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Review

Checks and balances in membrane phospholipid class and acyl chain homeostasis, the yeast perspective

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ABSTRACT

Glycerophospholipids are the most abundant membrane lipid constituents in most eukaryotic cells. As a consequence, phospholipid class and acyl chain homeostasis are crucial for maintaining optimal physical properties of membranes that in turn are crucial for membrane function. The topic of this review is our current understanding of membrane phospholipid homeostasis in the reference eukaryote *Saccharomyces cerevisiae*. After introducing the physical parameters of the membrane that are kept in optimal range, the properties of the major membrane phospholipids and their contributions to membrane structure and dynamics are summarized. Phospholipid metabolism and known mechanisms of regulation are discussed, including potential sensors for monitoring membrane physical properties. Special attention is paid to processes that maintain the phospholipid class specific molecular species profiles, and to the interplay between phospholipid class and acyl chain composition when yeast membrane lipid homeostasis is challenged. Based on the reviewed studies, molecular species selectivity of the lipid metabolic enzymes, and mass action in acyl-CoA metabolism are put forward as important intrinsic contributors to membrane lipid homeostasis.

Contents

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1. Introduction

Cells and intracellular compartments are separated from their environment by a barrier with a thickness in the range of 5–9 nm. These barriers, commonly known as membranes, are composed of a matrix of lipid molecules arranged in two layers or leaflets. The hydrophobic part of the lipids in each layer is directed inward, while the hydrophilic headgroups are exposed to the aqueous environment. The hydrophobic core renders membranes virtually impermeable for polar compounds. Each cellular membrane harbors its own unique set of proteins that is required for the functions of the enclosed compartment. The proteins embedded in and associated with the lipid bilayer perform a plethora of functions including the transport of polar compounds across the membrane, energy transduction, and the transfer of information in response to external stimuli.

Biological membranes not only vary in the proteins associated with them, but also in their lipid composition. Thousands of different lipid molecules have been identified in the lipidome of cellular membranes, each with its own unique properties, indicating that lipids do not only serve as passive membrane building blocks. They for instance create the appropriate environment for optimal catalytic activity and/or stability of membrane proteins. Therefore, knowledge of the mechanisms maintaining membrane lipid homeostasis is of paramount importance for understanding membrane functions and processes.

Membrane lipids can be divided in different families based on their molecular structure, with glycerophospholipids, glycoglycerolipids (in chloroplasts), sterols and sphingolipids being most abundant in eukaryotes. This review focuses on the regulation of membrane lipid homeostasis in the reference eukaryote *Saccharomyces cerevisiae*, a unicellular eukaryote commonly known as baker's yeast with longstanding and widely appreciated applications in bakeries and breweries. *S. cerevisiae*'s short cell cycle and the wide range of available molecular biology tools and genetic screens make it very suitable for biochemical and cell biological research. It is probably the best understood eukaryotic organism and an ideal model for higher eukaryotes because of the many homologies at the gene and protein levels.

In yeast, as in most other eukaryotes, the glycerophospholipids, from now on referred to as phospholipids, are the most abundant membrane lipids constituting some 70% of the membranes' lipid matrix [1]. Ergosterol (the yeast sterol) and the sphingolipids inositolphosphorylceramide (IPC), mannosyl-inositolphosphorylceramide (MIPC) and mannosyl-diinositolphosphorylceramide ($M(IP)_2C$) account for the remainder and are particularly abundant in the plasma membrane [2]. Phospholipid composition and synthesis are similar in yeast and mammalian cells. The phospholipids are subdivided in classes according to the nature of their polar headgroup with each class consisting of numerous molecular species, i.e. phospholipid molecules varying in the length and number of double bonds in their fatty acyl chains. Baker's yeast has a simple fatty acid profile compared to higher eukaryotes and lacks alkyl glycerolipids and sphingomyelin, facilitating membrane lipid analysis. Moreover, studies on mutant strains have shown that yeast can tolerate huge variations in membrane lipid composition rendering it the eukaryote of choice for investigating mechanisms and underlying principles that safeguard membrane integrity and function.

In the following, we will seek a better understanding of the mechanisms shaping bulk membrane phospholipid and acyl chain composition in yeast. First the physical properties of membranes determined by lipid composition will be discussed, followed by a concise overview of the properties and functions of the individual phospholipid classes. The biosynthesis of the phospholipids and its regulation will be briefly summarized. The processes and enzymes governing acyl chain homeostasis will be reviewed, followed by a short overview of (putative) membrane sensors. Next, the processes that determine phospholipid class specific acyl chain composition will be reviewed. Against this background, we will describe examples of the interplay between phospholipid class and acyl chain composition, including implications for the regulation of membrane lipid homeostasis.

2. Phospholipid composition determines the physical properties of the membrane

The lipid matrix of biological membranes is a complex assembly of different lipid molecules, tailored to the function of the particular membrane. The lipids constituting the bulk of the membrane lipid matrix determine generic physical properties of the membrane including membrane surface charge, membrane thickness, membrane fluidity, and membrane intrinsic curvature. For proper membrane structure and dynamics it is essential that these parameters are maintained in the appropriate range, also under varying environmental conditions. In addition, comparatively small quantities of lipid molecules are present or can be generated in the membrane, which serve functions in molecular recognition events

Table 1
Gel to liquid crystalline (T_m) phase transition temperatures (°C) of PC and PE and liquid crystalline to hexagonal H_{II} (T_H) phase transition temperatures of PE for a set of fully hydrated PC and PE molecular species relevant in yeast.[a]

*sn*1/*sn*2 acyl chains	T_m PC	T_m PE	T_H PE
18:0/18:0	54.5 ± 1.5	73.7 ± 1.4	101.8 ± 4.3
16:0/16:0	41.3 ± 1.8	62.3 ± 5.0	120.6 ± 3.6
16:1/16:1	−35.5[b]	−33.5	43.4 ± 0.2
16:0/18:1	−2.5 ± 2.4	24.4 ± 1.6	70.8 ± 2.5
18:1/18:1	−18.3 ± 3.6	−7.3 ± 3.8	8.5 ± 1.9

[a]Data for PC taken from [262] and [b][263]; data for PE taken from [264].

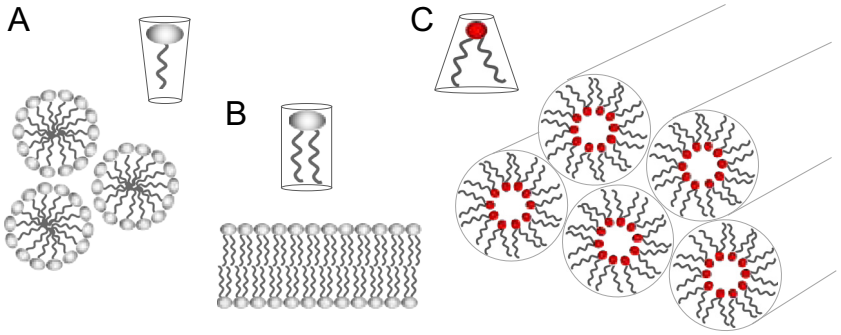

Fig. 1. Schematic representation of the molecular shapes of lipids. (A) Molecules with an overall inverted conical shape, e.g. lysophospholipids, assemble as structures with positive curvature such as micelles. (B) Cylindrical-shaped lipid molecules preferentially adopt bilayer structure. (C) Lipids with a conical molecular shape assemble as structures with negative curvature such as the inverted hexagonal (H_{II}) phase. Adapted from [13].

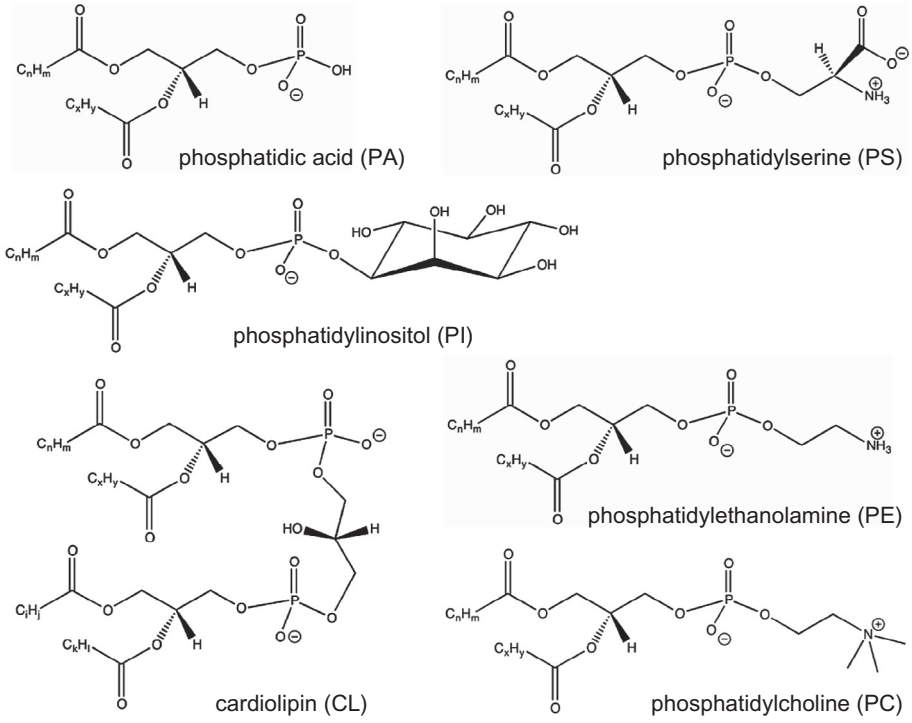

Fig. 2. Structures of glycerophospholipids, with C_xH_y, C_nH_m, C_iH_j, C_kH_l corresponding to the acyl chains.

(e.g. phosphoinositides, phosphatidic acid, diacylglycerol, ceramide, lyso-phospholipids).

Membrane surface charge depends on the membrane's content of negatively charged phospholipids, which mediates functional interactions with positively charged regions of peripheral and integral membrane proteins.

Membrane thickness is determined by the effective length of the lipid acyl chains, which in turn is modulated by the sterol content. Sterols have an ordering/stretching effect on the acyl chains present in phospholipids, increasing membrane thickness. Sphingolipids usually contain long to very long saturated acyl chains and due to the preferential interaction between sphingolipids and sterols form liquid ordered domains [3]. The enrichment of sterols and sphingolipids along the secretory pathway renders endosomes and plasma membranes thicker and sturdier as compared to other membranes [4,5].

Membrane fluidity at a given temperature depends on the lipid class and acyl chain composition and on the sterol content [6]. Poikilothermic organisms adjust their membrane lipid composition when exposed to changes in temperature to maintain optimal membrane fluidity. As the temperature drops, the organism may respond by increasing the content of unsaturated acyl chains, and/or decreasing the average acyl chain length, and/or decreasing the sterol content. Gel-to-liquid-crystalline phase transition temperatures (T_m) have been determined in bilayers consisting of individual synthetic phospholipid species. T_m values increase with increasing length of the acyl chains, and decrease in the order of disaturated to mono-unsaturated to di-unsaturated lipids (Table 1). T_m values for phosphatidylcholine (PC, introduced in Section 3.2) are lower than for the corresponding species of phosphatidylethanolamine (PE, introduced in Section 3.1) due to the tighter packing of the acyl chains in a PE bilayer resulting from the smaller dimensions of the PE headgroup. The membranes of yeast contain mostly di- and mono-unsaturated phospholipids composed of C16:1, C18:1, C16:0 and C18:0 acyl chains [7], and are in the liquid crystalline state at physiological temperatures. In these membranes,

sterols decrease membrane fluidity by their chain ordering effect. Yeast primarily responds to a drop in temperature by decreasing the average length of the acyl chains [8,9].

The ratio between bilayer- and non-bilayer preferring lipids in a membrane determines membrane intrinsic curvature [10], a property that should not be mistaken for membrane curvature. According to the shape–structure concept of lipid polymorphism [11], lipids with an overall cylindrical molecular shape, i.e. with the cross-sectional area of the headgroup matching that of the two acyl chains, will assemble in bilayers upon hydration. If the effective cross-sectional area of the headgroup is larger than that of the acyl chains (type I lipids), hydration results in the formation of structures with positive curvature, e.g. micelles. Cone-shaped lipids of which the effective cross-sectional area of the headgroup is smaller than that of the acyl chains (type II lipids) tend to adopt aggregate structures with negative curvature such as the inverted hexagonal phase (H_{II}) when hydrated (Fig. 1).

