. Martin, 1996 Fatty acid-responsive control of mRNA stability. Unsaturated fatty acid-induced degradation of the *Saccharomyces OLE1* transcript. J. Biol. Chem. 271: 25801–25809.
Goodmann, J. M., 2009 Demonstrated and inferred metabolism associated with cytosolic lipid droplets. J. Lipid Res. 50: 2148–2156.
Greenberg, M. L., and J. M. Lopes, 1996 Genetic regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae*. Microbiol. Rev. 60: 1–20.
Greenberg, M. L., B. Reiner, and S. A. Henry, 1982a Regulatory mutations of inositol biosynthesis in yeast: isolation of inositol excreting mutants. Genetics 100: 19–33.
Greenberg, M., P. Goldwasser, and S. Henry, 1982b Characterization of a regulatory mutant constitutive for inositol-1-phosphate synthetase. Mol. Gen. Genet. 186: 157–163.
Greenberg, M. L., L. S. Klig, V. A. Letts, B. Shicker-Loewy, and S. A. Henry, 1983 Yeast mutant defective in phosphatidylcholine synthesis. J. Bacteriol. 153: 791–799.
Griac, P., 2007 Sec14 related proteins in yeast. Biochim. Biophys. Acta 1771: 737–745.
Griac, P., M. J. Swede, and S. A. Henry, 1996 The role of phosphatidylcholine biosynthesis in the regulation of the *INO1* gene of yeast. J. Biol. Chem. 271: 25692–25698.
Gu, Z., F. Valianpour, S. Chen, F. M. Vaz, G. A. Hakkaart *et al.*, 2004 Aberrant cardiolipin metabolism in the yeast taz1 mutant: a model for Barth syndrome. Mol. Microbiol. 51: 149–158.
Guan, X. L., and M. R. Wenk, 2006 Mass spectrometry-based profiling of phospholipids and sphingolipids in extracts from *Saccharomyces cerevisiae*. Yeast 23: 465–477.
Guerinot, M. L., and D. Eide, 1999 Zeroing in on zinc uptake in yeast and plants. Curr. Opin. Plant Biol. 2: 244–249.
Guo, S., L. E. Stolz, S. M. Lemrow, and J. D. York, 1999 *SAC1*-like domains of yeast *SAC1*, I*NP52*, and *INP53* and of human synaptojanin encode polyphosphoinositide phosphatases. J. Biol. Chem. 274: 12990–12995.
Guo, Y., K. R. Cordes, R. V. Farese Jr. and T. C. Walther, 2009 Lipid droplets at a glance. J. Cell Sci. 122: 749–752.
Habeler, G., K. Natter, G. G. Thallinger, M. E. Crawford, S. D. Kohlwein *et al.*, 2002 YPL.db: the Yeast Protein Localization database. Nucleic Acids Res. 30: 80–83.
Ham, H. J., H. J. Rho, S. K. Shin, and H. J. Yoon, 2010 The *TGL2* gene of *Saccharomyces cerevisiae* encodes an active acylglycerol lipase located in the mitochondria. J. Biol. Chem. 285: 3005–3013.
Han, G., K. Gable, S. D. Kohlwein, F. Beaudoin, J. A. Napier *et al.*, 2002 The *Saccharomyces cerevisiae YBR159w* gene encodes the 3-ketoreductase of the microsomal fatty acid elongase. J. Biol. Chem. 277: 35440–35449.
Han, G.-S., C. N. Johnston, X. Chen, K. Athenstaedt, G. Daum *et al.*, 2001 Regulation of the *Saccharomyces cerevisiae DPP1*-encoded diacylglycerol pyrophosphate phosphatase by zinc. J. Biol. Chem. 276: 10126–10133.
Han, G.-S., A. Audhya, D. J. Markley, S. D. Emr, and G. M. Carman, 2002 The *Saccharomyces cerevisiae LSB6* gene encodes phosphatidylinositol 4-kinase activity. J. Biol. Chem. 277: 47709–47718.
Han, G.-S., C. N. Johnston, and G. M. Carman, 2004 Vacuole membrane topography of the *DPP1*-encoded diacylglycerol pyrophosphate phosphatase catalytic site from *Saccharomyces cerevisiae*. J. Biol. Chem. 279: 5338–5345.
Han, G.-S., W.-I. Wu, and G. M. Carman, 2006 The *Saccharomyces cerevisiae* lipin homolog is a Mg^{2+}-dependent phosphatidate phosphatase enzyme. J. Biol. Chem. 281: 9210–9218.
Han, G.-S., S. Siniossoglou, and G. M. Carman, 2007 The cellular functions of the yeast lipin homolog Pah1p are dependent on its phosphatidate phosphatase activity. J. Biol. Chem. 282: 37026–37035.
Han, G.-S., L. O'Hara, G. M. Carman, and S. Siniossoglou, 2008a An unconventional diacylglycerol kinase that regulates phospholipid synthesis and nuclear membrane growth. J. Biol. Chem. 283: 20433–20442.
Han, G.-S., L. O'Hara, S. Siniossoglou, and G. M. Carman, 2008b Characterization of the yeast *DGK1*-encoded CTP-dependent diacylglycerol kinase. J. Biol. Chem. 283: 20443–20453.
Han, S.-H., G.-S. Han, W. M. Iwanyshyn, and G. M. Carman, 2005 Regulation of the *PIS1*-encoded phosphatidylinositol synthase in *Saccharomyces cerevisiae* by zinc. J. Biol. Chem. 280: 29017–29024.
Hancock, L. C., R. P. Behta, and J. M. Lopes, 2006 Genomic analysis of the Opi- phenotype. Genetics 173: 621–634.
Hanson, B. A., and R. L. Lester, 1980 Effects of inositol starvation on phospholipid and glycan syntheses in *Saccharomyces cerevisiae*. J. Bacteriol. 142: 79–89.
Harbison, C. T., D. B. Gordon, T. I. Lee, N. J. Rinaldi, K. D. Macisaac *et al.*, 2004 Transcriptional regulatory code of a eukaryotic genome. Nature 431: 99–104.
Harington, A., E. Schwarz, P. P. Slonimski, and C. J. Herbert, 1994 Subcellular relocalization of a long-chain fatty acid CoA ligase by a suppressor mutation alleviates a respiration deficiency in *Saccharomyces cerevisiae*. EMBO J. 13: 5531–5538.
Hasslacher, M., A. S. Ivessa, F. Paltauf, and S. D. Kohlwein, 1993 Acetyl-CoA carboxylase from yeast is an essential enzyme and is regulated by factors that control phospholipid metabolism. J. Biol. Chem. 268: 10946–10952.
Heier, C., U. Taschler, S. Rengachari, M. Oberer, H. Wolinski *et al.*, 2010 Identification of Yju3p as functional orthologue of mammalian monoglyceride lipase in the yeast *Saccharomyces cerevisiae*. Biochim. Biophys. Acta 1801: 1063–1071.
Henry, S. A., 1982 Membrane lipids of yeast: biochemical and genetic studies, pp. 101–158 in *The Molecular Biology of the Yeast Saccharomyces. Metabolism and Gene Expression*, edited by J. N. Strathern, E. W. Jones, and J. R. Broach. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Henry, S. A., and J. L. Patton-Vogt, 1998 Genetic regulation of phospholipid metabolism: yeast as a model eukaryote. Prog.Nucleic Acid Res. 61: 133–179.

Herman, P. K., and S. D. Emr, 1990 Characterization of *VPS34*, a gene required for vacuolar protein sorting and vacuole segregation in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 10: 6742–6754.

Herrick, D., R. Parker, and A. Jacobson, 1990 Identification and comparison of stable and unstable mRNAs in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 10: 2269–2284.

