

# Phosphatidate phosphatase regulates membrane phospholipid synthesis via phosphatidylserine synthase



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## ABSTRACT

The yeast *Saccharomyces cerevisiae* serves as a model eukaryote to elucidate the regulation of lipid metabolism. In exponentially growing yeast, a diverse set of membrane lipids are synthesized from the precursor phosphatidate via the liponucleotide intermediate CDP-diacylglycerol. As cells exhaust nutrients and progress into the stationary phase, phosphatidate is channeled via diacylglycerol to the synthesis of triacylglycerol. The *CHO1*-encoded phosphatidylserine synthase, which catalyzes the committed step in membrane phospholipid synthesis via CDP-diacylglycerol, and the *PAH1*-encoded phosphatidate phosphatase, which catalyzes the committed step in triacylglycerol synthesis are regulated throughout cell growth by genetic and biochemical mechanisms to control the balanced synthesis of membrane phospholipids and triacylglycerol. The loss of phosphatidate phosphatase activity (e.g., *pah1Δ* mutation) increases the level of phosphatidate and its conversion to membrane phospholipids by inducing *Cho1* expression and phosphatidylserine synthase activity. The regulation of the *CHO1* expression is mediated through the inositol-sensitive upstream activation sequence ( $UAS_{INO}$ ), a *cis*-acting element for the phosphatidate-controlled Henry (*Ino2–Ino4/Opi1*) regulatory circuit. Consequently, phosphatidate phosphatase activity regulates phospholipid synthesis through the transcriptional regulation of the phosphatidylserine synthase enzyme.

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## 1. Introduction

The yeast *Saccharomyces cerevisiae*<sup>1</sup> is used as a model eukaryotic organism to elucidate the metabolism, cell biology, and regulation of glycerolipids. The strong homology of yeast proteins, pathways, and regulatory networks with those of higher eukaryotes has provided numerous insights into the genetics and biochemistry of lipid-related diseases (Henry et al., 2012; Kohlwein, 2010; Kurat et al., 2006; Natter and Kohlwein, 2013). The synthesis of lipids is a dynamic process that yeast cells engage in throughout their growth (Carman and Han, 2011; Chang and Carman, 2008; Henry et al., 2012). In exponentially growing yeast, a diverse set of membrane phospholipids (e.g., phosphatidylserine and its derivatives phosphatidylethanolamine and phosphatidylcholine, phosphatidylinositol and its derivative phosphoinositides and sphingolipids, and phosphatidylglycerophosphate and its derivatives phosphatidylglycerol and cardiolipin) are synthesized from the precursor phosphatidate via the liponucleotide intermediate CDP-diacylglycerol (Henry et al., 2012). As the cells exhaust nutrients and progress into the stationary phase (e.g., quiescence), phosphatidate is channeled to the synthesis of triacylglycerol via its dephosphorylation to diacylglycerol (Hosaka and Yamashita, 1984; Pascual et al., 2013; Taylor and Parks, 1979). Upon growth resumption with fresh medium, the stored triacylglycerol is mobilized to diacylglycerol and free fatty acid for the synthesis of phosphatidate and its conversion to membrane phospholipids (Carman and Han, 2011; Fakas et al., 2011a; Gaspar et al., 2011; Henry et al., 2012; Kurat et al., 2009; Rajakumari et al., 2008). This review focuses on the *PAH1*-encoded phosphatidate phosphatase (PAP)<sup>2</sup> (EC 3.1.3.4) and the *CHO1*-encoded phosphatidylserine synthase (PSS)<sup>3</sup> (EC 2.7.8.8), which are highly regulated to control the synthesis of triacylglycerol and membrane phospholipids during cell growth.

PAP catalyzes the Mg<sup>2+</sup>-dependent dephosphorylation of phosphatidate to produce diacylglycerol (Han et al., 2006; Lin and Carman, 1989) (Fig. 1A), whereas PSS catalyzes the Mn<sup>2+</sup>-dependent formation of phosphatidylserine by displacing the CMP moiety from CDP-diacylglycerol with serine (Bae-Lee and Carman, 1984; Kiyono et al., 1987; Letts et al., 1983; Nikawa et al., 1987) (Fig. 2A). PAP activity is governed by a conserved DXDX(T/V) catalytic motif within its haloacid dehalogenase-like domain (Han et al., 2006, 2007; Koonin and Tatusov, 1994; Madera et al., 2004; Péterfy et al., 2001) (Fig. 1B), whereas PSS activity is governed by a conserved CDP-alcohol phosphotransferase motif DGX<sub>2</sub>ARX<sub>7,8</sub>GX<sub>3</sub>DX<sub>3</sub>D within a larger domain common to other phospholipid biosynthetic enzymes that catalyze similar types of reactions (Williams and McMaster, 1998) (Fig. 2B). For catalytic function *in vivo*, both PSS and PAP associate with the membrane to access their phospholipid substrates. PSS is an integral membrane enzyme in the endoplasmic reticulum (Habeler et al., 2002; Huh et al., 2003; Kumar et al., 2002; Natter et al., 2005), whereas PAP as a peripheral membrane enzyme that translocates from the cytosol to the nuclear/endoplasmic reticulum membrane (Barbosa et al., 2015; Karanasios et al., 2010, 2013).