Type II lipids are major constituents of biological membranes and confer flexibility to the membrane that is important in fusion and fission reactions and for the functioning of membrane proteins. PE is a typical non-bilayer lipid, its propensity to adopt the H_{II} phase increases with temperature and acyl chain desaturation, while it decreases with acyl chain length (Table 1). C16:1/C18:1 (34:2) PE is the most abundant non-bilayer lipid in yeast. The impact of non-bilayer lipids on membrane flexibility can be defined using different concepts. Increasing amounts of a non-bilayer lipid in the leaflets of a membrane, give rise to curvature stress (“frustrated bilayer”) that can be relaxed e.g. by transition to the H_{II} phase, or by adsorbing peripheral membrane proteins. The first scenario is accompanied by loss of membrane barrier function and unlikely to occur in vivo. However, locally high concentrations of non-bilayer preferring lipids do facilitate membrane fusion [12]. In the second scenario the binding of peripheral membrane proteins is triggered by lipid packing defects that result from the increased level of non-bilayer lipids.

The effect of non-bilayer lipids on the physical properties of a membrane can also be described in terms of the lateral pressure profile, i.e. the lateral pressure varying with depth across the membrane. Type II lipids decrease the lateral pressure at the level of the lipid headgroups while increasing lateral pressure in the acyl chain region and shifting it towards the center of the bilayer. Changes in lateral pressure profile can easily be envisioned to affect membrane protein function. For an elaborate review on the influence of non-bilayer lipids on membrane proteins the reader is referred to Ref. [13].

3. Properties and functions of membrane phospholipids

Glycerophospholipids consist of a glycerol backbone with two hydrophobic acyl chains esterified at the *sn*-1 and *sn*-2 positions and a phosphate or phosphorylalcohol moiety esterified at the *sn*-3 position (Fig. 2). The mitochondrial lipid cardiolipin (CL) is an exception to this rule, as it contains 4 acyl chains. Table 2 shows the typical phospholipid composition of wild type yeast cultured on the non-fermentable carbon source lactate. Phospholipid and acyl chain composition in yeast are highly dependent on the growth phase of the cells and the composition of the culture medium [14–16]. Phospholipids are classified according to the structure of their headgroup, which is zwitterionic in phosphatidylcholine (PC) and phosphatidylethanolamine (PE), and anionic in phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidic acid (PA), and cardiolipin (CL) (Fig. 2). The phospholipid classes each comprise a multitude of molecular species defined by the nature of the acyl chains esterified at the *sn*-1 and *sn*-2 positions. In the following, the properties and functions of the main phospholipid classes found in yeast are described.

Table 2
Typical phospholipid composition of yeast cell homogenate. Wild type strain D273-10B was cultured on semi-synthetic lactate medium to the late logarithmic phase, and phospholipid composition was determined. ‘Other’ phospholipids include PA, PG, PMME and PDME. Data taken from [14].

Phospholipid class	mol%
PC	38
PE	30
PS	4
PI	16
CL	5
Other	7

3.1. Phosphatidylethanolamine

Whereas PC, PI, PS, CL and PA spontaneously assemble as lipid bilayers (liposomes), when hydrated at physiological pH ($5 < pH < 8$) and osmolarity, PE may organize as an inverted hexagonal (H_{II}) phase. The non-bilayer propensity of PE depends on its acyl chain composition with double bonds and longer acyl chains promoting the tendency toward negative membrane curvature (Table 1) [11,17].

PE is an essential phospholipid in yeast [18]. The importance of PE for mitochondrial function in yeast is well established, as depletion of PE in mitochondria leads to impaired respiration, defects in the assembly of mitochondrial protein complexes and loss of mitochondrial DNA [19,20]. PE is also required for delivery of cytoplasmic proteins to the vacuole by autophagy, since covalent binding of PE to Atg8p is essential for the assembly of autophagosomes, double membrane vesicles that enclose cytoplasmic proteins [21]. Furthermore, PE has been implicated in the transport of the amino acid transporters to the plasma membrane [22,23].

3.2. Phosphatidylcholine

PC is the most abundant phospholipid in most eukaryotic cells including yeast (Table 2). PC (or *N*-mono- or *N*-dimethylated PE) is essential for yeast growth [24–27]. PC is structurally related to PE, since both lipids carry an aminoalcohol in their headgroup. As a result, these lipids are zwitterionic, having a positive and negative charge on the amine and phosphate, respectively. However, the three methyl groups attached to the amine in PC render the volume of the PC headgroup larger than that of PE. Moreover, the PC headgroup is not capable of forming H-bonds with e.g. the phosphoryl groups of neighboring lipids [28]. As a result, the hydration level of PC is higher than that of PE.

All three effects cause weaker lipid–lipid interactions in PC bilayers [28,29], as illustrated by the fact that the gel-to-liquid transition temperature of PC is about 20 °C lower than that of PE with similar acyl chains (Table 1). The chemical structure and the hydration render the cross-sectional area occupied by the PC headgroup comparable to that of the acyl chains, i.e. PC is a typical bilayer lipid with a ‘cylindrical’ shape.

An important function of PC is to form a stable matrix for (intra)cellular membranes, which is facilitated by the geometrical shape and physico-chemical properties of PC discussed above. The importance of PC for stabilizing the membrane bilayer is underscored by the cellular response to depletion of PC in yeast that involves integration of shorter and more saturated acyl chains in PE to increase its bilayer propensity [26].

Together with other membrane constituents, PC shapes the lateral pressure profile within membranes, i.e. the repulsive/attractive forces between lipids as a function of the depth in the membrane [13]. By changing the forces at the appropriate depths

in the membrane, the stability and/or activity of membrane proteins and membrane protein complexes might be influenced. A mechanosensitive Ca^{2+}-influx mechanism in the yeast plasma membrane may present an example. Although the protein(s) involved are unknown, PC depletion was shown to sensitize them, resulting in an increased Ca^{2+} influx after osmotic down shock [30].

A completely different function of PC is to serve as a reservoir of lipid messengers. Many signaling cascades involve a step in which PC is hydrolyzed to yield PA, DAG, lyso-PC and/or free fatty acids [31]. An important enzyme in this regard is the yeast phospholipase D Spo14p that generates PA required for sporulation, reviewed in [32]. Defects in PC biosynthesis in yeast lead to respiratory deficiency implicating PC in mitochondrial function [33]. PC has been implicated in the functioning of the mitochondrial glycerol-3-phosphate dehydrogenase Gut2p [34].

3.3. Phosphatidylinositol

PI is another essential and abundant membrane phospholipid. PI serves as biosynthetic precursor for the phosphoinositides that are essential in signal transduction, as they recruit proteins containing phosphoinositide-binding domains and serve as precursor of second messengers in intracellular signaling pathways [35]. Moreover PI is the precursor of GPI-proteins [36] and yeast sphingolipids [37], additional membrane constituents essential for viability. With a pK_a value of the phosphodiester moiety around 2.5 [38], PI is negatively charged at physiological pH. The PI content of yeast membranes strongly depends on the availability of inositol [39,40], suggesting that yeast easily copes with large variations in membrane surface charge. Compared to the other phospholipid classes, PI is most enriched in saturated acyl chains [1,4].

3.4. Phosphatidylserine

PS is a negatively charged phospholipid in the physiological pH range with apparent pK_a values around 10, 4.5, and <3 for the amino-, carboxyl-, and phosphate groups, respectively [41,42]. PS is a quantitatively minor phospholipid (Table 2), but has a pivotal function in phospholipid biosynthesis as precursor of PE and PC in the CDP-DAG pathway (see forthcoming Section 4.1). PS performs crucial functions in the cell biology of higher eukaryotes, e.g. as specific activator of protein kinase C [43]. PS is not essential in yeast provided that the synthesis of PE and PC is sustained [19,44]. However, PS is required for correct targeting and activation of the small GTPase Cdc42p during cell division and mating [45].

3.5. Cardiolipin

CL is a special phospholipid in many respects. Whereas other phospholipids are found in most organellar membranes, CL is exclusively localized to mitochondria, primarily but not exclusively in the mitochondrial inner membrane [46–48]. Cardiolipin is a phospholipid that is composed of two 1,2-diacylphosphatidate moieties that are connected to the 1- and 3-hydroxyl groups of a single glycerol headgroup (Fig. 2). Thus, cardiolipin is essentially a fusion of two regular phospholipids, which results in unique properties. Due to the two phosphate groups, CL could have a net charge of −2. However, in a titration experiment CL appeared to have a pK_a of 2.8 and a second pK_a of 7.5–9.5, implying that CL is a monovalent anion under physiological conditions [49]. Accordingly, a recent analysis of CL-vesicles by IR spectroscopy yielded pK_a values of 4.7 and 7.9 [50]. Since the second pK_a was much lower in the absence of the hydroxyl group of the central glycerol group, it was postulated that the second proton is trapped in a resonance structure involving this hydroxyl group and the two phosphate groups [49]. Proton trapping by CL has been proposed to play a role in oxidative phosphorylation [51].

The cross-sectional area of the CL headgroup is relatively small compared to that of the acyl chains, rendering CL inclined to form inverted hexagonal membrane structures, but only when the repulsive forces between the phosphates are decreased in the presence of (divalent) cations, including protons (i.e. low pH) [52]. Increasing unsaturation and length of the acyl chains promote formation of the H_{II} phase in cardiolipin [53,54], similar to PE (Sections 2 and 3.1). The repertoire of acyl chains found in CL is very limited and enriched in unsaturated acyl chains, comprising only C16:1 and/or C18:1 in *S. cerevisiae* [55], suggesting that the non-bilayer propensity of CL is important for function. The ability of CL to switch between lamellar and non-lamellar structures might play a role in mitochondrial fusion and fission processes [56,57], and in shaping the mitochondrial cristae [58], but it might also facilitate the presence of CL in membrane domains with different curvatures.

Although CL is not essential in yeast [59], it is important for mitochondrial structure and function in various ways (reviewed in [60]), for example by supporting the stability and activity of respiratory supercomplexes [61]. In a yeast mutant lacking CL, its biosynthetic precursor phosphatidylglycerol (PG), and PE presumably replace it. PG and CL share a negatively charged headgroup while PE and CL share non-bilayer propensity, explaining the functional redundancy. In this context it is worth noting that deletion of *CRD1* encoding CL synthase and *PSD1* encoding the main PE-synthesizing enzyme is synthetically lethal [62]. Furthermore, the synthesis of mitochondrial PE and CL has recently been shown to be coordinately regulated involving newly discovered proteins (reviewed in [63]), discussion of which is beyond the scope of this review.

3.6. Phosphatidic acid

Although quantitatively negligible at 1 mol% of membrane lipids, PA is a key intermediate in glycerolipid synthesis and has important regulatory functions impacting membrane lipid synthesis and composition. Unlike the other negatively charged phospholipids, PA contains a phosphomonoester headgroup that has a first pK_a value around 3.2 and a second pK_a in the range of 6.9–7.9, the exact value depending on the locally available concentration of hydrogen bond donors [64]. The primary amine group of PE engages in hydrogen-bonding with the phosphomonoester lowering the second pK_a of PA by destabilizing the intramolecular hydrogen bond within the phosphomonoester headgroup, whereas the quaternary amine group of PC cannot. As a consequence the negative charge of the PA headgroup will not only depend on pH but also on the locally prevailing ratio between the bulk membrane lipids PC and PE, and increase with decreasing PC/PE ratios. In turn the recruitment of effector proteins by the signaling lipid PA depends on the hydrogen-bond status of PA [65], and on intracellular pH as was shown for Opi1p, the repressor of the phospholipid biosynthetic genes [66].

Like CL, di-unsaturated PA can switch to the inverted hexagonal phase upon charge neutralization and headgroup dehydration. This transition already occurs at mildly acidic pH in the presence of submillimolar concentrations of divalent cations [67]. The packing properties of PA conferred by its type II lipid nature further contribute to the functions of PA in the cell. Recently, the physicochemical properties of PA in relation to its functions were discussed in two excellent reviews [68,69].