Hiltunen, J. K., K. J. Autio, M. S. Schonauer, V. A. Kursu, C. L. Dieckmann *et al.*, 2010 Mitochondrial fatty acid synthesis and respiration. Biochim. Biophys. Acta 1797: 1195–1202.

Hirsch, J. P., and S. A. Henry, 1986 Expression of the *Saccharomyces cerevisiae* inositol-1-P synthetase gene (*INO1*) gene is regulated bt factors that affect phospholipid synthesis. Mol. Cell. Biol. 6: 3320–3328.

Hjelmstad, R. H., and R. M. Bell, 1987 Mutants of *Saccharomyces cerevisiae* defective in sn-1,2- diacylglycerol cholinephosphotransferase: Isolation, characterization, and cloning of the *CPT1* gene. J. Biol. Chem. 262: 3909–3917.

Hjelmstad, R. H., and R. M. Bell, 1988 The sn-1,2-diacylglycerol ethanolaminephosphotransferase of *Saccharomyces cerevisiae*. Isolation of mutants and cloning of the *EPT1* gene. J. Biol. Chem. 263: 19748–19757.

Hjelmstad, R. H., and R. M. Bell, 1990 The sn-1,2-diacylglycerol cholinephosphotransferase of *Saccharomyces cerevisiae*. Nucleotide sequence, transcriptional mapping, and gene product analysis of the *CPT1* gene. J. Biol. Chem. 265: 1755–1764.

Hjelmstad, R. H., and R. M. Bell, 1991 sn-1,2-diacylglycerol choline- and ethanolaminephosphotransferases in *Saccharomyces cerevisiae*. Nucleotide sequence of the *EPT1* gene and comparison of the *CPT1* and *EPT1* gene products. J. Biol. Chem. 266: 5094–5103.

Holthuis, J. C., and T. P. Levine, 2005 Lipid traffic: floppy drives and a superhighway. Nat. Rev. Mol. Cell Biol. 6: 209–220.

Homann, M. J., A. M. Bailis, S. A. Henry, and G. M. Carman, 1987 Coordinate regulation of phospholipid biosynthesis by serine in *Saccharomyces cerevisiae*. J. Bacteriol. 169: 3276–3280.

Hoppe, T., K. Matuschewski, M. Rape, S. Schlenker, H. D. Ulrich *et al.*, 2000 Activation of a membrane-bound transcription factor by regulated ubiquitin/proteasome-dependent processing. Cell 102: 577–586.

Hosaka, K., T. Kodaki, and S. Yamashita, 1989 Cloning and characterization of the yeast *CKI* gene encoding choline kinase and its expression in *Escherichia coli*. J. Biol. Chem. 264: 2053–2059.

Huh, W. K., J. V. Falvo, L. C. Gerke, A. S. Carroll, R. W. Howson *et al.*, 2003 Global analysis of protein localization in budding yeast. Nature 425: 686–691.

Huisinga, K. L., and B. F. Pugh, 2004 A genome-wide housekeeping role for TFIID and a highly regulated stress-related role for SAGA in Saccharomyces cerevisiae. Mol. Cell 13: 573–585.

Hung, W., K. A. Olson, A. Breitkreutz, and I. Sadowski, 1997 Characterization of the basal and pheromone-stimulated phosphorylation states of Ste12p. Eur. J. Biochem. 245: 241–251.

Hyde, M., L. Block-Alper, J. Felix, P. Webster, and D. J. Meyer, 2002 Induction of secretory pathway components in yeast is associated with increased stability of their mRNA. J. Cell Biol. 156: 2002.

Iwanyshyn, W. M., G. S. Han, and G. M. Carman, 2004 Regulation of phospholipid synthesis in *Saccharomyces cerevisiae* by zinc. J. Biol. Chem. 279: 21976–21983.

Jacquier, N., and R. Schneiter, 2010 Ypk1, the yeast orthologue of the human serum- and glucocorticoid-induced kinase, is required for efficient uptake of fatty acids. J. Cell Sci. 123: 2218–2227.

Jain, S., N. Stanford, N. Bhagwat, B. Seiler, M. Costanzo *et al.*, 2007 Identification of a novel lysophospholipid acyltransferase in *Saccharomyces cerevisiae*. J. Biol. Chem. 282: 30562–30569.

Jandrositz, A., J. Petschnigg, R. Zimmermann, K. Natter, H. Scholze *et al.*, 2005 The lipid droplet enzyme Tgl1p hydrolyzes both steryl esters and triglycerides in the yeast, *Saccharomyces cerevisiae*. Biochim. Biophys. Acta 1735: 50–58.

Jani, N. M., and J. M. Lopes, 2009 Regulated transcription of the Saccharomyces cerevisiae phosphatidylinositol biosynthetic gene, *PIS1*, yields pleiotropic effects on phospholipid synthesis. FEM. Yeast Res. 9: 552–564.

Janitor, M., and J. Subik, 1993 Molecular cloning of the *PEL1* gene of *Saccharomyces cerevisiae* that is essential for the viability of petite mutants. Curr. Genet. 24: 307–312.

Janitor, M., M. Obernauerova, S. D. Kohlwein, and J. Subik, 1996 The *pel1* mutant of *Saccharomyces cerevisiae* is deficient in cardiolipin and does not survive the disruption of the *CHO1* gene encoding phosphatidylserine synthase. FEMS Microbiol. Lett. 140: 43–47.

Jaschke, Y., J. Schwarz, D. Clausnitzer, C. Muller, and H. J. Schuller, 2011 Pleiotropic corepressors Sin3 and Ssn6 interact with repressor Opi1 and negatively regulate transcription of genes required for phospholipid biosynthesis in the yeast *Saccharomyces cerevisiae*. Mol. Genet. Genomics 285: 91–100.

Jesch, S. A., and S. A. Henry, 2005 Yeast inositol lipids: synthesis, regulation, and involvement in membrane trafficking and lipid signaling, pp. 105–242 in *Cell Biology and Dynamics of Yeast Lipids*, Vol. 36/661, edited by G. Daum. Research Signpost, Kerala, India.

Jesch, S. A., X. Zhao, M. T. Wells, and S. A. Henry, 2005 Genome wide analysis reveals inositol, not choline, as the major effector of Ino2p-Ino4p and unfolded protein response target gene expression in yeast. J. Biol. Chem. 280: 9106–9118.

Jesch, S. A., P. Liu, X. Zhao, M. T. Wells, and S. A. Henry, 2006 Multiple endoplasmic reticulum-to-nucleus signaling pathways coordinate phospholipid metabolism with gene expression by distinct mechanisms. J. Biol. Chem. 281: 24070–24083.

Jesch, S. A., M. L. Gaspar, C. J. Stefan, M. A. Aregullin, and S. A. Henry, 2010 Interruption of inositol sphingolipid synthesis triggers Stt4p-dependent protein kinase C signaling. J. Biol. Chem. 285: 41947–41960.

Jiang, F., H. S. Rizavi, and M. L. Greenberg, 1997 Cardiolipin is not essential for the growth of *Saccharomyces cerevisiae* on fermentable or non-fermentable carbon sources. Mol. Microbiol. 26: 481–491.

Johnson, D. R., L. J. Knoll, D. E. Levin, and J. I. Gordon, 1994a *Saccharomyces cerevisiae* contains four fatty acid activation (FAA) genes: an assessment of their role in regulating protein N-myristoylation and cellular lipid metabolism. J. Cell Biol. 127: 751–762.

Johnson, D. R., L. J. Knoll, N. Rowley, and J. I. Gordon, 1994b Genetic analysis of the role of *Saccharomyces cerevisiae* acyl-CoA synthetase genes in regulating protein N-myristoylation. J. Biol. Chem. 269: 18037–18046.