The PAP reaction is the committed step for the synthesis of the neutral lipid triacylglycerol, whereas the PSS reaction is the committed step in the CDP-diacylglycerol pathway for the *de novo* synthesis of the major membrane phospholipids phosphatidylcholine and phosphatidylethanolamine (Carman and Han, 2011; Henry et al., 2012) (Fig. 3). The diacylglycerol produced from the PAP reaction can also be used for the synthesis of phosphatidylcholine and phosphatidylethanolamine, respectively, in the CDP-choline and CDP-ethanolamine branches of the Kennedy pathway when supplemented with choline or ethanolamine, and this biosynthetic pathway becomes essential for the cells defective in PSS and other enzymes in the CDP-diacylglycerol pathway of phospholipid synthesis (Carman and Han, 2011; Henry et al., 2012) (Fig. 3). The PAP and PSS reactions have a close relationship with phosphatidate in that it is the substrate of PAP and the precursor of the CDP-diacylglycerol used as the substrate of PSS (Fig. 3). The partitioning of phosphatidate between diacylglycerol and CDP-diacylglycerol is a major regulatory step that bifurcates lipid synthesis into branches that lead to triacylglycerol and membrane phospholipids (Fakas et al., 2011b; Han et al., 2006; Pascual et al., 2013) (Fig. 3). The PAP enzyme plays a major role in this metabolism by exerting a negative regulatory effect on the level of phosphatidate used for the *de novo* synthesis of membrane phospholipids. The regulations of the PAP and PSS enzymes and their connections in controlling lipid synthesis are discussed below.

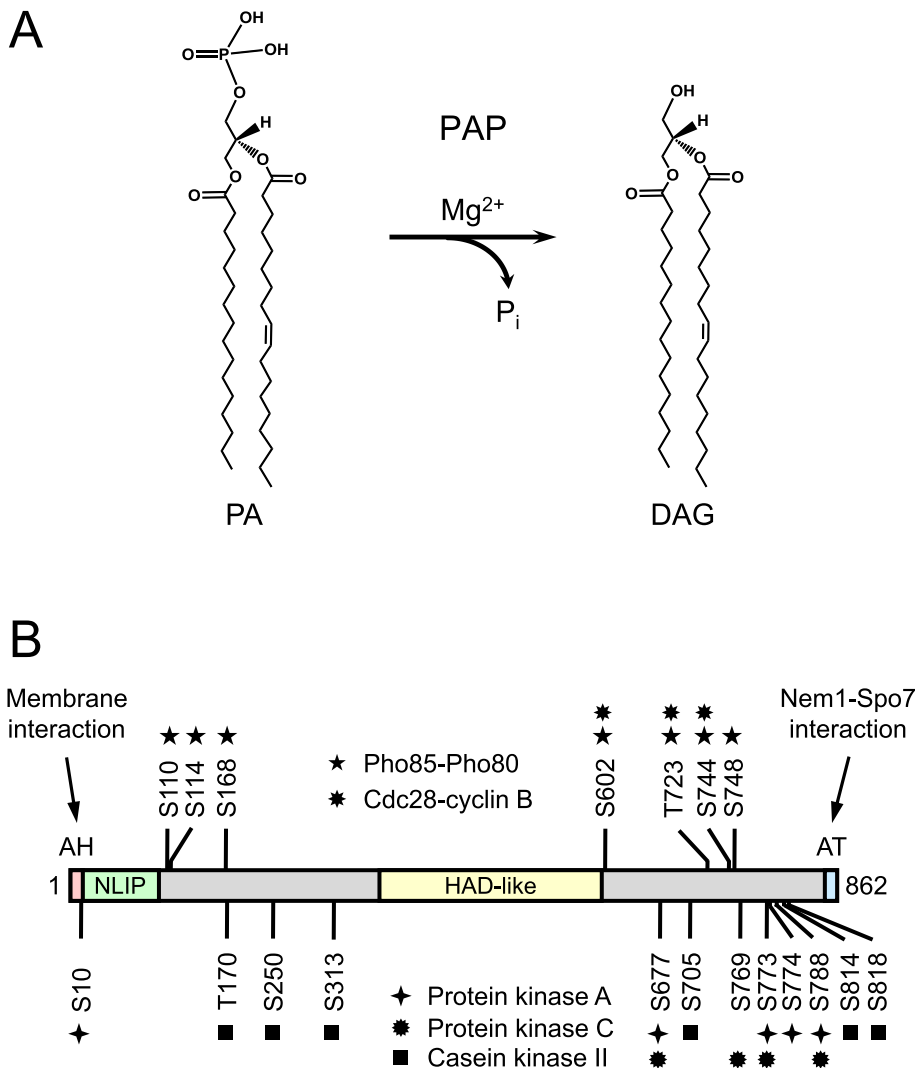
## 2. Importance of PAP and PSS in lipid metabolism and cell physiology