4. Biosynthesis of membrane phospholipids and its regulation

In the following a brief overview of the biosynthesis of phospholipids is presented, followed by a summary of the main

regulatory mechanisms governing phospholipid synthesis in yeast. For a more complete coverage of these topics the reader is referred to recent reviews [70,71].

4.1. Pathways of phospholipid synthesis

The biosynthesis of glycerophospholipids in yeast is similar to that in higher eukaryotes. The formation of PA initiates the synthesis of all glycerophospholipids and of triacylglycerol (TAG) (Fig. 3). The first committed step is the transfer of an acyl chain from acyl-CoA to the *sn*-1 position of glycerol-3-phosphate by Gpt2p/Gat1p or Sct1p/Gat2p to produce lyso-PA [72]. Sct1p and Gpt2p also use dihydroxyacetone phosphate as substrate to produce acyl-DHAP, which is converted to lyso-PA by Ayr1p [73]. Subsequently a second acyl chain is transferred from acyl-CoA to the *sn*-2 position by Slc1p or Ale1p to yield PA [74]. The acyl-CoAs used derive from *de novo* synthesis described in Section 5.1, from exogenous fatty acids, or from hydrolysis of phospholipids, triacylglycerol and sterylesters. Free fatty acids are activated to acyl-CoAs by acyl-CoA synthetases (reviewed in [75]).

PA can either be dephosphorylated to diacylglycerol (DAG) by Pah1p [76], or converted to CDP-DAG by Cds1p (Fig. 3, [77]), both important intermediates for distinct branches of phospholipid synthesis. DAG is substrate for TAG synthesis by Dga1p [78,79] or Lro1p [80], and contributes to PE and PC synthesis through the CDP-ethanolamine and CDP-choline branches of the Kennedy pathway, respectively. Yeast contains three additional phosphatidate phosphatases, which in contrast to Pah1p do not contribute to *de novo* lipid biosynthesis. Lpp1p [81] and Dpp1p [82] are thought to locally control PA, DAG pyrophosphate and lyso-PA levels in the Golgi and vacuole, respectively, whereas the recently identified App1p has been proposed to regulate PA and DAG concentrations in endocytic vesicle formation [83]. The regulation of Pah1p activity is crucial in lipid homeostasis (reviewed in [84]). In addition to the PA phosphatases, the DAG kinase Dgk1p catalyzing the reverse reaction plays an important role in regulating cellular levels of DAG and PA [85], and is required for resumption of growth from stationary phase [86].

Interestingly, Cds1p has a dual localization in the ER and mitochondria [77,87], implying that PA is transported from the ER to mitochondria. In the ER CDP-DAG can be converted to PI by Pis1p [88], or to PS by Cho1p [89], while in mitochondria it is converted to phosphatidylglycerol phosphate (PGP) by Pgs1p [90] (Fig. 3). The inositol used in the synthesis of PI is either produced from glucose-6-phosphate involving the enzymes Ino1p and Inm1p, or imported from the culture medium.

PS, synthesized by Cho1p in the ER, is decarboxylated to PE, which requires interorganelle lipid transport. Psd1p in the inner mitochondrial membrane is the major PS decarboxylase [91,92], while Psd2p [93] in the Golgi is a minor contributor to cellular PE. PE can subsequently be methylated in three steps to yield PC by two methyltransferases localized to the ER that use *S*-adenosyl-L-methionine (SAM) as methyl donor. Interestingly, methylation of Psd2p-derived PE was shown to be preferred over that of PE synthesized by Psd1p [95]. Cho2p/Pem1p produces phosphatidylmonomethylethanolamine (PMME) and Opi3/Pem2p carries out the second and third methylation, yielding phosphatidyldimethylethanolamine (PDME) and PC [24,96–98].

The formation of PGP in mitochondria by Pgs1p is the committed step in the synthesis of PG and CL. Gep4p subsequently dephosphorylates PGP to produce PG [99], which reacts with another CDP-DAG molecule catalyzed by the CL synthase Crd1p in the inner mitochondrial membrane to yield CL [59,100,101].

In the choline branch of the Kennedy pathway, a.k.a. the CDP-choline pathway, choline is first phosphorylated by cytosolic Cki1p [102]. The subsequent conversion of phosphocholine to CDP-choline by Pct1p localized to the nuclear periphery [103] is the rate-limiting step of the CDP-choline pathway under conditions of choline limitation [104,105]. Cpt1p of which the localization remains ill-defined, finally transfers the phosphocholine moiety from CDP-choline to DAG yielding PC [106]. The enzymes Ek i1p, Ect1p and Ept1p catalyze three similar steps in the CDP-ethanolamine route of PE synthesis [107–109]. The CDP-ethanolamine branch of the Kennedy pathway is partially redundant with the CDP-choline branch, in that Eki1p and Ept1p share overlapping substrate specificities with Cki1p and Cpt1p, respectively [107,110], and may also contribute to PC synthesis. Ect1p and Pct1p on the other hand are specific to PE and PC synthesis, respectively [104,108]. In the absence of exogenous choline the CDP-choline route is active as a recycling pathway that salvages choline produced by turnover of PC [112].

The two PC biosynthetic routes can substitute for one another (reviewed in [27]). The decarboxylation by Psd1p is the main source of cellular PE; *psd1Δ* cells cultured on a non-fermentable carbon source are ethanolamine auxotrophs [19]. In the absence of exogenous ethanolamine, the CDP-ethanolamine route relies on ethanolaminephosphate from sphingolipid catabolism, which yields sufficient PE to sustain growth of *psd1Δpsd2Δ* cells on a fermentable carbon source in the absence of exogenous ethanolamine supplementation [19].

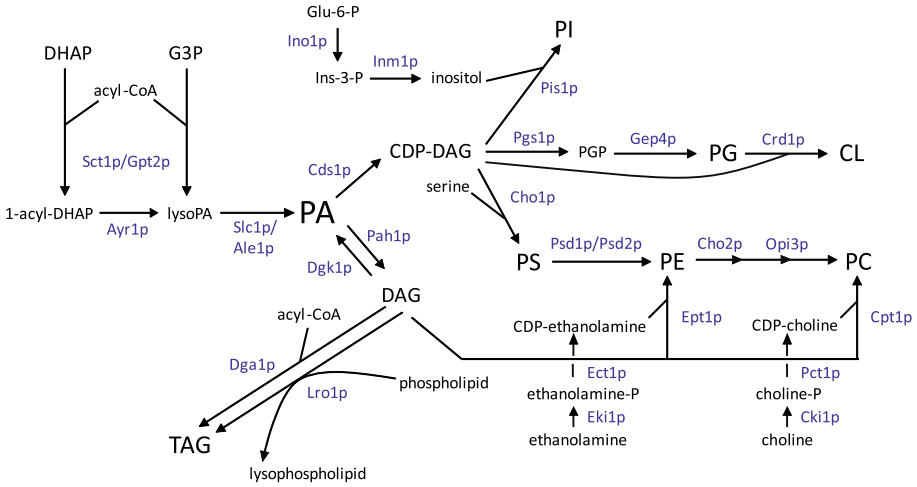

Fig. 3. Glycerolipid synthesis in yeast, with the enzymes depicted in blue. See text for details.

4.2. Regulation of phospholipid biosynthesis in yeast

The biosynthesis of phospholipids is regulated at the levels of gene expression, posttranslational modification, and enzyme activity via complex mechanisms and involving multiple factors. An overview of the key regulatory mechanisms in yeast is presented.

4.2.1. UAS_{INO} regulation

PA content plays a crucial role in the transcriptional regulation of many phospholipid biosynthesis genes as high levels of PA in the ER induce the transcription of genes possessing an inositol-responsive element (UAS_{INO}) [113]. When PA levels are high, the suppressor of transcription Opi1p is trapped in the ER by binding to the ER membrane protein Scs2p and PA and prevented from entering the nucleus [114]. Under these conditions, the Ino2–Ino4 heterodimer binds to the UAS_{INO} elements of different genes, and activates transcription. When PA levels are reduced, Opi1p is released and enters into the nucleus where it binds Ino2p, thus inhibiting transcriptional activation of the UAS_{INO} containing genes. The phospholipid biosynthesis genes controlled by an inositol-responsive element are: *INO1*, *CDS1*, *CHO1*, *PSD1*, *CHO2*, *OPI3*, *EKI1*, *EPT1*, *CKI1* and *CPT1* [70].

The *OPI1* gene was first identified in a screen for mutants that caused an OverProduction of Inositol (Opi^-) phenotype characterized by the excretion of inositol into the growth medium [115], due to the derepression of the *INO1* gene that shows the strongest upregulation of all UAS_{INO} genes. Secretion of inositol and inositol auxotrophy are convenient tools for diagnosing aberrant regulation of phospholipid synthesis [116].

Supplementation of inositol to culture media increases the utilization of CDP-DAG for PI synthesis, decreasing the amount of PA available for binding Opi1p and resulting in the repression of UAS_{INO} containing genes. The additional supplementation of choline enhances the UAS_{INO} repression by inositol, whereas choline by itself only has a limited effect [116]. Conversely, interference with the synthesis of PC through the CDP-DAG pathway in *cho2* and *opi3* mutants leads to derepression of *INO1* irrespective of the presence of inositol, which is suppressed by supplying choline [33]. In the absence of choline, Opi1p is sequestered at the ER membrane due to the increased level of PA and to the increased negative charge of PA resulting from the accumulation of PE and/or PMME (Section 3.6). Choline induces the CDP-choline route to consume DAG that draws upon the pool of PA, restoring the repression of *INO1*.

In addition to repressing phospholipid synthesis at the transcriptional level, inositol reduces PS synthesis by shifting the partitioning of the available CDP-DAG to PI, and by a direct inhibitory effect on Cho1p [39].

Recently, the *NTE1*-encoded phospholipase B that degrades PC (see forthcoming Section 7.3.1) was shown to attenuate the repression of the UAS_{INO} genes by Opi1p under conditions of high rates of PC synthesis (37 °C in the presence of choline), without accompanying measurable changes in PA level [117]. The authors proposed that Nte1p by degrading PC affects the lateral packing of the lipids in the ER membrane promoting Opi1p binding. As an alternative explanation, we propose that the increased Opi1p binding is accounted for by the increased negative charge of PA that results from Nte1p decreasing the PC/PE ratio.

4.2.2. Regulation by zinc

The expression of the *PIS1* gene is regulated by the zinc-responsive *cis*-acting element (UAS_{ZRE}) and the zinc-sensing transcriptional activator Zap1p [118]. Zinc depletion results in an increase in PI synthesis, which causes a drop in PA content with similar effects on transcription of UAS_{INO} genes as inositol supplementation. The ultimate effects of zinc depletion are an increase in PI content and a decrease in PE content [119]. The PC level does not decrease, because upregulation of the CDP-choline route compensates for the repression of the CDP-DAG pathway, due to the activation of *CKI1* and *EKI1* expression by Zap1 (overruling *CKI1* and *EKI1* repression via the UAS_{INO} element) [120,121]. Recently, the expression of the *PAH1*-encoded PA phosphatase was found to be induced by zinc depletion mediated by Zap1p [122]. Increased expression of Pah1p adds to the repression of the UAS_{INO} genes, and further stimulates the Kennedy pathway by extra supply of DAG.

4.2.3. Regulation by CTP and S-adenosyl-L-homocysteine

CTP is another phospholipid precursor with a regulatory role in phospholipid synthesis. CTP is the direct precursor of CDP-DAG, CDP-choline, and CDP-ethanolamine [123] and also serves as phosphate donor for PA synthesis by Dgk1p [124]. An increase in CTP synthesis induces an increased rate of PA synthesis and derepression of UAS_{INO} containing genes. As the production of CDP-choline from CTP and phosphocholine by Pct1p is the rate-limiting step of PC synthesis via the Kennedy pathway, changes in CTP concentration directly influence the rate of PC synthesis. Furthermore, CTP directly inhibits Cho1p activity further contributing to increased flux through the Kennedy pathway [123].