Joshi, A. S., J. Zhou, V. M. Gohil, S. Chen, and M. L. Greenberg, 2009 Cellular functions of cardiolipin in yeast. Biochim. Biophys. Acta 1793: 212–218.

Kaffman, A., N. M. Rank, E. M. O'Neill, L. S. Huang, and E. K. O'Shea, 1998 The receptor Msn5 exports the phosphorylated transcription factor Pho4 out of the nucleus. Nature 396: 482–486.

Kagiwada, S., and R. Zen, 2003 Role of the yeast VAP homolog, Scs2p, in *INO1* expression and phospholipid metabolism. J. Biochem. 133: 515–522.

Karanasios, E., G.-S. Han, Z. Xu, G. M. Carman, and S. Siniossoglou, 2010 A phosphorylation-regulated amphipathic helix controls the membrane translocation and function of the yeast phosphatidate phosphatase. Proc. Natl. Acad. Sci. USA 107: 17539–17544.

Karin, M., and T. Hunter, 1995 Transcriptional control by protein phosphorylation: signal transmission from the cell surface to the nucleus. Curr. Biol. 5: 747–757.

Kelley, M. J., and G. M. Carman, 1987 Purification and characterization of CDP-diacylglycerol synthase from *Saccharomyces cerevisiae*. J. Biol. Chem. 262: 14563–14570.

Kelley, M. J., A. M. Bailis, S. A. Henry, and G. M. Carman, 1988 Regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae* by inositol. Inositol is an inhibitor of phosphatidylserine synthase activity. J. Biol. Chem. 263: 18078–18085.

Kent, C., and G. M. Carman, 1999 Interactions of pathways for phosphatidylcholine metabolism, CTP synthesis, and secretion through the Golgi apparatus. Trends Biochem. Sci. 24: 146–150.

Kersting, M. C., and G. M. Carman, 2006 Regulation of the *Saccharomyces cerevisiae EKI1*-encoded ethanolamine kinase by zinc depletion. J. Biol. Chem. 281: 13110–13116.

Kihara, A., H. Sakuraba, M. Ikeda, A. Denpoh, and Y. Igarashi, 2008 Membrane topology and essential amino acid residues of Phs1, a 3-hydroxyacyl-CoA dehydratase involved in very long-chain fatty acid elongation. J. Biol. Chem. 283: 11199–11209.

Kim, K., K.-H. Kim, M. K. Storey, D. R. Voelker, and G. M. Carman, 1999 Isolation and characterization of the *Saccharomyces cerevisiae EKI1* gene encoding ethanolamine kinase. J. Biol. Chem. 274: 14857–14866.

Kiyono, K., K. Miura, Y. Kushima, T. Hikiji, M. Fukushima *et al.*, 1987 Primary structure and product characterization of the *Saccharomyces cerevisiae CHO1* gene that encodes phosphatidylserine synthase. J. Biochem. 102: 1089–1100.

Klig, L. S., and S. A. Henry, 1984 Isolation of the yeast *INO1* gene: located on an autonomously replicating plasmid, the gene is fully regulated. Proc. Natl. Acad. Sci. USA 81: 3816–3820.

Klig, L. S., M. J. Homann, G. M. Carman, and S. A. Henry, 1985 Coordinate regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae*: Pleiotropically constitutive *opi1* mutant. J. Bacteriol. 162: 1135–1141.

Kodaki, T., and S. Yamashita, 1987 Yeast phosphatidylethanolamine methylation pathway: Cloning and characterization of two distinct methyltransferase genes. J. Biol. Chem. 262: 15428–15435.

Kodaki, T., and S. Yamashita, 1989 Characterization of the methyltransferases in the yeast phosphatidylethanolamine methylation pathway by selective gene disruption. Eur. J. Biochem. 185: 243–251.

Koffel, R., R. Tiwari, L. Falquet, and R. Schneiter, 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, S. D., 2010a Obese and anorexic yeasts: experimental models to understand the metabolic syndrome and lipotoxicity. Biochim. Biophys. Acta 1801: 222–229.

Kohlwein, S. D., 2010b Triacylglycerol homeostasis: insights from yeast. J. Biol. Chem. 285: 15663–15667.

Kohlwein, S. D., S. Eder, C. S. Oh, C. E. Martin, K. Gable *et al.*, 2001 Tsc13p is required for fatty acid elongation and localizes to a novel structure at the nuclear-vacuolar interface in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 21: 109–125.

Komeili, A., and E. K. O'Shea, 1999 Roles of phosphorylation sites in regulating activity of the transcription factor Pho4. Science 284: 977–980.

Kornmann, B., E. Currie, S. R. Collins, M. Schuldiner, J. Nunnari *et al.*, 2009 An ER-mitochondria tethering complex revealed by a synthetic biology screen. Science 325: 477–481.

Kooijman, E.E., D.P. Tieleman, C. Testerink, T. Munnik, and D. T. Rijkers *et al*., 2007 An electrostatic/hydrogen bond switch as the basis for the specific interaction of phosphatidic acid with proteins. J. Biol. Chem. 282: 11356–11364.

Koshkin, V., and M. L. Greenberg, 2002 Cardiolipin prevents rate-dependent uncoupling and provides osmotic stability in yeast mitochondria. Biochem. J. 364: 317–322.

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

Kumar, A., S. Agarwal, J. A. Heyman, S. Matson, M. Heidtman *et al.*, 2002 Subcellular localization of the yeast proteome. Genes Dev. 16: 707–719.

Kurat, C. F., H. Wolinski, J. Petschnigg, S. Kaluarachchi, B. Andrews *et al.*, 2009 Cdk1/Cdc28-dependent activation of the major triacylglycerol lipase Tgl4 in yeast links lipolysis to cell-cycle progression. Mol. Cell 33: 53–63.

Kurat, C. F., K. Natter, J. Petschnigg, H. Wolinski, K. Scheuringer *et al.*, 2006 Obese yeast: triglyceride lipolysis is functionally conserved from mammals to yeast. J. Biol. Chem. 281: 491–500.

Kuziora, M. A., J. A. J. Chalmers, M. G. Douglas, R. A. Hitzeman, J. S. Mattick *et al.*, 1983 Molecular cloning of fatty acid synthase genes from *Saccharomyces cerevisiae*. J. Biol. Chem. 258: 11648–11653.

Kvam, E., K. Gable, T. M. Dunn, and D. S. Goldfarb, 2005 Targeting of Tsc13p to nucleus-vacuole junctions: a role for very-long-chain fatty acids in the biogenesis of microautophagic vesicles. Mol. Biol. Cell 16: 3987–3998.

Le Guedard, M., J. J. Bessoule, V. Boyer, S. Ayciriex, G. Velours *et al.*, 2009 PSI1 is responsible for the stearic acid enrichment that is characteristic of phosphatidylinositol in yeast. FEBS J. 276: 6412–6424.

Lee, K. S., J. L. Patton, M. Fido, L. K. Hines, S. D. Kohlwein *et al.*, 1994 The *Saccharomyces cerevisiae PLB1* gene encodes a protein required for lysophospholipase and phospholipase B activity. J. Biol. Chem. 269: 19725–19730.

Lee, T. I., N. J. Rinaldi, F. Robert, D. T. Odom, Z. Bar-Joseph *et al.*, 2002 Transcriptional regulatory networks in *Saccharomyces cerevisiae*. Science 298: 799–804.

Letts, V. A., L. S. Klig, M. Bae-Lee, G. M. Carman, and S. A. Henry, 1983 Isolation of the yeast structural gene for the membrane-associated enzyme phosphatidylserine synthase. Proc. Natl. Acad. Sci. USA 80: 7279–7283.

Levine, T., and C. Loewen, 2006 Inter-organelle membrane contact sites: through a glass, darkly. Curr. Opin. Cell Biol. 18: 371–378.