The analyses of yeast mutants lacking PAP and PSS have shed light on the importance of the enzymes in lipid metabolism and cell physiology. The *pah1Δ* mutant exhibits increased levels of the PAP substrate phosphatidate, but decreased levels of the enzyme product diacylglycerol and its derivative triacylglycerol (Fakas et al., 2011b; Han et al., 2006; Han et al., 2007). The *pah1Δ* mutation results in a variety of phenotypes that include the induction of phospholipid synthesis genes, the increase of phospholipid synthesis, the expansion of the nuclear/endoplasmic reticulum membrane, the susceptibility to fatty acid-

<sup>1</sup> In this review, *Saccharomyces cerevisiae* is used interchangeably with yeast.

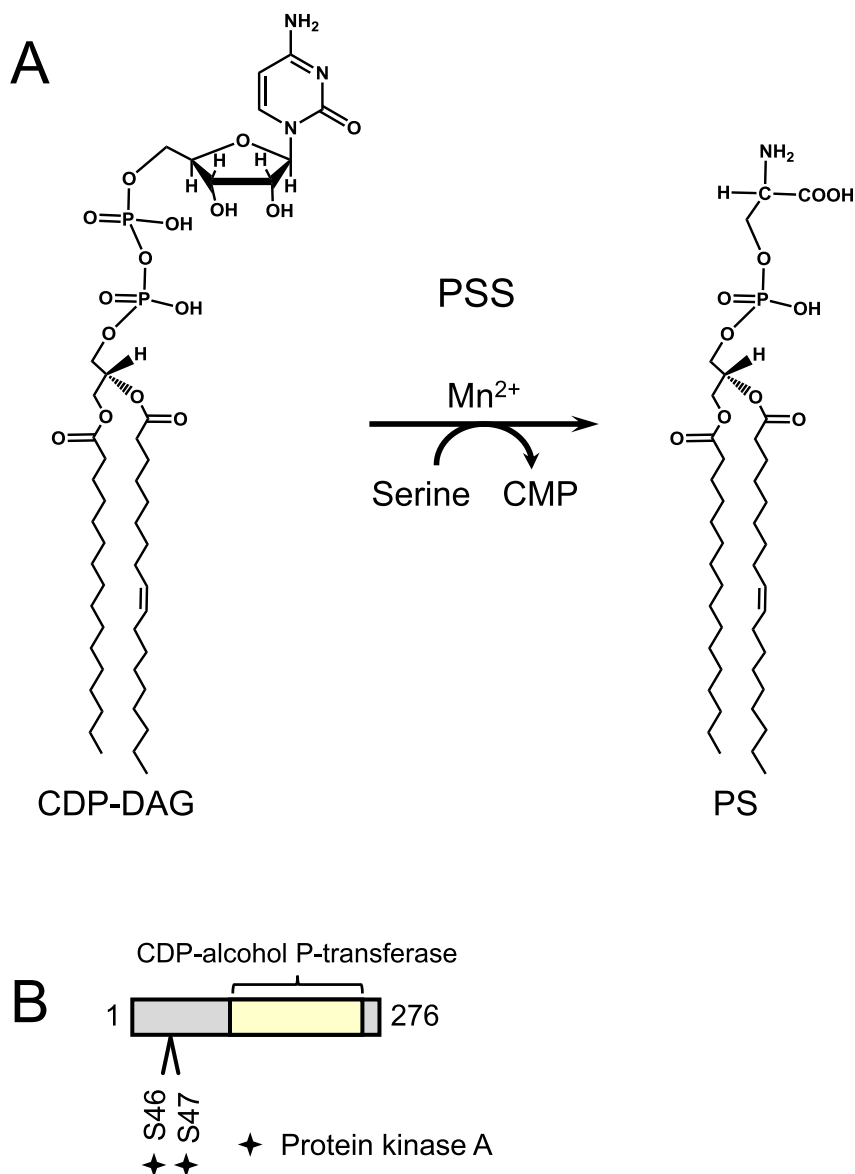
<sup>2</sup> The PAP orthologs in various organisms are known by different acronyms that are based on the names of genes that encode the enzyme. For example, in *S. cerevisiae*, the protein product of the *PAH1* gene is known as Pah1 (Han et al., 2006), whereas in human and mouse, the protein products of the *LPIN1* and *Lpin1* genes, respectively, are known as lipin 1 (Péterfy et al., 2001). The PAP encoded by *PAH1* differs from the lipid phosphate phosphatase enzymes encoded by *APP1* (Chae et al., 2012; Chae and Carman, 2013), *DPP1* (Toke et al., 1998) and *LPP1* (Toke et al., 1999), which dephosphorylate a broad spectrum of substrates (e.g., phosphatidate, lysophosphatidate, diacylglycerol pyrophosphate) and are not involved in *de novo* lipid synthesis.