S-adenosyl-L-homocysteine (AdoHcy) regulates PC synthesis through competitive inhibition of the PE methyltransferases Cho2p and Opi3p [125]. AdoHcy is a side product of these enzymes and is removed by the AdoHcy hydrolase Sah1p. Downregulation of Sah1p therefore leads to the inhibition of PC synthesis, an increase in PA levels and derepression of the UAS_{INO} containing genes, and causes an increase in TAG synthesis and lipid droplet content [126].

4.2.4. Regulation by phosphorylation

Phosphorylation of phospholipid biosynthetic enzymes by protein kinase A, protein kinase C, casein kinase II, and the cyclin-dependent protein kinase have been shown to be involved in the regulation of phospholipid synthesis. Substrates of these protein kinases include Cho1p, Pah1p, Cki1p, the *URA7* and *URA8*-encoded CTP synthetase, and the repressor Opi1p. For a review on the effects of (de)phosphorylation of these proteins on phospholipid biosynthesis the reader is referred to Ref. [70].

It was recently shown that the *CDC28* (*CDK1*)-encoded cyclin-dependent kinase inhibits the membrane association and activity of Pah1p [127], and activates the lipase Tgl4p that mobilizes free fatty acids from triacylglycerol, linking lipid metabolism to the cell cycle [128]. Recently, the G3P acyltransferases Sct1p and Gpt2p were shown to be phosphorylated [129], presumably to downregulate the enzymes' activity (see forthcoming Section 5.1).

Rapid advances in phosphoproteomics (see e.g. [130]) are expected to lead to many new, testable hypotheses of the role of phosphorylation networks in the regulation of membrane lipid composition.

5. Membrane acyl chain homeostasis

5.1. Fatty acid synthesis and elongation

The yeast fatty acyl chain profile is rather limited compared to its mammalian or plant counterparts that harbor a wide variation of fatty acids differing in length and degree of unsaturation. The fatty acid profile in yeast is strain and carbon source dependent [15,131]. It consists mainly of C16 and C18 fatty acids bearing either one or no double bond, named palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), and oleic acid (C18:1), that are present at percentages in the bulk part of 10, 40, 5, and 40,

respectively, the remainder consisting of C14:0, C14:1, and C26:0 [9,131].

Fatty acid synthesis in *S. cerevisiae* is initiated by the acetyl-CoA carboxylase Acc1p and continued by the cytosolic fatty acid synthase (FAS) multienzyme complex consisting of Fas1p and Fas2p that predominantly produces acyl-CoAs with an acyl chain length of 16 or 18 carbon atoms [132]. Using purified yeast FAS, the ratio between C16- and C18-fatty acids produced was shown to increase with decreasing temperature [133], consistent with regulation of membrane fluidity by adapting acyl chain length. The *ACC1*, *FAS1* and *FAS2* genes contain a UAS_{INO} element in their promoter regions [132], ensuring that fatty acid synthesis keeps pace with phospholipid biosynthesis.

The ER-based fatty acid elongases Elo1p, Elo2p and Elo3p together with the enzymes encoded by *YBR159w*, *PHS1*, and *TSC13* can elongate the acyl-CoAs up to C26 (reviewed in [71,132]). Although very low in abundance, the resulting very long acyl chains are essential as components of sphingolipids and GPI-anchors [134,135].

5.2. Fatty acid desaturation

Monounsaturated fatty acids account for approximately 70–80% of total fatty acids in yeast and are synthesized from saturated fatty acyl-CoA precursors by the endoplasmic reticulum (ER)-resident Δ9-fatty acid desaturase Ole1p in an oxygen-dependent reaction [9]. Unsaturated fatty acids are essential in yeast, as an *ole1* knock-out strain is only viable when unsaturated fatty acids are supplied in the medium [136]. The expression of Ole1p is tightly regulated at the level of transcription in response to carbon source, oxygen levels, and the presence of fatty acids in the growth medium, with unsaturated fatty acids repressing transcription [9]. Saturated fatty acids supplied in the medium increase the transcription of the *OLE1* gene [137] and Ole1p enzyme activity [138].

Transcriptional activation and mRNA stability of Ole1p requires proteolysis of the ER membrane proteins Spt23p and Mga2p. Disruption of Mga2p and Spt23p separately has little effect on growth, but disruption of both causes unsaturated fatty acid auxotrophy that can be suppressed by expressing *OLE1* from a promoter different from the endogenous one [139]. Spt23p dimers are ubiquitinated by the E3 ubiquitin ligase Rsp5p, which promotes cleavage of a C-terminal fragment of the ubiquitinated protein by the 26S proteasome and the subsequent release of the soluble 90 kDa fragment by the ubiquitin-selective chaperone/segregase Cdc48p/Npl4p/Ufd1p into the nucleus, where it activates the transcription of *OLE1* [140,141]. It has been proposed that changes in membrane fluidity induce fatty acid mediated repression of *OLE1* transcription and transient adaptations to cooling [140]. Spt23p may serve as membrane sensor in this regulation, since proteolysis of Spt23p is sensitive to the presence of unsaturated fatty acids, suggesting that its dimerization, ubiquitination, or proteolysis is sensitive to changes in membrane fluidity [140,142]. Unsaturated fatty acids do not affect the processing of Mga2p, but they do repress the activation of *OLE1* transcription by Mga2p, as was demonstrated using a truncated soluble form of Mga2p [142]. Mga2p also functions in destabilizing and stabilizing *OLE1* mRNA in the presence and absence of unsaturated fatty acids in the culture medium, respectively [143].

Inactivation of epitope-tagged Ole1p induced the fast, selective relocalization of the enzyme from the ER to a peripheral punctuate localization, that is reversed by supplying unsaturated fatty acids [144]. The functional significance of Ole1p relocalization remains to be elucidated. Epitope-tagged Ole1p was shown to be a short-lived protein that is degraded by ubiquitin/proteasome-dependent ER-associated degradation [145]. Whether Ole1p degradation is regulated by fatty acyl chain composition is not known.

Deletion of both the *FAA1* and *FAA4* genes encoding the two major acyl-CoA synthetases increases the *OLE1* mRNA level, irrespective of the presence of unsaturated fatty acids [137]. Although the cellular fatty acid composition of *faa1Δfaa4Δ* cells was unchanged compared to wild type, the size of the endogenous acyl-CoA pool was strongly reduced, in particular the levels of C16:1-CoA and C18:1-CoA [146], suggesting regulation of *OLE1* expression by acyl-CoA [75]. Deletion of *ACB1* encoding the single acyl-CoA binding protein, increases *OLE1* transcript levels and the cellular content of unsaturated acyl chains (see also Section 5.3), while leaving repression of *OLE1* transcription by unsaturated fatty acids intact [137,147].

5.3. Acyl-CoA metabolism and mass action

Once an acyl chain has been transferred from acyl-CoA to a lipid backbone, it is no longer susceptible to elongation or desaturation. As a consequence acyltransferases exhibiting acyl-CoA substrate specificity may affect cellular acyl chain composition. Inactivation or overexpression of acyltransferases that consume significant quantities of acyl-CoAs with substrate specificity, leads to accumulation or depletion of specific acyl-CoAs, respectively, and thus affects the mass action ratio of the preceding acyl-CoA synthesizing reactions. Changes in mass action ratio may alter the flux through the acyl-CoA synthesizing reactions, e.g. by affecting the rate of the reverse reaction, by affecting enzyme activity, or by shifting the concentrations of reactants and products in substrate-limited reactions. In the following we will argue that mass action accounts for the impact on cellular acyl chain composition by the activities of the glycerol-3-phosphate acyltransferase (GPAT) Sct1p and the acyl-CoA:diacylglycerol acyltransferase (DGAT) Dga1p [148,149].

The homologous GPATs Gpt2p/Gat1p and Sct1p/Gat2p attach an acyl chain from acyl-CoA at the *sn*-1 position of glycerol-3-phosphate. Gpt2p and Sct1p are responsible for the synthesis of the bulk of glycerolipids, as deletion of both encoding genes is synthetically lethal [72]. In vitro activity assays using cell lysates from strains overexpressing either GPAT and C16:0-CoA, C16:1-CoA, C18:0-CoA or C18:1 CoA as acyl donors, revealed that whereas Gpt2p only showed somewhat reduced activity toward C18:0-CoA, Sct1p had a clear preference for C16- over C18-acyl-CoAs [72]. De Smet et al. [148] reported that deletion of *SCT1* (but not *GPT2*) reduced the cellular content of C16:0 by 40%, whereas overexpression of catalytically active Sct1p resulted in a strong increase of C16:0 at the expense of C16:1 and C18:1 that was reflected to various extent in the molecular species profiles of all phospholipid classes. Moreover, by drawing on the pool of C16:0-CoA, overexpression of *SCT1* increased total fatty acid synthesis [148]. Co-overexpression of the *OLE1* gene and *SCT1* reduced the rise in C16:0, indicating that Sct1p and Ole1p compete for the shared substrate C16:0-CoA. The preferential incorporation of saturated C16:0 acyl chains into lipids by Sct1p protects them from desaturation by Ole1p, thus increasing the cellular content of saturated acyl chains (Fig. 4) [148].

Previously, a screen for yeast mutants sensitive to exogenous C18:1 identified the *gpt2Δ* strain but not the *sct1Δ* strain [150]. More recently, the accumulation of lipid droplets (and TAG) that is induced by supplying C18:1 in the yeast culture medium was shown to depend on active Gpt2p [151]. These data show that Sct1p by itself does not have sufficient capacity to process excess C18:1 into lipids, in agreement with Sct1p's preference for C16:0-CoA. As a consequence *gpt2Δ* cells loose viability when exposed to exogenous C18:1 [151], similar to the quadruple *are1Δare2Δdga1Δlro1Δ* mutant that is incapable of storing fatty acids as TAG or steryl esters [152,153].

Both Sct1p and Gpt2p are phosphoproteins, with the level of phosphorylation increasing with the enzymes' expression level, suggesting that phosphorylation inhibits activity [129]. Both Gpt2p and Sct1p were dephosphorylated upon exposure to C18:1 [151], whereas supplementation of C16:0 in the culture medium enhanced the phosphorylation of chromosomally expressed Sct1p [148], indicating a physiological role of the regulation of Sct1p in determining yeast fatty acid composition. The overexpression of *SCT1* confers a growth defect that was used to screen for suppressors. Deletion of the *CST26* (*PSI1*) gene that encodes an acyltransferase required for the incorporation of C18:0 into PI (see forthcoming Section 7.3.2) [154], completely suppressed the growth defect and the rise in cellular C16:0 content induced by the overexpression of Sct1p [148]. Deletion of *CST26* induced hyperphosphorylation of Sct1p, and decreased the level of Sct1p overexpression, suggesting that Cst26p is required for dephosphorylated, active Sct1p. The nature of the involvement of Cst26p in Sct1p phosphorylation remains to be investigated.

The finding that Cst26p affects the phosphorylation state and the activity of Sct1p may shed new light on the observation by Shui et al. [155] that the 1-acyl-lysoPA acyltransferase (LPAAT) activity utilizing saturated saturated acyl chains is decreased upon deletion of *CST26*. Based on this result Cst26p was assigned a role as lyso-PA acyltransferase (LPAAT) selective for saturated acyl-CoA, in addition to its function as lyso-PI acyltransferase. We propose an alternative explanation: due to the reduction in Sct1p activity resulting from increased phosphorylation in the absence of Cst26p, the availability of saturated acyl chains may become limiting for PA synthesis by established LPAATs.

Yeast cells lacking Dga1p (Fig. 4) exhibit an increased cellular level of C16:1 at the expense of C16:0 and C18:1 [149]. Cells lacking Pah1p, in which the supply of DAG for conversion to TAG by Dga1p is impaired, show a similar increase in C16:1/C18:1 ratio [156]. Dga1p was shown to efficiently use C18:1-CoA and C16:0-CoA as acyl donors in vitro [78]. To explain these findings we propose that in the absence of Dga1p-mediated TAG synthesis C18:1-CoA accumulates and decreasing its rate of synthesis by the desaturase through a mass action ratio-dependent feedback mechanism. As further consequences of the changes in mass action, the elongation of C16:0-CoA to C18:0-CoA is reduced and the conversion to C16:1-CoA enhanced.