Li, G., S. Chen, M. N. Thompson, and M. L. Greenberg, 2007 New insights into the regulation of cardiolipin biosynthesis in yeast: implications for Barth syndrome. Biochim. Biophys. Acta 1771: 432–441.

Li, X., S. A. Gerber, A. D. Rudner, S. A. Beausoleil, W. Haas *et al.*, 2007 Large-scale phosphorylation analysis of alpha-factor-arrested *Saccharomyces cerevisiae*. J. Proteome Res. 6: 1190–1197.

Lin, Y.-P., and G. M. Carman, 1989 Purification and characterization of phosphatidate phosphatase from *Saccharomyces cerevisiae*. J. Biol. Chem. 264: 8641–8645.

Liu, C., Z. Yang, J. Yang, Z. Xia, and S. Ao, 2000 Regulation of the yeast transcriptional factor *PHO2* activity by phosphorylation. J. Biol. Chem. 275: 31972–31978.

Lockshon, D., L. E. Surface, E. O. Kerr, M. Kaeberlein, and B. K. Kennedy, 2007 The sensitivity of yeast mutants to oleic acid implicates the peroxisome and other processes in membrane function. Genetics 175: 77–91.

Loewen, C. J. R., A. Roy, and T. P. Levine, 2003 A conserved ER targeting motif in three families of lipid binding proteins and in Opi1p binds VAP. EMBO J. 22: 2025–2035.

Loewen, C. J. R., M. L. Gaspar, S. A. Jesch, C. Delon, N. T. Ktistakis *et al.*, 2004 Phospholipid metabolism regulated by a transcription factor sensing phosphatidic acid. Science 304: 1644–1647.

Loewy, B. S., and S. A. Henry, 1984 The *INO2* and *INO4* loci of yeast are pleiotropic regulatory genes. Mol. Cell. Biol. 4: 2479–2485.

Lyons, T. J., A. P. Gasch, L. A. Gaither, D. Botstein, P. O. Brown *et al.*, 2000 Genome-wide characterization of the Zap1p zinc-responsive regulon in yeast. Proc. Natl. Acad. Sci. USA 97: 7957–7962.

Malanovic, N., I. Streith, H. Wolinski, G. Rechberger, S. D. Kohlwein *et al.*, 2008 S-adenosyl-L-homocysteine hydrolase, key enzyme of methylation metabolism, regulates phosphatidylcholine synthesis and triacylglycerol homeostasis in yeast: implications for homocysteine as a risk factor of atherosclerosis. J. Biol. Chem. 283: 23989–23999.

Martin, C. E., C. S. Oh, and Y. Jiang, 2007 Regulation of long chain unsaturated fatty acid synthesis in yeast. Biochim. Biophys. Acta 1771: 271–285.

Mattiazzi, M., A. Jambhekar, P. Kaferle, J. L. Derisi, I. Krizaj *et al.*, 2010 Genetic interactions between a phospholipase A2 and the Rim101 pathway components in S. cerevisiae reveal a role for this pathway in response to changes in membrane composition and shape. Mol. Genet. Genomics 283: 519–530.

Mayr, J. A., S. D. Kohlwein, and F. Paltauf, 1996 Identification of a novel, Ca(2+)-dependent phospholipase D with preference for phosphatidylserine and phosphatidylethanolamine in *Saccharomyces cerevisiae*. FEBS Lett. 393: 236–240.

McDonough, V. M., J. E. Stukey, and C. E. Martin, 1992 Specificity of unsaturated fatty acid-regulated expression of the *Saccharomyces cerevisiae OLE1* gene. J. Biol. Chem. 267: 5931–5936.

McDonough, V. M., R. J. Buxeda, M. E. C. Bruno, O. Ozier-Kalogeropoulos, M.-T. Adeline *et al.*, 1995 Regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae* by CTP. J. Biol. Chem. 270: 18774–18780.

McGraw, P., and S. A. Henry, 1989 Mutations in the *Saccharomyces cerevisiae OPI3* gene: Effects on phospholipid methylation, growth, and cross pathway regulation of phospholipid synthesis. Genetics 122: 317–330.

McMaster, C. R., and R. M. Bell, 1994 Phosphatidylcholine biosynthesis in *Saccharomyces cerevisiae*. Regulatory insights from studies employing null and chimeric *sn*-1,2-diacylglycerol choline- and ethanolaminephosphotransferases. J. Biol. Chem. 269: 28010–28016.

Merkel, O., M. Fido, J. A. Mayr, H. Pruger, F. Raab *et al.*, 1999 Characterization and function in vivo of two novel phospholipases B/lysophospholipases from *Saccharomyces cerevisiae*. J. Biol. Chem. 274: 28121–28127.

Merkel, O., O. V. Oskolkova, F. Raab, R. El-Toukhy, and F. Paltauf, 2005 Regulation of activity in vitro and in vivo of three phospholipases B from Saccharomyces cerevisiae. Biochem. J. 387: 489–496.

Mileykovskaya, E., M. Zhang, and W. Dowhan, 2005 Cardiolipin in energy transducing membranes. Biochemistry (Mosc.) 70: 154–158.

Min-Seok, R., Y. Kawamata, H. Nakamura, A. Ohta, and M. Takagi, 1996 Isolation and characterization of *ECT1* gene encoding CTP:phosphoethanolamine cytidylyltransferase of *Saccharomyces cerevisiae*. J. Biochem. 120: 1040–1047.

Morash, S. C., C. R. McMaster, R. H. Hjelmstad, and R. M. Bell, 1994 Studies employing *Saccharomyces cerevisiae cpt1* and *ept1* null mutants implicate the *CPT1* gene in coordinate regulation of phospholipid biosynthesis. J. Biol. Chem. 269: 28769–28776.

Muccioli, G. G., A. Sia, P. J. Muchowski, and N. Stella, 2009 Genetic manipulation of palmitoylethanolamide production and inactivation in *Saccharomyces cerevisiae*. PLoS ONE 4: e5942.

Murphy, D. J., and J. Vance, 1999 Mechanisms for lipid-body formation. Trends Biochem. Sci. 24: 109–115.

Murray, M., and M. L. Greenberg, 2000 Expression of yeast *INM1* encoding inositol monophosphatase is regulated by inositol, carbon source and growth stage and is decreased by lithium and valproate. Mol. Microbiol. 36: 651–661.

Nadkarni, A. K., V. M. McDonough, W.-L. Yang, J. E. Stukey, O. Ozier-Kalogeropoulos *et al.*, 1995 Differential Biochemical Regulation of the *URA7*- and *URA8*-encoded CTP synthetases from *Saccharomyces cerevisiae*. J. Biol. Chem. 270: 24982–24988.

Nakanishi, H., P. de los Santos, and A. M. Neiman, 2004 Positive and negative regulation of a SNARE protein by control of intracellular localization. Mol. Biol. Cell 15: 1802–1815.

Natter, K., P. Leitner, A. Faschinger, H. Wolinski, S. McCraith *et al.*, 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.

Nikawa, J., and S. Yamashita, 1984 Molecular cloning of the gene encoding CDP-diacylglycerol- inositol 3-phosphatidyl transferase in *Saccharomyces cerevisiae*. Eur. J. Biochem. 143: 251–256.

Nikawa, J., Y. Tsukagoshi, and S. Yamashita, 1986 Cloning of a gene encoding choline transport in *Saccharomyces cerevisiae*. J. Bacteriol. 166: 328–330.

Nikawa, J., T. Kodaki, and S. Yamashita, 1987a Primary structure and disruption of the phosphatidylinositol synthase gene of *Saccharomyces cerevisiae*. J. Biochem. 262: 4876–4881.