<sup>3</sup> The *S. cerevisiae* PSS should differ from the PSS from Gram-negative bacteria (e.g., *Escherichia coli*), which catalyzes its CDP-DAG-dependent reaction via a metal cofactor-independent ping-pong reaction mechanism (Larson and Dowhan, 1976) or the PSS enzyme from mammalian cells, which catalyzes an exchange reaction between phosphatidylcholine or phosphatidylethanolamine with serine (Vance, 1998).



**Fig. 1.** Reaction catalyzed by yeast PAP and the domain structure and phosphorylation sites in Pah1. **A**, The figure shows the structures of phosphatidate (PA) and diacylglycerol (DAG) and the reaction catalyzed by PAP. **B**, The diagram shows the positions of the amphipathic helix (AH, pink) required for endoplasmic reticulum membrane interaction (Karanasios et al., 2010), the NLIP (green) and HAD-like (yellow) domains that are required for PAP activity (Han et al., 2007), the acidic tail (AT) required for interaction with Nem1-Spo7 (Karanasios et al., 2013), and the serine (S) and threonine (T) residues that are phosphorylated by Pho85-Pho80 (Choi et al., 2012), Cdc28-cyclin B (Choi et al., 2011), protein kinase A (Su et al., 2012), protein kinase C (Su et al., 2014a), and casein kinase II (Hsieh et al., 2016). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

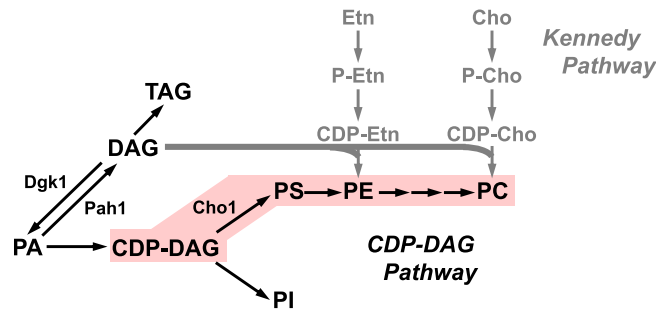
induced toxicity, and the reduction of lipid droplet formation (Adeyo et al., 2011; Fakas et al., 2011b; Han et al., 2006; Han et al., 2007; Santos-Rosa et al., 2005). The impact of the PAP deficiency on overall cell physiology is further exemplified by the fact that the *pah1*Δ mutant cannot grow on non-fermentable carbon sources (Han et al., 2006; Irie et al., 1993) as well as at elevated temperatures (Han et al., 2006; Irie et al., 1993; Santos-Rosa et al., 2005). Moreover, the mutant is hypersensitive to oxidative stress and has a shortened chronological life span (Park et al., 2015), and exhibits defects in cell wall integrity (Lussier et al., 1997; Ruiz et al., 1999) and vacuole fusion (Sasser et al., 2011). Some of the *pah1*Δ phenotypes require the function of Dgk1 (Adeyo et al., 2011; Fakas et al., 2011b; Han et al., 2008), the CTP-dependent diacylglycerol kinase that phosphorylates diacylglycerol to form phosphatidate (Fig. 3). The *cho1*Δ mutant lacks the ability to synthesize phosphatidylserine (Atkinson et al., 1980a, 1980b), and thus requires the supplementation of choline or ethanolamine to synthesize phosphatidylcholine or phosphatidylethanolamine by way of the Kennedy pathway (Carman and Han, 2011; Henry et al., 2012). Studies with cells lacking PSS activity have revealed that the product phosphatidylserine is required for vacuole function and morphogenesis (Hamamatsu et al., 1994), directing endocytic proteins to the plasma membrane (Sun and Drubin, 2012), tryptophan transport (Nakamura et al., 2000), and protein kinase C function (Dey et al., 2017; Nomura et al., 2017). Overall, these observations highlight the importance of understanding how the PAP and PSS enzymes are regulated.