Consistent with the notion of mass action in acyl-CoA metabolism affecting cellular acyl chain composition, an *ole1Δ* strain (cultured on C14:1- or C16:1-supplemented medium) does not accumulate C18:0 [157]. Another example is the accumulation of short acyl chains in a *hem1Δ* mutant that is incapable of desaturation by Ole1p due to heme deficiency [23]. Conversely, overexpression of Ole1p increases the level of C18:1 rather than that of C16:1 [148,158], suggesting that Ole1p prefers C18:0-CoA as substrate over C16:0-CoA, and by drawing on the pool of C18:0-CoA promotes an increase in average acyl chain length.

Depletion of the acyl-CoA binding protein Acb1p interferes with the synthesis of C26 fatty acids required for sphingolipid synthesis, and was shown to result in the accumulation of C18:0-CoA, while the total acyl-CoA content was unaffected [159]. The fatty acid and phospholipid molecular species profiles of *acb1Δ* and Acb1p-depleted cells are characterized by a shortening of the average acyl chain length and increased unsaturation as compared to wild type [137,160,161]. While the increased unsaturation has been attributed to increased transcription of *OLE1* (Section 5.2), the shortening could be due to accumulating C18:0-CoA reducing acyl-CoA elongation through mass action-mediated feedback inhibition, and/or to impaired transport of acyl-CoAs to the elongases as proposed previously [159].

6. Membrane sensors and signaling in the regulation of lipid synthesis

Only very few membrane sensors monitoring physical parameters and/or the lipid composition of membranes and transmitting signals to the lipid biosynthetic machinery have been identified and characterized. After describing the established membrane sensors, (putative) membrane sensors and signaling pathways that have been implicated in regulating membrane lipid composition in *S. cerevisiae* will be discussed.

Currently, the control of fatty acid desaturation by the thermosensor DesK from *Bacillus subtilis* is probably mechanistically best understood. Using a simplified version of the histidine kinase DesK, consisting of only a single N-terminal membrane spanning helix attached to the C-terminal cytosolic kinase/phosphatase domain, Cybulski et al. [162] provided strong evidence that the sensor senses the thickening of the membrane as the temperature drops. The thickening of the membrane is proposed to force a cluster of

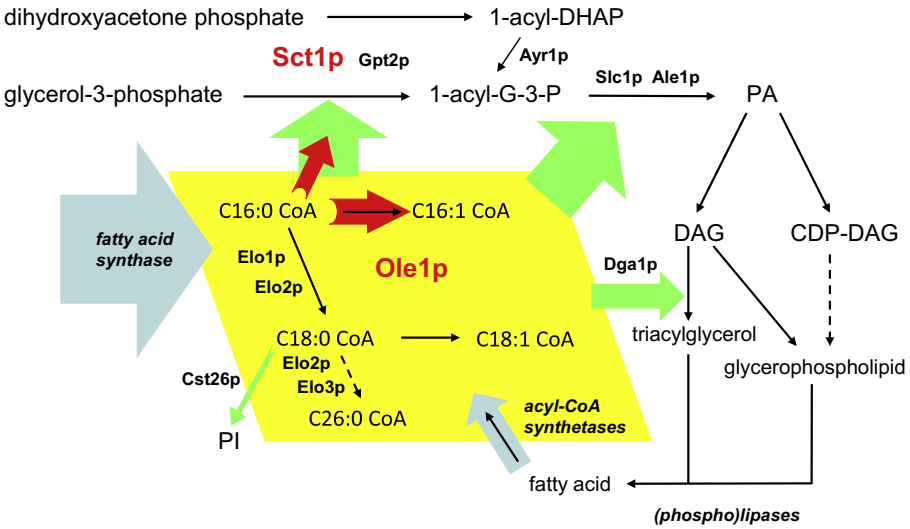

Fig. 4. The interface of acyl-CoA and glycerolipid metabolism. The yellow plane depicts the pool of acyl-CoAs. Fluxes of acyl chains into (blue arrows) and out of the acyl-CoA pool into glycerolipids (green arrows) are indicated. The red arrows highlight the competition between Ole1p and Sct1p for C16:0-CoA as explained in the text. To reduce complexity, metabolic fates of acyl-CoAs other than incorporation into glycerolipids have been omitted.

three hydrophilic amino acids into a more apolar environment, leading to a conformational change that triggers the autokinase activity of DesK. Subsequently, phosphorylated DesK activates DesR, the transcriptional activator of the desaturase [163].

In the cyanobacterium *Synechocystis*, the cold sensor histidine kinase Hik33, an integral membrane protein, has been identified as sensor of membrane fluidity (reviewed in [6]), however the mechanism of sensing remains a mystery [164,165]. Based on the finding that Hik33 also controls the expression of genes that are induced by hyperosmotic stress in *Synechocystis* [166], Panadero et al. [167] hypothesized that yeast Sln1p, the histidine kinase osmosensor in the plasma membrane, serves as sensor monitoring membrane fluidity. The HOG pathway was found to be activated via Sln1p in response to a downshift in temperature, and in response to treating the cells with DMSO, which rigidifies membranes [168], consistent with the hypothesis. However, activation of Sln1p did not enhance the transcription of *OLE1* [167], and the HOG pathway has not yet been linked to lipid biosynthesis.

In the feedback regulation of mammalian cholesterol biosynthesis at the level of transcription by SREBP, the integral membrane protein Scap (1276 amino acids) senses the sterol content of the ER membrane (reviewed in [169]). Recently, a 245 amino acid loop localized to the ER lumen was identified as the sterol sensor, binding cholesterol with similar specificity as the entire membrane domain of the protein [170]. How this loop recognizes and binds cholesterol present in the membrane is not yet known.

Which proteins are known or suspected to sense membrane properties in yeast? As already discussed, the peripheral membrane protein and suppressor of transcription of phospholipid biosynthetic genes Opi1p is a sensor of the PA content of the ER (Section 4.4.1), and the ER integral membrane protein Spt23p and activator of *OLE1* transcription was suggested to serve as sensor of membrane fluidity (Section 4.2).

In 1989, the cytosolic protein Sec14p was found to be essential in the transport of secretory proteins from the Golgi complex [171]. Soon thereafter, Cleves et al. [172] uncovered a link between Sec14p and PC synthesis via the CDP-choline pathway, and it was demonstrated that Sec14p bound to PC inhibits the activity of Pct1p [173], and hence the flux through the CDP-choline pathway in vivo [174]. In the current view Sec14p has a dual role as PC sensor and PI-presenting nanoreactor [175]. Sec14p coordinates PC biosynthesis and membrane trafficking by keeping the local levels of DAG, the non-bilayer preferring lipid precursor of PC in the CDP-choline pathway, in the optimal range for vesicle budding. In addition, by exchanging PI for PC, Sec14p stimulates PI-4-P production thus coupling input from PC metabolism to PIP biosynthesis and downstream phosphoinositide signaling [175]. The cellular functions of the five Sec14p homologues in yeast, Sfh1p-Sfh5p, remain poorly understood (reviewed in [176]), with the exception of Pdr17p (Sfh4p) that is essential for efficient access of PS to the site of Psd2p [177]. Recently, Sfh2p was found to prevent the accumulation of saturated fatty acids under conditions mimicking anaerobiosis, implicating Sfh2p in the regulation of fatty acid synthesis [178], and consistent with a role as membrane sensor.

The cross-talk between lipid metabolism and the unfolded protein response (UPR) is well established [179]. The integral membrane protein Ire1p is the ER stress sensor that detects misfolded proteins and activates UPR. UPR activates the transcription of lipid biosynthetic genes [180], and conversely, the transcription of UPR-regulated genes responds to changes in phospholipid metabolism and perturbation of lipid homeostasis [181,182]. Recently, the activation of UPR by lipid stress, including inositol starvation, was shown to occur independently of a functional luminal domain of Ire1p that associates with unfolded proteins [183]. Moreover, activation of UPR by inositol starvation was shown to occur in the absence of increasing levels of unfolded proteins [184]. Combined studies indicate that there is a separate mechanism for activating

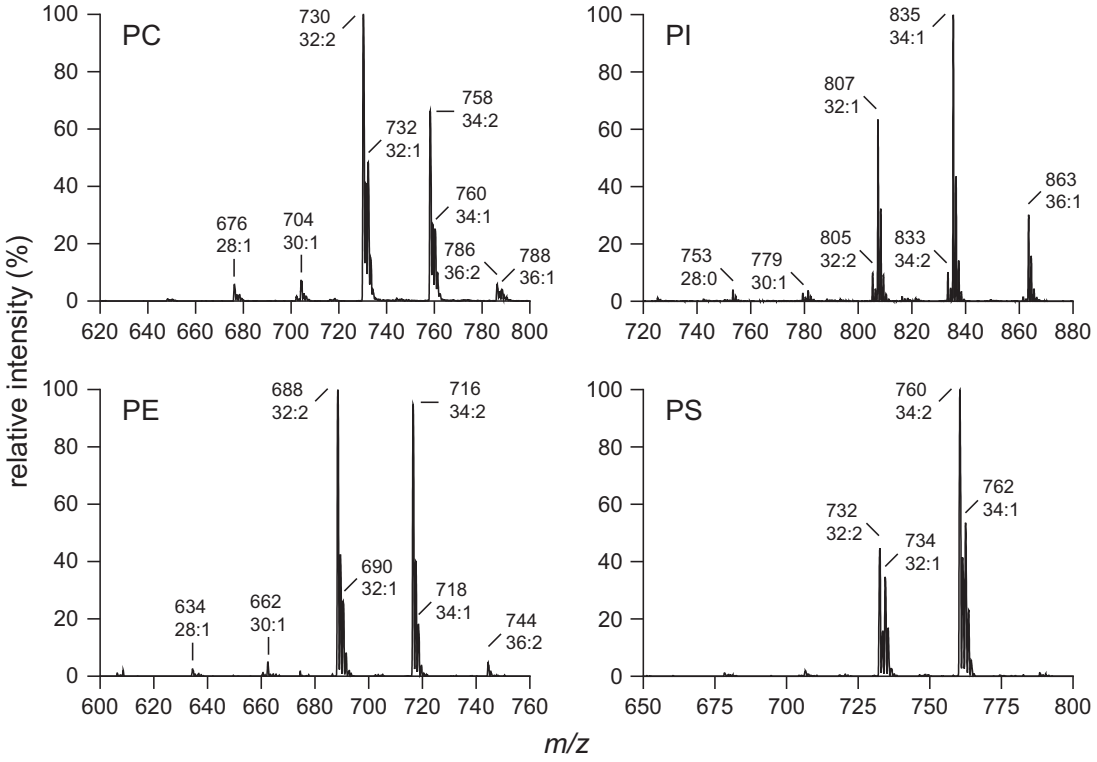

Fig. 5. Molecular species profiles of the 4 major membrane phospholipids in wild type yeast recorded by mass spectrometry. Strain BY4742 was cultured to late log-phase in synthetic lactate medium. The major molecular species of PC, PE, PI and PS are indicated by their *m/z* values. The species labels indicate total acyl carbon atoms:total acyl double bonds. In each panel the intensity of the highest peak was set at 100%. For details see [161].

UPR in response to lipid stress, in which the cytosolic or transmembrane domain of Ire1p serves as membrane sensor.

Pct1p, the soluble rate-limiting enzyme of the CDP-choline route may combine sensor and effector function. The activity of Pct1p and its mammalian homologue CT in vitro strongly depends on membrane binding and insertion, which is determined by lipid composition and stimulated by the presence of anionic lipids [185]. The activity of CT (Pct1p was not tested) was shown to be further stimulated by increasing levels of non-bilayer lipids [186,187]. Thus, low PC levels may directly stimulate PC biosynthesis. Recently, the activity of Pct1p was found to be regulated by the Kap60–Kap95 karyopherin complex [188]. Both proteins are involved in the nuclear import of Pct1p. In addition, Kap95 (and to a lesser extent Kap60) was found to be essential for Pct1 activity in vivo via an as yet unknown mechanism.