Nikawa, J., Y. Tsukagoshi, T. Kodaki, and S. Yamashita, 1987b Nucleotide sequence and characterization of the yeast *PSS* gene encoding phosphatidylserine synthase. Eur. J. Biochem. 167: 7–12.

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

Nikawa, J., A. Murakami, E. Esumi, and K. Hosaka, 1995 Cloning and sequence of the SCS2 gene, which can suppress the defect of INO1 expression in an inositol auxotrophic mutant of Saccharomyces cerevisiae. J. Biochem. (Tokyo) 118: 39–45.

Nunez, L. R., S. A. Jesch, M. L. Gaspar, C. Almaguer, M. Villa-Garcia *et al.*, 2008 Cell wall integrity MAPK pathway is essential for lipid homeostasis. J. Biol. Chem. 283: 34204–34217.

Obermeyer, T., P. Fraisl, C. C. DiRusso, and P. N. Black, 2007 Topology of the yeast fatty acid transport protein Fat1p: mechanistic implications for functional domains on the cytosolic surface of the plasma membrane. J. Lipid Res. 48: 2354–2364.

Oelkers, P., A. Tinkelenberg, N. Erdeniz, D. Cromley, J. T. Billheimer *et al.*, 2000 A lecithin cholesterol acyltransferase-like gene mediates diacylglycerol esterification in yeast. J. Biol. Chem. 275: 15609–15612.

Oelkers, P., D. Cromley, M. Padamsee, J. T. Billheimer, and S. L. Sturley, 2002 The *DGA1* gene determines a second triglyceride synthetic pathway in yeast. J. Biol. Chem. 277: 8877–8881.

Oh, C. S., D. A. Toke, S. Mandala, and C. E. Martin, 1997 *ELO2* and *ELO3*, homologues of the *Saccharomyces cerevisiae ELO1* gene, function in fatty acid elongation and are required for sphingolipid formation. J. Biol. Chem. 272: 17376–17384.

Oshiro, J., G.-S. Han, and G. M. Carman, 2003 Diacylglycerol pyrophosphate phosphatase in *Saccarhomyces cerevisiae*. Biochim. Biophys. Acta 1635: 1–9.

Osman, C., M. Haag, C. Potting, J. Rodenfels, P. V. Dip *et al.*, 2009a The genetic interactome of prohibitins: coordinated control of cardiolipin and phosphatidylethanolamine by conserved regulators in mitochondria. J. Cell Biol. 184: 583–596.

Osman, C., C. Merkwirth, and T. Langer, 2009b Prohibitins and the functional compartmentalization of mitochondrial membranes. J. Cell Sci. 122: 3823–3830.

Osman, C., D. R. Voelker, and T. Langer, 2011 Making heads or tails of phospholipids in mitochondria. J. Cell Biol. 192: 7–16.

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

Ostrander, D. B., D. J. O'Brien, J. A. Gorman, and G. M. Carman, 1998 Effect of CTP synthetase regulation by CTP on phospholipid synthesis in *Saccharomyces cerevisiae*. J. Biol. Chem. 273: 18992–19001.

Ouyang, Q., M. Ruiz-Noriega, and S. A. Henry, 1999 The *REG1* gene product is required for repression of *INO1* and other inositol-sensitive upstream activating sequence-containing genes of yeast. Genetics 152: 89–100.

Ozier-Kalogeropoulos, O., F. Fasiolo, M.-T. Adeline, J. Collin, and F. Lacroute, 1991 Cloning, sequencing and characterization of the *Saccharomyces cerevisiae URA7* gene encoding CTP synthetase. Mol. Gen. Genet. 231: 7–16.

Ozier-Kalogeropoulos, O., M.-T. Adeline, W.-L. Yang, G. M. Carman, and F. Lacroute, 1994 Use of synthetic lethal mutants to clone and characterize a novel CTP synthetase gene in *Saccharomyces cerevisiae*. Mol. Gen. Genet. 242: 431–439.

Paltauf, F., S. D. Kohlwein, and S. A. Henry, 1992 Regulation and compartmentalization of lipid synthesis in yeast, pp. 415–500 in *The Molecular and Cellular Biology of the Yeast Saccharomyces: Gene Expression*, edited by E. W. Jones, J. R. Pringle, and J. R. Broach. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Pan, X., P. Roberts, Y. Chen, E. Kvam, N. Shulga *et al.*, 2000 Nucleus-vacuole junctions in *Saccharomyces cerevisiae* are formed through the direct interaction of Vac8p with Nvj1p. Mol. Biol. Cell 11: 2445–2457.

Parker, R., and H. Song, 2004 The enzymes and control of eukaryotic mRNA turnover. Nat. Struct. Mol. Biol. 11: 121–127.

Patton-Vogt, J., 2007 Transport and metabolism of glycerophosphodiesters produced through phospholipid deacylation. Biochim. Biophys. Acta 1771: 337–342.

Patton-Vogt, J. L., and S. A. Henry, 1998 *GIT1*, a gene encoding a novel transporter for glycerophosphoinositol in *Saccharomyces cerevisiae*. Genetics 149: 1707–1715.

Patton-Vogt, J. L., P. Griac, A. Sreenivas, V. Bruno, S. Dowd *et al.*, 1997 Role of the yeast phosphatidylinositol/phosphatidylcholine transfer protein (Sec14p) in phosphatidylcholine turnover and *INO1* regulation. J. Biol. Chem. 272: 20873–20883.

Paulus, H., and E. P. Kennedy, 1960 The enzymatic synthesis of inositol monophosphatide. J. Biol. Chem. 235: 1303–1311.

Peng, J., D. Schwartz, J. E. Elias, C. C. Thoreen, D. Cheng *et al.*, 2003 A proteomics approach to understanding protein ubiquitination. Nat. Biotechnol. 21: 921–926.

Petschnigg, J., H. Wolinski, D. Kolb, G. Zellnig, C. F. Kurat *et al.*, 2009 Good fat, essential cellular requirements for triacylglycerol synthesis to maintain membrane homeostasis in yeast. J. Biol. Chem. 284: 30981–30993.

Pittet, M., and A. Conzelmann, 2007 Biosynthesis and function of GPI proteins in the yeast *Saccharomyces cerevisiae*. Biochim. Biophys. Acta 1771: 405–420.

Pomorski, T., J. C. Holthuis, A. Herrmann, and G. van Meer, 2004 Tracking down lipid flippases and their biological functions. J. Cell Sci. 117: 805–813.

Poole, M. A., M. J. Homann, M. Bae-Lee, and G. M. Carman, 1986 Regulation of phosphatidylserine synthase from *Saccharomyces cerevisiae* by phospholipid precursors. J. Bacteriol. 168: 668–672.

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

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

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

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

Rattray, J. B., A. Schibeci, and D. K. Kidby, 1975 Lipids of yeast. Bacteriol. Rev. 39: 197–231.

Raychaudhuri, S., and W. A. Prinz, 2010 The diverse functions of oxysterol-binding proteins. Annu. Rev. Cell Dev. Biol. 26: 157–177.

Reddy, V. S., A. K. Singh, and R. Rajasekharan, 2008 The *Saccharomyces cerevisiae PHM8* gene encodes a soluble magnesium-dependent lysophosphatidic acid phosphatase. J. Biol. Chem. 283: 8846–8854.

Ren, G., P. Vajjhala, J. S. Lee, B. Winsor, and A. L. Munn, 2006 The BAR domain proteins: molding membranes in fission, fusion, and phagy. Microbiol. Mol. Biol. Rev. 70: 37–120.

Riekhof, W. R., J. Wu, M. A. Gijon, S. Zarini, R. C. Murphy *et al.*, 2007a Lysophosphatidylcholine Metabolism in *Saccharomyces cerevisiae*: The role of P-type ATPases in transport and a broad specificity acyltransferase in acylation. J. Biol. Chem. 282: 36853–36861.