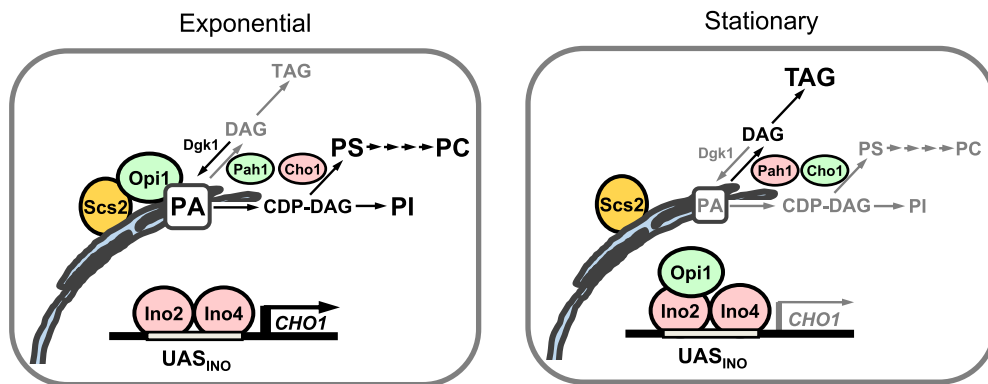
**Fig. 2.** Reaction catalyzed by yeast PSS and the domain structure and phosphorylation sites in Cho1. **A**, The figure shows the structures of CDP-diacylglycerol (CDP-DAG) and phosphatidylserine (PS) and the reaction catalyzed by PSS. **B**, The diagram shows the positions of the CDP-alcohol phosphotransferase motif (yellow) and the serine (S) residues that are phosphorylated by protein kinase A (Choi et al., 2010). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 3. Genetic and biochemical regulations of PSS

The PSS enzyme is regulated for its protein level and catalytic activity by genetic and biochemical mechanisms. The expression of the PSS gene, *CHO1*, is regulated by water-soluble phospholipid precursors (e.g., inositol, choline, ethanolamine, serine) (Bailis et al., 1987, 1992; Homann et al., 1987a; Klig et al., 1985; Poole et al., 1986), essential nutrients (e.g., zinc) (Iwanyshyn et al., 2004), and growth phase (Homann et al., 1987b; Lamping et al., 1995). These forms of regulation occur through the Henry regulatory circuit (Fig. 4), which involves the UAS<sub>INO</sub> cis-acting element in the *CHO1* promoter, the transcriptional activators Ino2 and Ino4, and the transcriptional repressor Opi1 (Carman and Han, 2009b; Carman and Henry, 1999; Carman and Henry, 2007; Greenberg and Lopes, 1996). The expression of *CHO1* is induced in the exponential phase when cells are grown in the absence of the phospholipid precursors (Bailis et al., 1987, 1992; Homann et al., 1987a; Klig et al., 1985; Poole et al., 1986) and in the presence of the essential nutrient zinc (Iwanyshyn et al., 2004). The elevation of the gene expression is mediated by an Ino2-Ino4 activator complex that drives transcription through its binding to the UAS<sub>INO</sub> element in the promoter (Carman and Henry, 1999; Chen et al., 2007; Greenberg and Lopes, 1996; Henry and Patton-Vogt, 1998). This



**Fig. 3.** Lipid synthesis in yeast. The pathways shown for the synthesis of lipids include the relevant steps discussed in this review. A more comprehensive figure for the synthesis of triacylglycerol and membrane phospholipids via the CDP-diacylglycerol and Kennedy pathways may be found in reference (Henry et al., 2012). The CDP-diacylglycerol pathway of phospholipid synthesis is highlighted in pink, whereas the Kennedy pathway is shown in grey to indicate its minor role in phospholipid synthesis in cells grown without choline (Cho) or ethanolamine (Etn). The reactions catalyzed by the CHO1-encoded PSS, PAH1-encoded PAP, and DKG1-encoded diacylglycerol kinase are indicated. Abbreviations: PA, phosphatidate; DAG, diacylglycerol; TAG, triacylglycerol; CDP-DAG, CDP-diacylglycerol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 4.** Model for the PAP-mediated regulation of PSS expression and lipid synthesis during growth. The diagram shows the Henry regulatory circuit that includes the repressor Opi1, the Ino2-Ino4 activator complex, and the UAS<sub>INO</sub> element in CHO1 (Henry et al., 2012), and the bifurcation of phosphatidate for the synthesis of triacylglycerol and phospholipids via the CDP-diacylglycerol pathway. Under growth conditions (e.g., exponential phase, left) whereby Pah1 expression (green highlight) and PAP activity are low (Pascual et al., 2013), the level of phosphatidate is elevated and the Opi1 repressor is tethered to the nuclear/endoplasmic reticulum membrane via its interactions with phosphatidate and Scs2. This allows for the transcriptional activation (bold arrow) of CHO1 by the Ino2-Ino4 complex and the inductions of Cho1 (pink highlight) and PSS activity for increased phospholipid synthesis (large letters) via the CDP-diacylglycerol pathway. The reduced rate of triacylglycerol synthesis in the exponential phase of growth (Pascual et al., 2013) is indicated with small grey letters. Under growth conditions (e.g., stationary phase, right) whereby Pah1 expression (pink highlight) and PAP activity are high (Pascual et al., 2013), the synthesis of triacylglycerol is elevated (large letters) and the phosphatidate level is reduced (small grey letters). This allows for the dissociation of Opi1 from the nuclear/endoplasmic reticulum membrane and its entry into the nucleus where it represses the transcriptional activation of CHO1 (thin grey arrow and letters) by inhibiting the function of the Ino2-Ino4 activator complex through its binding to Ino2. The repression of Cho1 (green highlight) and PSS activity results in the reduction of phospholipid synthesis (small grey letters). Abbreviations: PA, phosphatidate; DAG, diacylglycerol; TAG, triacylglycerol; CDP-DAG, CDP-diacylglycerol; PS, phosphatidylserine; PC, phosphatidylcholine; PI, phosphatidylinositol. The figure was taken from Han and Carman (2017). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