A recent screen for yeast deletion strains whose growth is differentially affected by C16:1 and C18:1 supplemented in the growth medium, identified components of the cell wall integrity (CWI) signaling pathway, a Map kinase pathway dependent on Pkc1p and Rho1p [189]. The deletion mutants that differed in sensitivity to C16:1 and C18:1 showed changes in acyl chain content and membrane fluidity, indicating that they are impaired in the regulation of membrane fluidity. While the membrane fluidity homeostasis pathway shares components with the CWI pathway, its sensor(s) and effector(s) remain to be identified. Importantly, this is the first report of a signaling pathway controlling membrane fluidity in eukaryotes. Interestingly, the CWI-MAPK pathway has previously been implicated in lipid homeostasis. The CWI pathway is activated in wild type cells cultured in the absence of inositol, and mutants in the CWI pathway exhibit inositol auxotrophy and alterations in lipid metabolism that are enhanced in the presence of choline [190].

7. Processes determining the acyl chain composition of phospholipid classes

Although the phospholipid class and acyl chain composition of yeast membranes varies depending on growth phase and culture conditions, the phospholipid classes retain characteristic differences in acyl chain composition, with e.g. the content of saturated acyl chains decreasing in the order of PI > PS > PC > PE–CL (Fig. 5) [1,4]. In this section the processes will be summarized that are responsible for establishing the acyl chain composition of the particular phospholipid classes in addition to the composition of the pool of acyl chains available for incorporation. These include the substrate selectivity of the biosynthetic enzymes with respect to lipid molecular species, the availability of lipid precursor species at the intracellular site of the enzyme, and molecular species selective lipid turnover and acyl chain remodeling.

7.1. Substrate selectivity of the lipid biosynthetic enzymes

The substrate specificity of a number of yeast lipid biosynthetic enzymes has been measured in in vitro enzyme assays using radiolabeled substrates and in vitro or in vivo using stable isotope labeled substrates and detection by mass spectrometry. In addition, the substrate preference of several enzymes has been deduced from changes in the lipidome upon deleting the encoding gene.

PA is a short-lived intermediate in glycerolipid metabolism. Its steady state molecular species profile depends for the most part on the substrate preferences of the PA synthesizing and consuming enzymes in glycerolipid synthesis (Fig. 3). The substrate selectivity of the yeast GPATs has been described in Section 5.3. The lyso-PA acyltransferases (LPAATs) catalyze the attachment of the second acyl chain at the *sn*-2 position of the glycerol backbone. *SLC1* and *ALE1* encode the major LPAATs, and simultaneous deletion of both genes is synthetically lethal [74]. Slc1p has a strong preference for acylating lyso-PA, but also uses lyso-PS and lyso-PI as substrates in vitro [74]. In contrast, Ale1p is capable of acylating a broad range of lyso-phospholipids with similar efficiency [74,191–193]. Deletion of either *SLC1* or *ALE1* hardly affects the phospholipid class composition of yeast cells [74]. Deletion of *ALE1* hardly affects the molecular species profiles of the phospholipid classes, while deletion of *SLC1* slightly increased the level of C32 phospholipid species at the expense of C34 [74,155], indicating decreased incorporation of C18:1 and consistent with the in vitro preference of Slc1p for C18:1-CoA [155]. Whether deletion of *SLC1* affects the overall cellular fatty acid profile is not known. Several additional enzymes with LPAAT activity have been identified in yeast: Ict1p that is expressed in the presence of organic solvents [194], Loa1p that has a preference for C18:1-CoA and has been implicated in lipid droplet formation [195], and the multiple function triacylglycerol lipases Tgl4p and Tgl5p localized to the lipid droplets [196,197].

Cds1p and Pah1p, the PA-consuming enzymes producing CDP-DAG and DAG, respectively, have not been characterized for molecular species selectivity. The same applies to the enzymes consuming CDP-DAG, Cho1p, Pis1p and Pgs1p. Pis1p may prefer CDP-DAG molecular species containing saturated acyl chains in view of the relatively high content of saturated acyl chains in PI. Alternatively this could result from the composition of the local CDP-DAG pool in the ER that Pis1p has access to [4]. The substrate specificity of the cardiolipin synthase Crd1p was tested in vitro showing a slight preference for molecular species of PG and CDP-DAG containing unsaturated acyl chains [161].

The decarboxylation of PS by Psd1p is the main source of PE in yeast [19], and in the absence of choline supplementation, PE is the main lipid precursor of PC. Since PE and PC are much more abundant than PS, the steady state PS species composition can be considered to reflect the left-overs of the PS decarboxylation reactions. Compared to PS, PC and particularly PE are enriched in unsaturated acyl chains [198], indicative for the preferential decarboxylation of unsaturated PS species, which could originate from substrate specificity of the decarboxylases. Alternatively, it could result from the species selectivity of the intermembrane transport steps required for PS to reach the site of Psd1p (forthcoming Section 7.2). The PS molecular species specificity of Psd1p and Psd2p has so far not been tested. In agreement with species-selective decarboxylation, lipidome analysis showed that a *psd1Δpsd2Δ* strain accumulates the di-unsaturated species 32:2 and 34:2 in its steady state PS profile. In contrast, the single *psd1Δ* deletion strain showed a decrease in the level of PS unsaturation [198], showing the limitations of the analysis of steady state lipid compositions of gene deletion strains for elucidating lipid substrate specificities.

To investigate the molecular species-selectivity of the PE methyltransferases Cho2p and Opi3p, cells were pulse labeled with (*methyl*-D_3)-methionine. Subsequently, ESI-MS/MS enabled distinction between the population of newly synthesized PC molecules from the pre-existing population [199]. The molecular species profile of newly synthesized PC was found to be dominated by the di-unsaturated PC species with an enrichment of the 32:2 species in comparison to the precursor PE. The preferential conversion of 32:2 PE to PC by the methyltransferases was recapitulated in vitro in isolated microsomes, indicating that it is an intrinsic property of the methyltransferases, of Cho2p in particular [200]. Thus, the PE methyltransferases could be viewed as regulators of membrane intrinsic curvature: by preferentially methylating 32:2 PE over 34:2 PE they increase the average length of the acyl chains in the remaining PE and enhance its non-bilayer propensity [27].

Similarly, pulse labeling yeast cells with deuterium-labeled choline and ethanolamine, revealed the molecular species profiles of PC and PE synthesized by the CDP-choline and CDP-ethanolamine pathways, respectively. The molecular species profile of CDP-choline derived PC is more diverse than that produced by PE methylation and contains increased proportions of the mono-unsaturated species [199]. Cpt1p and Ept1p (Fig. 3) produce the 4 major lipid species (32:2, 32:1, 34:2, 34:1) in different ratios, with the C32 cluster being more abundant than the C34 cluster in the PC profile and less abundant in the PE profile [201,202]. The small pool-size and rapid turnover of DAG do not allow analysis of precursor-product relationships. However, the differences in species compositions were consistent with results from in vitro assays using defined DAG species [203], suggesting that distinct sets of DAG species are consumed by Cpt1p and Ept1p due to differences in substrate preference. Alternatively, the two enzymes due to differences in subcellular localization may have access to separate pools of DAG varying in species composition as discussed elsewhere [201].

7.2. Availability of lipid substrates at the site of the enzymes

Whereas the synthesis of PA, PS, PI and PC is localized to the ER and Golgi (Cpt1p), the synthesis of CL and the conversion of PS to PE require interorganelle transport of the precursor lipids, a process that could be molecular species selective. The synthesis of CL is localized to the mitochondrial inner membrane and depends on mitochondrial import of PA from the ER (reviewed in [63]). Intermembrane transport of PS to the mitochondrial inner membrane and the Golgi precedes its decarboxylation by Psd1p and Psd2p, respectively. Subsequently, part of the PE produced by Psd1p is exported to the ER. Transport of PS from the ER to the Golgi has been proposed to involve a multiprotein transport complex requiring Pdr17p (Sfh4p) and the C2 domain of Psd2p in the Golgi membrane [204]. The mitochondrial import of PS is thought to occur at membrane contact sites between the mitochondria-associated membrane, an ER subfraction that is enriched in Cho1p, and the mitochondrial outer membrane [205,206], and proceeds by unknown mechanisms regulated by ubiquitination [204,207]. The role proposed for the Mmm1p/Mdm10p/Mdm12p/Mdm34p ER-mitochondria encounter structure (ERMES) in phospholipid exchange is controversial [208,209].

Experimental evidence in favor of molecular species selective mitochondrial import derives from experiments in mammalian cells. Heikinheimo and Somerharju [210,211] studied the molecular species dependence of the decarboxylation of PS in baby hamster kidney cells in pulse labeling experiments. The rate of decarboxylation was found to inversely correlate with the hydrophobicity of the PS molecular species as determined from their retention time on a reverse phase column. Since all PS species were decarboxylated with similar efficiency by rat liver PSD enzyme in the presence of detergent, it was concluded that the translocation of PS molecular species from ER to mitochondria diminished with increasing hydrophobicity. Whether the concept of hydrophilic, i.e. shorter and more unsaturated, molecular species exchanging more readily between ER and mitochondria than their more hydrophobic counterparts applies to yeast, remains to be tested. An analysis of the steady state molecular species profiles of PS, PE and PC in ER and mitochondrial fractions did not argue in favor [198]. However, the concept would account for the enrichment of saturated acyl chains in PS relative to PE and PC (Fig. 5), and for the absence of C14:1 chains in PS as opposed to PE, PC and PI in wild type cells cultured in C14:1-supplemented medium [157].

Even within the ER, there may be restrictions to the accessibility of lipid substrates. Recently, the membrane topology of the LPAATs Slc1p and Ale1p was elucidated, revealing that the active sites face the ER lumen [212]. Similarly, the most conserved acyltransferase signature motifs of the GPAT Gpt2p were found to localize to the ER lumen [213]. These results suggest that acyl-CoAs have to be transported across the ER membrane adding another potential layer for regulation.

7.3. Phospholipid turnover and acyl chain exchange

In addition to the turnover of phospholipids during *de novo* synthesis (Section 7.1), phospholipids are subject to degradation by phospholipases and exchange of acyl chains in reactions catalyzed by phospholipases, acyltransferases and transacylases, contributing to the acyl chain profiles of the phospholipid classes. Current insights into the post-synthetic metabolism of membrane phospholipids will be reviewed. The focus will be on turnover and acyl chain exchange of PC, the end product of phospholipid biosynthesis, for which these processes have been most extensively investigated. Acyl chain remodeling is defined as the post-synthetic modification of a phospholipid's molecular species profile. Turnover of PC via the CDP-choline route, species-selective degradation and acyl chain exchange may contribute to PC remodeling.

7.3.1. (Phospho)lipase mediated turnover of phospholipids

McMaster and Bell [112] discovered that the CDP-choline route utilizes choline derived from PC turnover for PC synthesis in the absence of choline in the culture medium. Strains in which reutilization is (partially) blocked by a defective CDP-choline route, revealed that PC turnover produces choline that is excreted. The choline excretion is reduced by the deletion of *SPO14*, establishing a function for a phospholipase D in PC turnover (Fig. 6) [214].

Dowd et al. [215] showed that the turnover of PC to glycerophosphocholine (GPC) increases when choline is added to the culture medium or when cells are shifted from 30 to 37 °C, which is accompanied by increased flux through the CDP-choline pathway. Mutants defective in the CDP-choline route failed to produce GPC under these conditions, whereas mutants defective in the PE methylation route displayed normal induction of GPC production, suggesting a metabolic link between phospholipase B-mediated PC turnover and PC synthesis via the CDP-choline pathway. Deletion of the three genes encoding phospholipases B, *PLB1*, *PLB2* and *PLB3*, that had been identified at that time did not affect the production of intracellular GPC [215]. A fourth phospholipase B, the ER-resident neuropathy esterase homologue Nte1p, was identified by Zaccheo et al. [216], who found that PC turnover to intracellular

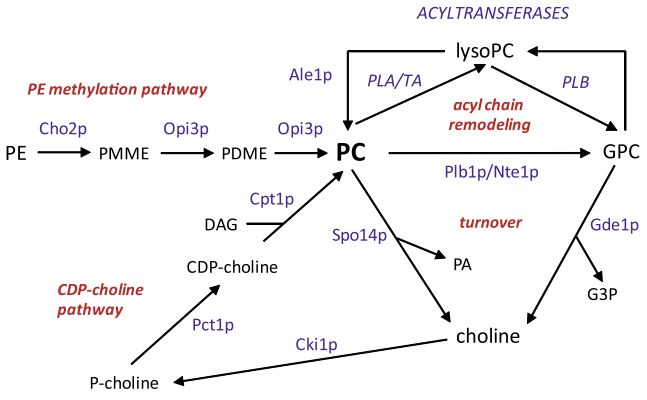

Fig. 6. PC metabolism in yeast. See text for details about PC biosynthesis and turnover. Acyl chain remodeling of PC may proceed by deacylation to lysoPC or GPC by Plb1p, Nte1p, or other phospholipases A and/or B, and subsequent reacylation by acyltransferases. Ale1p transfers acyl chains to the *sn*-2 position of 1-acyl lysoPC. Alternatively, the de- and reacylation reactions in acyl chain remodeling can be catalyzed by transacylases (TA).