Riekhof, W. R., J. Wu, J. L. Jones, and D. R. Voelker, 2007b Identification and characterization of the major lysophosphatidylethanolamine acyltransferase in *Saccharomyces cerevisiae*. J. Biol. Chem. 282: 28344–28352.

Rijken, P. J., R. H. Houtkooper, H. Akbari, J. F. Brouwers, M. C. Koorengevel *et al.*, 2009 Cardiolipin molecular species with shorter acyl chains accumulate in Saccharomyces cerevisiae mutants lacking the acyl coenzyme A-binding protein Acb1p: new insights into acyl chain remodeling of cardiolipin. J. Biol. Chem. 284: 27609–27619.

Rockenfeller, P., J. Ring, V. Muschett, A. Beranek, S. Buettner *et al.*, 2010 Fatty acids trigger mitochondrion-dependent necrosis. Cell Cycle 9: 2836–2842.

Rose, K., S. A. Rudge, M. A. Frohman, A. J. Morris, and J. Engebrecht, 1995 Phospholipase D signaling is essential for meiosis. Proc. Natl. Acad. Sci. USA 92: 12151–12155.

Roth, A. F., J. Wan, A. O. Bailey, B. Sun, J. A. Kuchar *et al.*, 2006 Global analysis of protein palmitoylation in yeast. Cell 125: 1003–1013.

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

Santiago, T. C., and C. B. Mamoun, 2003 Genome expression analysis in yeast reveals novel transcriptional regulation by inositol and choline and new regulatory functions for Opi1p, Ino2p, and Ino4p. J. Biol. Chem. 278: 38723–38730.

Santos-Rosa, H., J. Leung, N. Grimsey, S. Peak-Chew, and S. Siniossoglou, 2005 The yeast lipin Smp2 couples phospholipid biosynthesis to nuclear membrane growth. EMBO J. 24: 1931–1941.

Scharnewski, M., P. Pongdontri, G. Mora, M. Hoppert, and M. Fulda, 2008 Mutants of *Saccharomyces cerevisiae* deficient in acyl-CoA synthetases secrete fatty acids due to interrupted fatty acid recycling. FEBS J. 275: 2765–2778.

Schjerling, C. K., R. Hummel, J. K. Hansen, C. Borsting, J. M. Mikkelsen *et al.*, 1996 Disruption of the gene encoding the acyl-CoA-binding protein (ACB1) perturbs acyl-CoA metabolism in *Saccharomyces cerevisiae*. J. Biol. Chem. 271: 22514–22521.

Schnabl, M., O. V. Oskolkova, R. Holic, B. Brezna, H. Pichler *et al.*, 2003 Subcellular localization of yeast Sec14 homologues and their involvement in regulation of phospholipid turnover. Eur. J. Biochem. 270: 3133–3145.

Schneiter, R., and S. D. Kohlwein, 1997 Organelle structure, function, and inheritance in yeast: a role for fatty acid synthesis? Cell 88: 431–434.

Schneiter, R., B. Brugger, R. Sandhoff, G. Zellnig, A. Leber *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.

Schneiter, R., C. E. Guerra, M. Lampl, V. Tatzer, G. Zellnig *et al.*, 2000 A novel cold-sensitive allele of the rate-limiting enzyme of fatty acid synthesis, acetyl coenzyme A carboxylase, affects the morphology of the yeast vacuole through acylation of Vac8p. Mol. Cell. Biol. 20: 2984–2995.

Schu, P. V., K. Takegawa, M. J. Fry, J. H. Stack, M. D. Waterfield *et al.*, 1993 Phosphatidylinositol 3-kinase encoded by yeast VPS34 gene essential for protein sorting. Science 260: 88–91.

Schuck, S., W. A. Prinz, K. S. Thorn, C. Voss, and P. Walter, 2009 Membrane expansion alleviates endoplasmic reticulum stress independently of the unfolded protein response. J. Cell Biol. 187: 525–536.

Schuiki, I., M. Schnabl, T. Czabany, C. Hrastnik, and G. Daum, 2010 Phosphatidylethanolamine synthesized by four different pathways is supplied to the plasma membrane of the yeast *Saccharomyces cerevisiae*. Biochim. Biophys. Acta 1801: 480–486.

Schuller, H. J., A. Hahn, F. Troster, A. Schutz, and E. Schweizer, 1992 Coordinate genetic control of yeast fatty acid synthase genes FAS1 and FAS2 by an upstream activation site common to genes involved in membrane lipid biosynthesis. EMBO J. 11: 107–114.

Schuller, H. J., A. Schutz, S. Knab, B. Hoffmann, and E. Schweizer, 1994 Importance of general regulatory factors Rap1p, Abf1p and Reb1p for the activation of yeast fatty acid synthase genes FAS1 and FAS2. Eur. J. Biochem. 225: 213–222.

Schwabe, J. W., and A. Klug, 1994 Zinc mining for protein domains. [news; comment] Nat. Struct. Biol. 1: 345–349.

Schweizer, M., L. M. Roberts, H. J. Holtke, K. Takabayashi, E. Hollerer *et al.*, 1986 The pentafunctional *FAS1* gene of yeast: its nucleotide sequence and order of the catalytic domains. Mol. Gen. Genet. 203: 479–486.

Shelton, S. N., B. Barylko, D. D. Binns, B. F. Horazdovsky, J. P. Albanesi *et al.*, 2003 *Saccharomyces cerevisiae* contains a Type II phosphoinositide 4-kinase. Biochem. J. 371: 533–540.

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

Shirra, M. K., and K. M. Arndt, 1999 1999 Evidence for the involvement of the Glc7-Reg1 phosphatase and the Snf1-Snf4 kinase in the regulation of *INO1* transcription in *Saccharomyces cerevisiae*. Genetics 152: 73–87.

Shirra, M. K., J. Patton-Vogt, A. Ulrich, O. Liuta-Tehlivets, S. D. Kohlwein *et al.*, 2001 Inhibition of acetyl coenzyme A carboxylase activity restores expression of the INO1 gene in a snf1 mutant strain of *Saccharomyces cerevisiae*. Mol. Cell. Biol. 21: 5710–5722.

Shirra, M. K., S. E. Rogers, D. E. Alexander, and K. M. Arndt, 2005 The Snf1 protein kinase and Sit4 protein phosphatase have opposing functions in regulating TATA-binding protein association with the *Saccharomyces cerevisiae INO1* promoter. Genetics 169: 1957–1972.

Simockova, M., R. Holic, D. Tahotna, J. Patton-Vogt, and P. Griac, 2008 Yeast Pgc1p (YPL206c) controls the amount of phosphatidylglycerol via a phospholipase C-type degradation mechanism. J. Biol. Chem. 283: 17107–17115.

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

Sorger, D., and G. Daum, 2002 Synthesis of triacylglycerols by the acyl-coenzyme A:diacyl-glycerol acyltransferase Dga1p in lipid particles of the yeast *Saccharomyces cerevisiae*. J. Bacteriol. 184: 519–524.

Sorger, D., K. Athenstaedt, C. Hrastnik, and G. Daum, 2004 A yeast strain lacking lipid particles bears a defect in ergosterol formation. J. Biol. Chem. 279: 31190–31196.

Soto, A., and G. M. Carman, 2008 Regulation of the *Saccharomyces cerevisiae CKI1*-encoded choline kinase by zinc depletion. J. Biol. Chem. 283: 10079–10088.

Spellman, P. T., G. Sherlock, M. Q. Zhang, V. R. Iyer, K. Anders *et al.*, 1998 Comprehensive identification of cell cycle-regulated genes of the yeast Saccharomyces cerevisiae by microarray hybridization. Mol. Biol. Cell 9: 3273–3297.