results in an increase in CHO1 mRNA abundance, PSS protein, and its enzyme activity (Bailis et al., 1987, 1992; Homann et al., 1987a; Iwanyshyn et al., 2004; Klig et al., 1985; Poole et al., 1986). Overall, this regulation favors the use of CDP-diacylglycerol for the synthesis phosphatidylserine relative to phosphatidylinositol, and the synthesis of phosphatidylcholine and phosphatidylethanolamine via the CDP-diacylglycerol pathway (Carman and Henry, 1999, 2007) (Fig. 3).

In contrast, the expression of CHO1 is reduced in the exponential phase by inositol supplementation, and this regulation is enhanced by the inclusion of choline, ethanolamine, or serine in the growth medium (Bailis et al., 1987, 1992; Homann et al., 1987a; Klig et al., 1985; Poole et al., 1986). In the absence of inositol supplementation, CHO1 expression is also reduced in exponential phase cells when zinc is depleted from the growth medium (Iwanyshyn et al., 2004) or when cells progress from the exponential to the stationary phases of growth (Homann et al., 1987b; Lamping et al., 1995). The regulations by zinc and growth phase occur in the absence of inositol supplementation (Homann et al., 1987b; Iwanyshyn et al., 2004; Lamping et al., 1995). The reduction of CHO1 expression is mediated by the repressor Opi1, which interacts with Ino2 to attenuate transcription for reduced abundance of mRNA and protein, and enzymatic activity (Carman and Henry, 1999; Chen et al., 2007; Greenberg and Lopes, 1996; Henry and Patton-Vogt, 1998) (Fig. 4). Consequently, CDP-diacylglycerol is favorably partitioned

to phosphatidylinositol at the expense of phosphatidylserine and there is decrease in the synthesis of phosphatidylcholine and phosphatidylethanolamine via the CDP-diacylglycerol pathway (Carman and Henry, 1999, 2007). The attenuation of the CDP-diacylglycerol pathway for phosphatidylcholine and phosphatidylethanolamine synthesis by *CHO1* repression is compensated by the Kennedy pathway when cells are supplemented with choline or ethanolamine (Carman and Han, 2009b; Carman and Henry, 1999; Carman and Henry, 2007).

The expression of *CHO1* is also controlled by mechanisms that do not involve its UAS<sub>INO</sub> element and the transcription factors Ino2, Ino4, and Opi1. For example, the level of the *CHO1* transcript is controlled at the post-transcriptional level by its rate of decay (Choi et al., 2004; Choi and Carman, 2007). The *CHO1* transcript is primarily degraded through the general 5'-3' mRNA decay pathway that involves deadenylation, mRNA decapping, and 5'-3'- exonuclease activities (Choi and Carman, 2007). In wild type cells, the *CHO1* transcript is moderately stable with a half-life of 12 min (Choi and Carman, 2007). However, defects in mitochondrial respiration stabilize the *CHO1* transcript to a half-life of >45 min (Choi and Carman, 2007). This regulation results in increases in the abundance of *CHO1* mRNA and PSS protein, enzymatic activity, and the synthesis of phosphatidylserine (Choi and Carman, 2007).

With respect to its biochemical regulation, PSS is stimulated by phosphatidate (PAP substrate) by a mechanism that increases its affinity for CDP-diacylglycerol, whereas it is non-competitively inhibited by diacylglycerol (PAP product) (Bae-Lee and Carman, 1990) and inositol (Kelley et al., 1988). PSS is inhibited by the nucleotide CTP through its chelation effect on the divalent metal cofactor (McDonough et al., 1995). Stimulation of PSS activity favors phospholipid synthesis via the CDP-diacylglycerol pathway (Carman and Zeimet, 1996), whereas the enzyme inhibition favors the synthesis of phosphatidylinositol relative to phosphatidylserine, and the synthesis of phospholipids via the Kennedy pathway provided that cells are supplemented with choline or ethanolamine (Carman and Han, 2009b; Carman and Zeimet, 1996).