GPC is virtually absent in an *nte1Δ* deletion strain. Remarkably, Nte1p specifically degrades PC produced by the CDP-choline pathway, leaving PC synthesized by methylation of PE intact.

When the PC sensor Sec14p is inactivated, Nte1p-mediated PC degradation is reduced [217,218], whereas PC degradation by Spo14p is activated [214,219]. In the presence of choline, the introduction of inositol in the culture medium induces rapid Nte1p-mediated degradation of PC and concomitant synthesis of PI [40]. Inositol deprivation increases the intracellular pool of GPC, dependent on the presence of Nte1p [117]. Whether Spo14p and Nte1p display substrate preference at the level of PC molecular species is not known. The finding that the species profile of the unlabeled preexisting PC did not change during a prolonged labeling experiment with deuterated PC precursors, argues that species selective degradation does not play a major role in determining the PC species composition [199].

Interestingly, deletion of *SCT1* and *GPT2* encoding the GPATs has opposite effects on the turnover of CDP-choline-derived PC and the rate of TAG synthesis. Whereas deletion of the *SCT1* gene causes a 10-fold decrease in turnover of CDP-choline-derived PC to GPC and a 50% decrease in the rate of TAG synthesis, deletion of *GPT2* causes a 5-fold increase in flux through the CDP-choline route and a 50% increase in the rate of TAG production [220]. These data led to the hypothesis that CDP-choline-derived PC originates from lyso-PA synthesized by Sct1p, and is preferentially deacylated by Nte1p [218]. There is additional evidence for Sct1p and Gpt2p contributing to lyso-PA (and derived PA) pools that enter different lipid biosynthetic pathways. Deletion of *SCT1* (but not *GPT2*) rescues *pah1Δ* cells lacking the phosphatase that converts PA into diacylglycerol (DAG) [85]. Moreover, a genome-wide screen identified the *sct1Δ* strain (but not *gpt2Δ*) as inositol auxotroph under conditions of enhanced flux through the CDP-choline route [221]. Combined observations suggest that the acyl-CoA preferences of the GPATs discussed in Section 5.3 impact on the fluxes through the lipid biosynthetic routes. Also taking into account the molecular species selectivity of the phosphatidylcholine (PC) biosynthetic routes (Section 7.1), we hypothesize that the Sct1p-derived subpopulation of lyso-PA molecules enriched in saturated acyl chains is preferentially converted into PC and TAG via DAG, rather than shuttled into the CDP-DAG pathway. As an extension we propose that the CDP-choline route and Nte1p-mediated PC-turnover serve for storing and mobilizing saturated acyl chains, respectively, which would make CDP-choline-derived PC a versatile buffer for saturated acyl chains. Many questions remain, including: How are the different pools of (lyso-)PA kept apart? Is this related to the differential enrichment of Sct1p and Gpt2p in distinct ER subfractions [129]? How does Nte1p distinguish between PE- and CDP-choline derived PC?

With respect to the phospholipases B encoded by *PLB1–3*, degradation of PC by Plb1p has been observed in vivo, with the GPC produced released into the culture medium [222]. Degradation of PC by Plb2p has so far only been observed in vitro, while Plb3p does not cleave PC at a detectable rate [223]. However, the fact that overexpression of Plb2p confers resistance to lysophosphatidylcholine suggests a role for this protein in PC homeostasis [224]. Plb1p and Plb3p have been localized to the plasma membrane and periplasmic space [222,223], and Plb2p to the plasma membrane, the periplasmic space, and the culture supernatant based on enzyme activity [223,224]. GFP-tagged versions of Plb1p and Plb2p were associated with the ER and intracellular vesicles, and of Plb3p with intracellular vesicles and the cytoplasm [103]. Recently, all three *PLB*'s were shown to have a role in metabolizing excess lyso-PC produced by overexpression of a plant phospholipase A_2 in yeast cells [225].

The GPC produced by phospholipases B may either be metabolized to choline by the phosphodiesterase Gde1p [226,227] for recycling via the CDP-choline pathway, or serve as substrate for the hitherto unidentified acyltransferase that synthesizes 1-acyl lyso-PC, which could be further acylated by the broad-specificity acyltransferase Ale1p (Fig. 4) [228].

Compared to PC, little is known about lipase-mediated turnover of the other membrane phospholipids. In addition to PC, Plb1p also deacylates PE in vivo [222]. Whether the resulting GPE is further degraded to ethanolamine for recycling via the CDP-ethanolamine route is not known. Deletion of *PLB3* reduces the degradation of PI and secretion of glycerophosphoinositol [223]. Based on in vitro experiments, Plb2p appears to be a broad specificity phospholipase B [223,229]. The phospholipid:diacylglycerol acyl transferase Lro1p uses PE and PC as substrate in vitro, with a preference for PE [80,230]. In vivo CDP-ethanolamine-derived PE was shown to be Lro1p's preferred substrate [231]. The metabolic fate of the lyso-PE produced is not known. Mitochondria contain a Ca^{2+}-dependent phospholipase D activity of which the encoding gene remains to be identified, and that preferentially converts PE and PS in vitro [232,233]. Deletion of *PGC1* results in the accumulation of PG, a biosynthetic precursor of CL. Mitochondrial Pgc1p has phospholipase C activity degrading PG into DAG and glycerol-3-phosphate [234].

7.3.2. Phospholipid remodeling by acyl chain exchange

W.E. Lands first described phospholipid acyl chain exchange [235], based on the finding that lysophosphatidylcholine formed by a phospholipase A_2 was reacylated in an acyl-CoA dependent manner in rat liver microsomes. Variations to the Lands' cycle include acyl chain exchange involving release of both acyl chains and glycerophosphodiesters as intermediate, as proposed for PC in yeast [228], and acyl-CoA independent acyl transfer catalyzed by transacylases that shuttle acyl chains between glycerolipids [236].

Yamada and co-authors were the first to report acyltransferase activities potentially involved in acyl chain remodeling of PC in yeast microsomes [237]. Selective acyl chain exchange by phospholipase A_2-mediated deacylation and subsequent reacylation in vivo was demonstrated by Wagner and Paltauf [7]. Boumann et al. [199] monitored acyl chain remodeling of PC by stable isotope labeling and detection by tandem mass spectrometry and showed that acyl chain exchange is required to generate the steady state molecular species profile of PC in yeast [199]. Acyl chain exchange at the *sn*-1 position of the glycerol backbone plays a major role in attaining the steady state PC species profile in *pct1Δ* strains that synthesize PC exclusively through PE methylation [199]. The remodeling of newly synthesized PC increases the level of the mono-unsaturated at the expense of the di-unsaturated species, indicative for incorporation of C16:0. Consistently, decreasing the cellular C16:0 level by deletion of *SCT1* strongly reduces the extent of PC acyl chain remodeling [148]. Overexpression of *SCT1* on the other hand strongly enhances the extent of acyl chain remodeling of PE-derived PC [238], again suggesting that PC may serve as store for excess C16:0. Under conditions of *SCT1* overexpression, Plb1p was implicated in acyl chain exchange of PE-derived PC. Deletion of the *PLB1* gene decreased the rate of PC remodeling by twofold, and reduced the cellular content of lyso-PC [238].

Tanaka et al. [239] investigated PC remodeling by studying the metabolic fate of deuterium-labeled short-chain (di-C8:0) PC molecules that supported growth of the choline auxotrophic *cho2Δopi3Δ* strain. The short acyl chains at the *sn*-1 and *sn*-2 position were substituted yielding PC with a wild type-like molecular species profile. The appearance of remodeling intermediates with one C8-chain substituted showed that the observed PC remodeling was not just the result of degradation of diC_8PC to choline and recycling via the CDP-choline route, but actually involved a deacylation-reacylation mechanism. The intermediates showed that C16:0

preferentially substitutes for C8:0 at the *sn*-1 position, whereas C16:1 substituted with similar efficiency at both positions of the glycerol backbone. The involvement of candidate remodeling enzymes was tested, suggesting a role for the acyltransferase Ale1p and arguing against involvement of the phospholipases Plb1p, Plb2p, and Plb3p. Ale1p catalyzes acyl transfer to the *sn*-2 position of 1-acyl lyso-PC, with a preference for unsaturated acyl-CoA species [74,191–193,240].

Using a similar approach the remodeling of di-C10:0 PE molecules supplied in the culture medium was examined in a *psd1Δpsd2Δ* strain under conditions of a repressed CDP-ethanolamine pathway [241]. The replacement of the short chain at the *sn*-2 position by C16:1 or C18:1 preceded the exchange at the *sn*-1 position in the remodeling of the exogenous PE, in contrast to PC where there is no clear order. The phospholipases Plb1p, Plb2p, Plb3p, Nte1p, and the potential phospholipases Spo1p and Yor022cp were not required for remodeling of exogenous PE. The acyltransferase Ale1p was involved in the incorporation of C16:1 and C18:1 at the *sn*-2 position.

Except for Plb1p [238], the enzymes responsible for the phospholipase and acyltransferase or transacylase activities in acyl chain exchange remain to be identified. Phospholipase A_1 and A_2 activities hydrolyzing PC have been described, however gene–enzyme relationships remain to be established [242,243]. The triacylglycerol lipase Tgl4p may be involved as it shows phospholipase A_2 activity toward PC in vitro and affects the PC molecular species profile [196]. The lysophospholipid intermediates could also be generated by transacylases, such as Lro1p discussed in Section 7.3.1, and the cardiolipin remodeling transacylase Taz1p, discussed below. The acyl-CoA synthetases Faa1p and Faa4p were proposed to recycle free fatty acids generated during phospholipid remodeling [244]. Apart from Ale1p, candidate acyltransferases include Taz1p, which was shown to exhibit acyl-CoA independent 1-acyl-lyso-PC acyltransferase activity [245], and Tgl3p, a dual function enzyme with TAG lipase and acyltransferase activity [197]. Tgl3p acylates 1-acyl-lyso-PE in vitro with a preference for C18:1-CoA as acyl donor. The changes in phospholipid molecular species profiles resulting from inactivating Tgl3p could result from the loss of its acyltransferase activity, its lipase activity supplying fatty acids and DAG, or both [246].

Cst26p/Psi1p is an acyltransferase involved in PI acyl chain remodeling in yeast: it transfers C18:0 chains to 2-acyl-lyso-PI in vitro in an acyl-CoA dependent manner and is required for the incorporation of C18:0 at the *sn*-1 position of PI in vivo [154]. Deletion of *CST26* decreases the cellular content of C18:0 [148], which is attributed to the loss of a sink for C18:0 acyl chains (Fig. 4).

Remodeling of the mitochondrial phospholipid cardiolipin is crucial for establishing the characteristic CL species profile, enriched in C16:1 and C18:1 [247]. This process has been studied extensively and involves the transacylase Taz1p (the yeast homologue of tafazzin) [248] and the phospholipase A Cld1p [249], both localized to mitochondria. Cld1p specifically clips off C16:0 from CL, and the generated monolyso-CL is reacylated by Taz1p that presumably does not display any acyl chain preference by itself, similar to its tafazzin counterpart in *Drosophila* [161,250]. Instead, the enrichment in unsaturated acyl chains conferred to CL by tafazzin has been proposed to originate from the membrane environment where the enzyme preferably acts, i.e. in membrane zones of high negative curvature enriched in non-bilayer preferring lipids [251].