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

Stålberg, K., A. C. Neal, H. Ronne, and U. Stahl, 2008 Identification of a novel GPCAT activity and a new pathway for phosphatidylcholine biosynthesis in *S. cerevisiae*. J. Lipid Res. 49: 1794–1806.

Stewart, L. C., and M. P. Yaffe, 1991 A role for unsaturated fatty acids in mitochondrial movement and inheritance. J. Cell Biol. 115: 1249–1257.

Stolz, L. E., C. V. Huynh, J. Thorner, and J. D. York, 1998a Identification and characterization of an essential family of inositol polyphosphate 5-phosphatases (*INP51*, *INP52* and *INP53* gene products) in the yeast *Saccharomyces cerevisiae*. Genetics 148: 1715–1729.

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

Stukey, J. E., V. M. McDonough, and C. E. Martin, 1989 Isolation and characterization of *OLE1*, a gene affecting fatty acid desaturation from *Saccharomyces cerevisiae*. J. Biol. Chem. 264: 16537–16544.

Summers, E. F., V. A. Letts, P. McGraw, and S. A. Henry, 1988 *Saccharomyces cerevisiae cho2* mutants are deficient in phospholipid methylation and cross-pathway regulation of inositol synthesis. Genetics 120: 909–922.

Szymanski, K. M., D. Binns, R. Bartz, N. V. Grishin, W. P. Li *et al.*, 2007 The lipodystrophy protein seipin is found at endoplasmic reticulum lipid droplet junctions and is important for droplet morphology. Proc. Natl. Acad. Sci. USA 104: 20890–20895.

Takata, Y., T. Yamada, Y. Huang, J. Komoto, T. Gomi *et al.*, 2002 Catalytic mechanism of S-adenosylhomocysteine hydrolase. Site-directed mutagenesis of Asp-130, Lys-185, Asp-189, and Asn-190. J. Biol. Chem. 277: 22670–22676.

Tamaki, H., A. Shimada, Y. Ito, M. Ohya, J. Takase *et al.*, 2007 *LPT1* encodes a membrane-bound O-acyltransferase involved in the acylation of lysophospholipids in the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. 282: 34288–34298.

Tanaka, K., R. Fukuda, Y. Ono, H. Eguchi, S. Nagasawa *et al.*, 2008 Incorporation and remodeling of extracellular phosphatidylcholine with short acyl residues in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta 1781: 391–399.

Tatzer, V., G. Zellnig, S. D. Kohlwein, and R. Schneiter, 2002 Lipid-dependent subcellular relocalization of the acyl chain desaturase in yeast. Mol. Biol. Cell 13: 4429–4442.

Taylor, G. S., T. Maehama, and J. E. Dixon, 2000 Myotubularin, a protein tyrosine phosphatase mutated in myotubular myopathy, dephosphorylates the lipid second messenger, phosphatidylinositol 3-phosphate. Proc. Natl. Acad. Sci. USA 97: 8910–8915.

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

Testet, E., J. Laroche-Traineau, A. Noubhani, D. Coulon, O. Bunoust *et al.*, 2005 Ypr140wp, 'the yeast tafazzin', displays a mi-