Phosphorylation is another biochemical mechanism by which PSS is regulated (Choi et al., 2010; Kinney and Carman, 1988). Protein kinase A (cAMP-dependent protein kinase) phosphorylates PSS at Ser-46 and Ser-47 (Fig. 2B). On the one hand, the phosphorylation at these sites inhibits PSS activity (Kinney and Carman, 1988), but on the other hand, the phosphorylation stabilizes its abundance for the net effect of stimulating the synthesis of phosphatidylserine relative to phosphatidylinositol (Choi et al., 2010).

#### 4. Genetic and biochemical regulations of PAP

Insight into the expression, mode of action, and biochemical regulation of PAP has been gained through studies on the yeast enzyme (Carman and Han, 2006, 2009a; Pascual and Carman, 2013; Siniosoglou, 2009, 2013). The expression of *PAH1* is regulated at the transcriptional level by growth phase and nutrient status (Pascual et al., 2013; Soto-Cardalda et al., 2011). The transcription of *PAH1* is induced throughout growth, and its induction in the stationary phase is enhanced by inositol supplementation (Pascual et al., 2013). This transcriptional regulation is mediated through Ino2, Ino4, Opi1, Gis1, and Rph1 (Pascual et al., 2013). The *PAH1* expression is also induced by zinc deficiency in the exponential phase through the Zap1-mediated transcriptional activation, resulting in an increase in the synthesis of phosphatidylcholine via the CDP-choline branch of the Kennedy pathway (Soto-Cardalda et al., 2011). In contrast, the induction of *PAH1* expression in zinc-replete stationary phase cells is responsible for increased synthesis and accumulation of triacylglycerol that occurs at the expense of phospholipid synthesis (Pascual et al., 2013). In response to growth phase and nutrient status, transcription factors that induce the *PAH1* expression have negative regulatory effects on phospholipid synthesis genes (e.g., *CHO1*, see below) (Carman and Han, 2011; Henry et al., 2012), and the opposing regulations collectively contribute to the balanced synthesis of membrane phospholipids and triacylglycerol.

On a biochemical level, PAP activity is stimulated by negatively charged phospholipids such as CDP-diacylglycerol (PSS substrate) that increase the enzyme affinity for phosphatidate (Wu and Carman, 1996), but is attenuated by positively charged sphingoid bases (e.g., sphinganine and phytosphingosine) that decrease its affinity for phosphatidate (Wu et al., 1993). The enzyme is also inhibited by ATP and CTP through a complex mechanism that affects both the  $V_{max}$  and the  $K_m$  for phosphatidate, and through a chelating effect on the cofactor  $Mg^{2+}$  (Wu and Carman, 1994). As a peripheral membrane protein (Han et al., 2006), Pah1 associates with the nuclear/endoplasmic reticulum membrane through its dephosphorylation that is catalyzed by an endoplasmic reticulum-associated protein phosphatase complex composed of Nem1 (catalytic subunit) and Spo7 (regulatory subunit) (Barbosa et al., 2015; Choi et al., 2011, 2012; Karanasios et al., 2010; Karanasios et al., 2013; O'Hara et al., 2006; Santos-Rosa et al., 2005; Siniosoglou et al., 1998; Su et al., 2012; Xu et al., 2011). The interaction of Pah1 with Nem1-Spo7 occurs through the C-terminal acidic tail (Karanasios et al., 2013), whereas its membrane association occurs through the N-terminal amphipathic helix (Karanasios et al., 2010). The dephosphorylation of Pah1 by Nem1-Spo7 also stimulates its PAP activity and degradation by the 20S proteasome (Choi et al., 2011, 2012; Hsieh et al., 2015; O'Hara et al., 2006; Pascual et al., 2014; Santos-Rosa et al., 2005; Su et al., 2012, 2014a, 2014b; Xu et al., 2011).

Pah1 is a phosphoprotein in the cytosol, and its phosphorylation is carried out by multiple protein kinases. Our laboratory has taken a systematic approach to identify protein kinases that phosphorylate Pah1, to determine its phosphorylation sites, and to reveal the physiological relevance of its phosphorylation through mutagenic analyses. We have shown that Pah1 is a *bona fide* substrate for Pho85-Pho80 (Choi et al., 2012), Cdc28-cyclin B (Choi et al., 2011), protein kinase A (Su et al., 2012), protein kinase C (Su et al., 2014a), and casein kinase II (Hsieh et al., 2016) by determining its phosphorylation sites. Some of the phosphorylation sites regulate the localization of Pah1, its PAP activity, or susceptibility to the 20S proteasomal degradation (Choi et al., 2011, 2012; Su et al., 2012; Su et al., 2014a). Moreover, the phosphorylation of Pah1 on some sites influences



its phosphorylation on other sites by the same protein kinase or different protein kinases, indicating that it is subject to hierarchical phosphorylation (Hsieh et al., 2016; Su et al., 2012, 2014a). In general, the phosphorylation of PAP attenuates its function by sequestering the enzyme to a cytosolic location apart from its substrate phosphatidate and by inhibiting its enzyme activity (Choi et al., 2011, 2012; O'Hara et al., 2006; Su et al., 2012), whereas the dephosphorylation has the opposite effect (Choi et al., 2011; Madeo et al., 1997; O'Hara et al., 2006; Su et al., 2012, 2014b). Overall, phosphorylation of PAP favors phospholipid synthesis at the expense of triacylglycerol synthesis, whereas dephosphorylation favors triacylglycerol synthesis at the expense of phospholipids synthesis (Pascual and Carman, 2013).