8. The interplay between phospholipid class and acyl chain composition

Prokaryotes such as *A. laidlawii* and *Escherichia coli* have been shown to be able to adjust their membrane lipid composition by varying lipid class and/or acyl chain composition to preserve the lamellar liquid crystalline phase when faced with varying culture temperatures, fatty acid supplements, or inactivation of lipid biosynthetic enzymes. These organisms avoid the formation of gel and non-bilayer phase that would impair membrane barrier function, while maintaining non-bilayer propensity (reviewed in [252]). Recently, chloroplasts were shown to reduce the content of non-bilayer preferring monogalactosyldiacylglycerol (MGDG) in response to freezing [253]. Higher eukaryotes have been suggested to control membrane lipid composition by similar regulatory mechanisms [254].

Insight into how phospholipid class and acyl chain composition affect one another in maintaining yeast membrane lipid homeostasis may provide a first glimpse of the underlying regulatory mechanisms. This requires studies in which the phospholipid class composition is analyzed in response to a measured change in fatty acid composition and vice versa, preferably at the level of the lipid molecular species. Unfortunately, the availability of such data is still rather limited. Here a number of relevant studies will be discussed and interpreted in terms of regulation of membrane lipid homeostasis.

8.1. Manipulation of acyl chain composition

Heme depletion in a *hem1Δ* mutant mimics anaerobiosis in yeast, and has been used as model to study the effects of accumulating saturated acyl chains. The increasing level of saturated acyl chains induced by heme depletion is accompanied by a shortening of the acyl chains (see Section 5.3 and [23]) and a rise in the level of PI at the expense of PS, PE and PC, with the saturated acyl chains preferentially being incorporated in PI and PC [255]. Overexpression of *PIS1* suppresses the growth defect of cells that accumulate saturated acyl chains due to a deficiency of the ubiquitin ligase Rsp5p required for *OLE1* transcription (Section 5.2) [256]. The suppression was explained by the rerouting of the saturated acyl chains from PE to PI to restore the acyl chain composition of PE. Overexpression of *SCT1* induces a rise in C16:0 and C18:0 acyl chains, decreases average acyl chain length, and increases the content of PI at the expense of PE; most of the saturated acyl chains are stored in PC and PI [148]. The above studies indicate that PI and PC serve as buffers for saturated acyl chains. With regard to membrane lipid homeostasis, the reduction of PE content with increased saturation may serve two purposes: (i) it counteracts the effect of increased saturation in maintaining fluidity, and (ii) it allows the PE remaining to preserve non-bilayer propensity.

Recent yeast lipidomics studies addressing the temperature dependence of the yeast lipidome have put the response to increased saturation into a more physiological perspective. With rising culture temperatures, gradual increases in the degree of saturation and the average length of the acyl chains were recorded, accompanied by a modest increase in PI at the expense of PE [1,16]. In terms of membrane lipid homeostasis, the most straight-forward interpretation is that the temperature-induced increases in membrane fluidity and intrinsic curvature are countered by reduced acyl chain desaturation and a decrease of the level of PE, respectively. Since the rise in PI at the expense of PE was shown to be a consequence of an increase in acyl chain saturation in the studies described above, the following mechanism emerges: first Ole1p activity is reduced in response to increased temperature resulting in decreased unsaturation of acyl chains; next, the suspected substrate selectivity of Pis1p for CDP-DAG containing saturated acyl chains (Section 7.1) enhances the flux of CDP-DAG toward PI at the expense of the flux toward PS and PE. A decrease in the conversion of PS to PE due to a reduced availability of unsaturated PS species may also contribute (see Sections 7.1 and 7.2).

Overexpression of *OLE1* increases overall acyl chain unsaturation and average acyl chain length [148,158] (see Section 5.3), however the effect on phospholipid composition has not been analyzed.

In Fig. 7 the effects of manipulating acyl chain desaturation have been summarized. In conclusion, an increase in the degree of acyl chain saturation evokes a response that seems to be primarily aimed at preserving membrane fluidity, and can to a large extent be explained by substrate preferences of the phospholipid biosynthetic enzymes, and accumulation of acyl-CoA intermediates affecting upstream reactions as argued in Section 5.3. The consequent inevitable loss of membrane intrinsic curvature is not taken for granted, since the PE remaining appears to be protected against losing non-bilayer propensity. Finally, we note that the effect of increased acyl chain saturation on phospholipid composition is reminiscent of the effect of zinc depletion (Section 4.2.2).

Fatty acids, including those that do not naturally occur in yeast, are efficiently taken up and incorporated into lipids when supplemented in the culture medium [7,138]. Only few studies have addressed the effects of exogenous fatty acids on both yeast acyl chain and phospholipid composition. When oleate (C18:1) replaces glucose as carbon source, the level of acyl chain unsaturation and C18:1-containing molecular species is strongly increased in all phospholipid classes, with PC storing most C18:1 [257]. However, membrane phospholipid composition is hardly affected, suggesting that the increased membrane fluidity and the enhanced membrane intrinsic curvature (resulting from the increased non-bilayer propensity of PE) under these conditions are still in the optimal range. In contrast, glucose-grown cells supplied with C14:1 display a shortening of average acyl chain length that as such would increase membrane fluidity and decrease PE non-bilayer propensity. Both effects appear to be counterbalanced by a strong rise in the proportion of PE [157].

8.2. Manipulation of phospholipid composition

In the complementary approach, phospholipid biosynthesis mutants allow manipulation of the phospholipid class composition and examination of the effect on acyl chain profiles. As the level of cellular PE is depleted by successively deleting *PSD1*, *PSD2*, and *ALE1*, the PE remaining is enriched in C18 acyl chains at the expense of C16 while the level of unsaturation is hardly affected [258], consistent with the molecular species selectivity of the CDP-ethanolamine route (Section 7.1; [201]). Interpreting these results in terms of membrane lipid homeostasis, it appears that the inevitable loss of membrane intrinsic curvature is somewhat compensated by enhancing the non-bilayer propensity of the PE remaining (by increased acyl chain length rather than by increased desaturation). Assuming that the changes in PE species profile reflect the overall changes in cellular fatty acid composition (these were not reported, but data from [198] support the assumption), the increase in membrane fluidity due to the loss of PE is counterbalanced by the increased C18/C16 ratio.

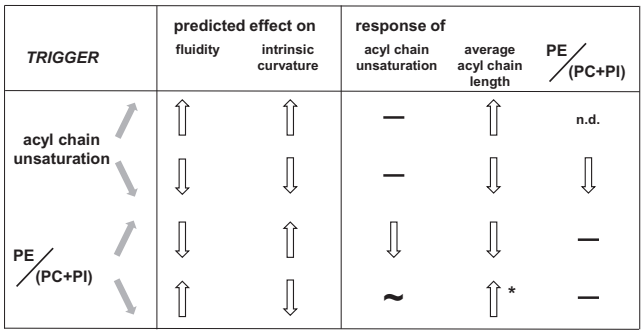

Fig. 7. Manipulation of membrane lipid composition. The predicted effects on membrane fluidity and intrinsic curvature of increasing and decreasing acyl chain unsaturation and PE-to-(PC + PI) ratio have been indicated, as well as the response of membrane lipid composition to these triggers (*based on PE species profile [258]). See text for details.

The choline auxotrophic *cho2Δopi3Δ* mutant has been used to study the effect of depleting PC. After choline deprivation, exponential growth continued until the PC content had dropped from the initial 40 mol% below 2 mol% of total phospholipids, consistent with growth requiring some (synthesis of) PC. PC depletion was accompanied by a shortening and increased saturation of cellular acyl chains, adaptations that do not reflect a unidirectional adjustment of membrane fluidity. The changes were largest in PE that took over as most abundant phospholipid, and decreased its non-bilayer propensity [26]. These results unequivocally demonstrated that yeast regulates the ratio between bilayer and non-bilayer preferring lipids [27]. The decrease in membrane fluidity conferred by increasing PE levels and acyl chain saturation may be balanced by the acyl chain shortening. During PC depletion, the PI level gradually increased most likely due to the overproduction of inositol (Opi^- phenotype). Also the level of *OLE1* transcript is increased, which was attributed to the increased acyl chain saturation [26]. Fig. 7 summarizes the effects of manipulating PE and PC levels. The regulatory mechanism(s) responsible for the adaptations in response to PC depletion/PE accumulation remain to be solved.

Interestingly, the *SCT1* (Suppressor of Choline Transport) gene was first identified as a multicopy suppressor of a mutant strain with a severe defect in PC synthesis [259]. The suppression can now be explained by the increased acyl chain saturation that is induced by overexpressing Sct1p. As a result, the non-bilayer propensity of PE is reduced preventing the loss of membrane integrity at high PE content. Reasoning along similar lines, we propose an explanation for the partial suppression of the choline auxotrophy of *cho2Δopi3Δ* cells by the *ale1Δ* mutation at 35 °C but not at 30 °C [193]. Previously, *cho2Δopi3Δ* cells cultured in the presence of choline were shown to degrade PE to GPE [215]. The absence of Ale1p might prevent recycling of GPE to PE (see Section 7.3) and thus mitigate the detrimental effects of increased membrane negative intrinsic curvature. However, this explanation only applies if the degradation of PE in the absence of choline (i.e. under conditions of PE accumulation) is more efficient at 35 °C than at 30 °C (as was found for PC in the presence of choline [215]), which remains to be shown.

9. Concluding remarks and future perspectives

With the exception of the PA-mediated regulation of UAS_{INO} genes, a detailed understanding of the regulatory mechanisms maintaining membrane lipid homeostasis in yeast remains elusive. Recurrent patterns in the response of membrane lipid composition to imposed changes in membrane fluidity or membrane intrinsic curvature (Fig. 7) provide the first bits and pieces of mechanistic clues about crosstalk between phospholipid class and acyl chain metabolism. The adaptation of the acyl chain composition varies between the major phospholipid classes. PC is the most versatile phospholipid in adapting its acyl chain composition to the prevailing conditions, in which PC synthesis via the CDP-choline route and acyl chain exchange are instrumental. PI maintains its enrichment in saturated acyl chains and can store extra saturated fatty acids as needed. The acyl chain composition of PE appears to be less prone to variation, most likely to preserve non-bilayer propensity.

Two new concepts relevant for the regulation of membrane lipid composition have emerged from this review: (i) the molecular

species selectivity of lipid biosynthetic enzymes and pathways, and (ii) mass action in acyl-CoA metabolism appear to be important intrinsic, passive contributors to the adaptation mechanisms in membrane lipid homeostasis. The most important players still missing are the sensors of the physical state of the membrane that signal the biosynthetic machinery. Spt23p is a prime candidate, however direct evidence is still lacking, let alone a molecular mechanism. Pct1p may serve as sensor-effector in adjusting membrane intrinsic curvature (Section 6).

In addition to identifying the mysterious membrane sensors, solving the molecular mechanism(s) of interorganelle bulk lipid transport presents a major hurdle in coming to grips with membrane lipid homeostasis. Future research will address the genes/enzymes still missing in lipid metabolism, particularly those involved in phospholipid turnover and acyl chain exchange. Other important research questions concern the role of phosphorylation of biosynthetic and turnover enzymes in tuning membrane lipid composition, and the underlying regulatory cascades. While this review has focused on the metabolism of bulk membrane phospholipids, the interplay with the metabolism of the other cellular lipid constituents, i.e. storage lipids, sterols and sphingolipids (see e.g. [246,260,261]), is of equal relevance and should be further explored. Finally, understanding essential features of membrane lipid homeostasis such as intracellular lipid sorting, membrane lipid asymmetry, and lateral segregation (domain formation) of lipids, largely ignored in this review, will add additional layers of complexity.

Important contributions are expected from lipidomic analyses. Dynamic lipidomics approaches using stable isotope-labeled lipid precursors, will map the fluxes of lipid molecular species through the metabolic pathways, under a variety of conditions. Ultimately this could lead to a systems biology-based computational model capable of predicting adaptations in yeast membrane lipid composition.

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