tochondrial lysophosphatidylcholine (lyso-PC) acyltransferase activity related to triacylglycerol and mitochondrial lipid synthesis. Biochem. J. 387: 617–626.
Thiele, C., and J. Spandl, 2008 Cell biology of lipid droplets. Curr. Opin. Cell Biol. 20: 378–385.
Toke, D. A., and C. E. Martin, 1996 Isolation and characterization of a gene affecting fatty acid elongation in Saccharomyces cerevisiae. J. Biol. Chem. 271: 18413–18422.
Toke, D. A., W. L. Bennett, D. A. Dillon, X. Chen, J. Oshiro *et al.*, 1998 Isolation and characterization of the *Saccharomyces cerevisiae DPP1* gene encoding for diacylglycerol pyrophosphate phosphatase. J. Biol. Chem. 273: 3278–3284.
Toke, D. A., W. L. Bennett, J. Oshiro, W. I. Wu, D. R. Voelker *et al.*, 1999 Isolation and characterization of the *Saccharomyces cerevisiae LPP1* gene encoding a Mg^{2+}-independent phosphatidate phosphatase. J. Biol. Chem. 273: 14331–14338.
Towler, D. A., S. P. Adams, S. R. Eubanks, D. S. Towery, E. Jackson-Machelski *et al.*, 1987 Purification and characterization of yeast myristoyl CoA:protein N-myristoyltransferase. Proc. Natl. Acad. Sci. USA 84: 2708–2712.
Travers, K. J., C. K. Patil, L. Wodicka, D. J. Lockhart, J. S. Weissman *et al.*, 2000 Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. Cell 101: 249–258.
Trotter, P. J., and D. R. Voelker, 1995 Identification of a non-mitochondrial phosphatidylserine decarboxylase activity (*PSD2*) in the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. 270: 6062–6070.
Trotter, P. J., J. Pedretti, and D. R. Voelker, 1993 Phosphatidylserine decarboxylase from *Saccharomyces cerevisiae*. Isolation of mutants, cloning of the gene, and creation of a null allele. J. Biol. Chem. 268: 21416–21424.
Trotter, P. J., J. Pedretti, R. Yates, and D. R. Voelker, 1995 Phosphatidylserine decarboxylase 2 of *Saccharomyces cerevisiae*. Cloning and mapping of the gene, heterologous expression, and creation of the null allele. J. Biol. Chem. 270: 6071–6080.
Tsukagoshi, Y., J. Nikawa, and S. Yamashita, 1987 Molecular cloning and characterization of the gene encoding cholinephosphate cytidylyltransferase in *Saccharomyces cerevisiae*. Eur. J. Biochem. 169: 477–486.
Tuller, G., C. Hrastnik, G. Achleitner, U. Schiefthaler, F. Klein *et al.*, 1998 YDL142c encodes cardiolipin synthase (Clslp) and is non-essential for aerobic growth of *Saccharomyces cerevisiae*. FEBS Lett. 421: 15–18.
Zaccheo, O., D. Dinsdale, P. A. Meacock, and P. Glynn, 2004 Neuropathy target esterase and its yeast homolog degrade phosphatidylcholine to glycerophosphocholine in living cells. J. Biol. Chem. 279: 24024–24033.
Vallee, B. L., and K. H. Falchuk, 1993 The biochemical basis of zinc physiology. Physiol. Rev. 73: 79–118.
van Gestel, R. A., P. J. Rijken, S. Surinova, M. O'Flaherty, A. J. Heck *et al.*, 2010 The influence of the acyl chain composition of cardiolipin on the stability of mitochondrial complexes; an unexpected effect of cardiolipin in alpha-ketoglutarate dehydrogenase and prohibitin complexes. J. Proteomics 73: 806–814.
Vicinanza, M., G. D'Angelo, A. Di Campli, and M. A. De Matteis 2008 Phosphoinositides as regulators of membrane trafficking in health and disease. Cell Mol. Life Sci. 65: 2833–2841.
Villa-García, M. J., M. S. Choi, F. I. Hinz, M. L. Gaspar, S. A. Jesch *et al.*, 2011 Genome-wide screen for inositol auxotrophy in *Saccharomyces cerevisiae* implicates lipid metabolism in stress response signaling. Mol. Genet. Genomics 285: 125–149.
Vemula, M., P. Kandasamy, C. S. Oh, R. Chellappa, C. I. Gonzalez *et al.*, 2003 Maintenance and regulation of mRNA stability of the *Saccharomyces cerevisiae OLE1* gene requires multiple elements within the transcript that act through translation-independent mechanisms. J. Biol. Chem. 278: 45269–45279.
Voelker, D. R., 2003 New perspectives on the regulation of intermembrane glycerophospholipid traffic. J. Lipid Res. 44: 441–449.
Wagner, C., M. Dietz, J. Wittmann, A. Albrecht, and H. J. Schuller, 2001 The negative regulator Opi1 of phospholipid biosynthesis in yeast contacts the pleiotropic repressor Sin3 and the transcriptional activator Ino2. Mol. Microbiol. 41: 155–166.
Waksman, M., Y. Eli, M. Liscovitch, and J. E. Gerst, 1996 Identification and characterization of a gene encoding phospholipase D activity in yeast. J. Biol. Chem. 271: 2361–2364.
Watkins, P. A., J. F. Lu, S. J. Steinberg, S. J. Gould, K. D. Smith *et al.*, 1998 Disruption of the *Saccharomyces cerevisiae FAT1* gene decreases very long-chain fatty acyl-CoA synthetase activity and elevates intracellular very long-chain fatty acid concentrations. J. Biol. Chem. 273: 18210–18219.
Wenz, P., S. Schwank, U. Hoja, and H. J. Schuller, 2001 A downstream regulatory element located within the coding sequence mediates autoregulated expression of the yeast fatty acid synthase gene FAS2 by the FAS1 gene product. Nucleic Acids Res. 29: 4625–4632.
White, M. J., J. P. Hirsch, and S. A. Henry, 1991 The *OPI1* gene of *Saccharomyces cerevisiae*, a negative regulator of phospholipid biosynthesis, encodes a protein containing polyglutamine tracts and a leucine zipper. J. Biol. Chem. 266: 863–872.
Wiradjaja, F., L. M. Ooms, J. C. Whisstock, B. McColl, L. Helfenbaum *et al.*, 2001 The yeast inositol polyphosphate 5-phosphatase Inp54p localizes to the endoplasmic reticulum via a C-terminal hydrophobic anchoring tail: regulation of secretion from the endoplasmic reticulum. J. Biol. Chem. 276: 7643–7653.
Wolinski, H., and S. D. Kohlwein, 2008 Microscopic analysis of lipid droplet metabolism and dynamics in yeast. Methods Mol. Biol. 457: 151–163.
Wolinski, H., K. Natter, and S. D. Kohlwein, 2009 The fidgety yeast: focus on high-resolution live yeast cell microscopy. Methods Mol. Biol. 548: 75–99.
Woods, A., M. R. Munday, J. Scott, X. Yang, M. Carlson *et al.*, 1994 Yeast SNF1 is functionally related to mammalian AMP-activated protein kinase and regulates acetyl-CoA carboxylase in vivo. J. Biol. Chem. 269: 19509–19515.
Wu, W.-I., and G. M. Carman, 1994 Regulation of phosphatidate phosphatase activity from the yeast *Saccharomyces cerevisiae* by nucleotides. J. Biol. Chem. 269: 29495–29501.
Wu, W.-I., and G. M. Carman, 1996 Regulation of phosphatidate phosphatase activity from the yeast *Saccharomyces cerevisiae* by phospholipids. Biochemistry 35: 3790–3796.
Wu, W.-I., Y.-P. Lin, E. Wang, A. H. Merrill Jr. and G. M. Carman, 1993 Regulation of phosphatidate phosphatase activity from the yeast *Saccharomyces cerevisiae* by sphingoid bases. J. Biol. Chem. 268: 13830–13837.
Wu, W.-I., Y. Liu, B. Riedel, J. B. Wissing, A. S. Fischl *et al.*, 1996 Purification and characterization of diacylglycerol pyrophosphate phosphatase from *Saccharomyces cerevisiae*. J. Biol. Chem. 271: 1868–1876.
Xie, Z., M. Fang, M. P. Rivas, A. J. Faulkner, P. C. Sternweis *et al.*, 1998 Phospholipase D activity is required for suppression of yeast phosphatidylinositol transfer protein defects. Proc. Natl. Acad. Sci. USA 95: 12346–12351.
Yamamoto, A., D. B. DeWald, I. V. Boronenkov, R. A. Anderson, S. D. Emr *et al.*, 1995 Novel PI(4)P 5-kinase homologue, Fab1p, essential for normal vacuole function and morphology in yeast. Mol. Biol. Cell 6: 525–539.
Yang, H., M. Bard, D. A. Bruner, A. Gleeson, R. J. Deckelbaum *et al.*, 1996 Sterol esterification in yeast: a two-gene process. Science 272: 1353–1356.
Yang, W.-L., V. M. McDonough, O. Ozier-Kalogeropoulos, M.-T. Adeline, M. T. Flocco *et al.*, 1994 Purification and characterization of CTP synthetase, product of the *URA7* gene in *Saccharomyces cerevisiae*. Biochemistry 33: 10785–10793.

Yoko-o, T., Y. Matsui, H. Yagisawa, H. Nojima, I. Uno *et al.*, 1993 The putative phosphoinositide-specific phospholipase C gene, *PLC1*, of the yeast *Saccharomyces cerevisiae* is important for cell growth. Proc. Natl. Acad. Sci. USA 90: 1804–1808.

Yoshida, S., Y. Ohya, M. Goebl, A. Nakano, and Y. Anraku, 1994a A novel gene, *STT4*, encodes a phosphatidylinositol 4-kinase in the *PKC1* protein kinase pathway of *Saccharomyces cerevisiae*. J. Biol. Chem. 269: 1166–1172.

Yoshida, S., Y. Ohya, A. Nakano, and Y. Anraku, 1994b Genetic interactions among genes involved in the *STT4–PKC1* pathway of *Saccharomyces cerevisiae*. Mol. Gen. Genet. 242: 631–640.

Youn, J. Y., H. Friesen, T. Kishimoto, W. M. Henne, C. F. Kurat *et al.*, 2010 Dissecting BAR Domain Function in the Yeast Amphiphysins Rvs161 and Rvs167 during Endocytosis. Mol. Biol. Cell 21: 3054–3069.

Young, B. P., J. J. Shin, R. Orij, J. T. Chao, S. C. Li *et al.*, 2010 Phosphatidic acid is a pH biosensor that links membrane biogenesis to metabolism. Science 329: 1085–1088.

Zaccheo, O., D. Dinsdale, P. A. Meacock, and P. Glynn, 2004 Neuropathy target esterase and its yeast homologue degrade phosphatidylcholine to glycerophosphocholine in living cells. J. Biol. Chem. 279: 24024–24033.

Zanghellini, J., K. Natter, C. Jungreuthmayer, A. Thalhammer, C. F. Kurat *et al.*, 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.

Zhang, M., X. Su, E. Mileykovskaya, A. A. Amoscato, and W. Dowhan, 2003 Cardiolipin is not required to maintain mitochondrial DNA stability or cell viability for Saccharomyces cerevisiae grown at elevated temperatures. J. Biol. Chem. 278: 35204–35210.

Zheng, Z., and J. Zou, 2001 The initial step of the glycerolipid pathway: identification of glycerol 3-phosphate/dihydroxyacetone phosphate dual substrate acyltransferases in *Saccharomyces cerevisiae*. J. Biol. Chem. 276: 41710–41716.

Zhong, Q., V. M. Gohil, L. Ma, and M. L. Greenberg, 2004 Absence of cardiolipin results in temperature sensitivity, respiratory defects, and mitochondrial DNA instability independent of pet56. J. Biol. Chem. 279: 32294–32300.

Zinser, E., C. D. Sperka-Gottlieb, E. V. Fasch, S. D. Kohlwein, F. Paltauf *et al.*, 1991 Phospholipid synthesis and lipid composition of subcellular membranes in the unicellular eukaryote Saccharomyces cerevisiae. J. Bacteriol. 173: 2026–2034.

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