## 5. PAP controls the expression of PSS for membrane phospholipid synthesis

Changes in PAP activity are directly related to triacylglycerol synthesis, but are inversely related to phospholipid synthesis during cell growth (Pascual et al., 2013). Hence, yeast cells have a lower PAP activity in the exponential phase when the rate of phospholipid synthesis is high, but have a higher PAP activity in the stationary phase when the rate of phospholipid synthesis is low (Pascual et al., 2013). The increased level of phospholipid synthesis by the lack of PAP, which causes the expansion of the nuclear/endoplasmic reticulum membrane, is related to the increased availability of phosphatidate by the defect of its conversion to diacylglycerol as well as by the phosphatidate-mediated transcriptional induction of phospholipid synthesis genes (Fakas et al., 2011b; Han et al., 2006; Santos-Rosa et al., 2005).

The expression of PSS is massively induced (~20-fold) in the *pah1Δ* mutant, especially in the stationary phase when the *CHO1* expression and PSS activity of wild type cells is normally reduced relative to exponential phase (Han and Carman, 2017). A promoter analysis has revealed that the induced expression of PSS in the *pah1Δ* mutant is mediated through the UAS<sub>INO</sub> element in the *CHO1* gene (Han and Carman, 2017). This supports the notion that the elevation of the phosphatidate level in the *pah1Δ* mutant cells has an inhibitory effect on the Opi1-mediated transcriptional repression of *CHO1* as per the Henry regulatory circuit (Fig. 4). Indeed, phosphatidate is known to sequester Opi1 at the nuclear/endoplasmic reticulum membrane (Henry et al., 2012; Loewen et al., 2004). The upregulation of PSS expression as a major driving force in the synthesis of membrane phospholipids in the *pah1Δ* mutant is supported by the observation that a *CHO1* UAS<sub>INO</sub> mutation abolishes the upregulation of PSS and suppresses the increase of phospholipid synthesis and nuclear/endoplasmic reticulum membrane as well as the decrease of triacylglycerol synthesis and lipid droplet formation that is characteristic of *pah1Δ* mutant cells (Han and Carman, 2017). Moreover, the suppressive effects of the *CHO1* UAS<sub>INO</sub> mutation on the *pah1Δ* phenotypes are pronounced in the exponential phase of growth (Han and Carman, 2017) when PSS activity is expressed at its highest level (Homann et al., 1987b). These observations highlight the role of PAP activity in the negative regulation of PSS expression for membrane phospholipid synthesis. A model showing the PAP-mediated regulation of PSS expression and lipid synthesis during growth is shown in Fig. 4 (Han and Carman, 2017).

It has been known that transcription of another UAS<sub>INO</sub>-containing phospholipid synthesis gene (e.g., *OPI3*) of the CDP-diacylglycerol pathway is also induced in the *pah1Δ* mutant (O'Hara et al., 2006; Santos-Rosa et al., 2005). Thus, the encoded phospholipid methyltransferase enzyme that is involved in the synthesis of phosphatidylcholine from phosphatidylethanolamine is also expected to play a role in this regulation. Phospholipid synthesis genes (e.g., *CK11* (Hosaka et al., 1990), *CPT1* (Morash et al., 1994), *EK11* (Kersting et al., 2004), *EPT1* (McMaster and Bell, 1994)) in the Kennedy pathway are also subject to transcriptional regulation through the UAS<sub>INO</sub> element, but their encoded activities are expected to play a major role in this regulation only if the growth medium is supplemented with choline and/or ethanolamine (Carman and Han, 2011; Henry et al., 2012).

## 6. Concluding remarks

The regulation of lipid synthesis in yeast, as well as in higher eukaryotes, is very complex. The enzymes responsible for lipid synthesis are regulated by genetic and biochemical mechanisms for the control of their cellular locations, activity, and stability. In this review, we have given the reader some insight into how two of the most highly regulated enzymes in yeast lipid metabolism are regulated. Clearly, the long-term genetic regulations of the PAP and PSS enzymes coupled with their short-term biochemical regulations are critical to controlling the balance between the synthesis of triacylglycerol for stasis and the synthesis of membrane phospholipids for cell growth.

## Conflicts of interest

None.

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