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Phosphatidate-mediated regulation of lipid synthesis at the nuclear/endoplasmic reticulum membrane☆

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Abstract

In yeast and higher eukaryotes, phospholipids and triacylglycerol are derived from phosphatidate at the nuclear/endoplasmic reticulum membrane. In *de novo* biosynthetic pathways, phosphatidate is channeled into membrane phospholipids via its conversion to CDP-diacylglycerol. Its dephosphorylation to diacylglycerol is required for the synthesis of triacylglycerol as well as for the synthesis of phosphatidylcholine and phosphatidylethanolamine via the Kennedy pathway. In addition to the role of phosphatidate as a precursor, it is a regulatory molecule in the transcriptional control of phospholipid synthesis genes via the Henry regulatory circuit. Pah1 phosphatidate phosphatase and Dgk1 diacylglycerol kinase are key players that function counteractively in the control of the phosphatidate level at the nuclear/endoplasmic reticulum membrane. Loss of Pah1 phosphatidate phosphatase activity not only affects triacylglycerol synthesis but also disturbs the balance of the phosphatidate level, resulting in the alteration of lipid synthesis and related cellular defects. The *pah1*Δ phenotypes requiring Dgk1 diacylglycerol kinase exemplify the importance of the phosphatidate level in the misregulation of cellular processes. The catalytic function of Pah1 requires its translocation from the cytoplasm to the nuclear/endoplasmic reticulum membrane, which is regulated through its phosphorylation in the cytoplasm by multiple protein kinases as well as through its dephosphorylation by the membrane-associated Nem1-Spo7 protein phosphatase complex. This article is part of a Special Issue entitled Endoplasmic Reticulum Platforms for Membrane Lipid Dynamics.

Graphical abstract

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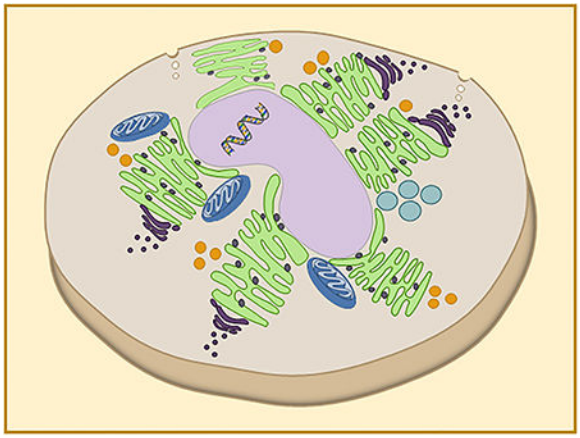

Yeast cell lacking the Pah1 phosphatidate phosphatase enzyme

Keywords

phospholipid; triacylglycerol; phosphatidate; diacylglycerol; phosphatidate phosphatase; diacylglycerol kinase

1. Introduction

Biological membranes are composed of many lipid species in which proteins are embedded. The eukaryotic cell membranes contain three major classes of lipids: phospholipids, sphingolipids, and sterols [1]. Phospholipids, amphipathic molecules consisting of hydrophobic tails (i.e., fatty acids) and hydrophilic heads, are major constituents of the membrane [2]. They also function as reservoirs of lipid mediators and serve to tether proteins at the membrane surface. Sphingolipids, which are also amphipathic in nature, are composed of a lipophilic amino alcohol (sphingosine) and hydrocarbon chains [1]. Additionally, they are involved in membrane structure, function, and signalling [1]. A subgroup of sphingolipids are glycosphingolipids and gangliosides, which play a key role in cell recognition and signalling [3]. Sterols are mainly structured by sterol rings: they contribute to membrane structure/fluidity and are precursors of fat-soluble vitamins and steroid hormones [1]. Lipids are not homogeneously distributed in cellular membranes and their composition within the membranes is highly regulated and mediated by proteins, ATP, or spontaneous diffusion [4,5]. The lipid composition has a strong influence on physical properties of the membrane such as fluidity, curvature, and lipid packing. It also plays a crucial role in the formation of the lipid domains [6], the membrane trafficking, fusion and fission events [7], formation of protein-protein and protein-lipid complexes [8]. Aberrant lipid composition or metabolism affects the function of cell organelles (e.g., endoplasmic reticulum stress [9]) or leads towards severe diseases (e.g., cancer, obesity, non-alcoholic fatty liver disease or fatty-acid induced lipotoxicity [10,11]).

Lipids in eukaryotic cells are primarily synthesized at the nuclear/endoplasmic reticulum (ER) membrane [1]. The ER is a dynamic network of flattened membrane, connected sacs and tubules, surrounding the nucleus and spreading across the cytoplasm [12]. The diverse

structure of the ER allows the organelle to perform specialized functions such as the synthesis of lipids [13] and proteins [14–16], and the regulation of calcium levels {3613}. The diverse functions of the ER are performed in different subdomains. The ER is continuous from the outer membrane of the nuclear envelope, which separates genetic material from the cell milieu. Proteins associated with the ER are synthesised, folded and post-translationally modified in the rough ER sheets embedded on the cytoplasmic surface [18,19]. Tomographic studies have revealed an additional portion of highly curved and smooth ribosomes on the membrane part of ER tubules [19]. The tubular system is highly dynamic, and its structure grows and continuously rearranges. Regions associated with ribosomes at the low density and smooth ER are considered as transitional ER. Due to its network structure, the ER associates/communicates with other organelle membranes that include those of the Golgi, lysosome/vacuole, and mitochondria [20–26]. The cortical ER, which is a part of the peripheral ER, interacts with the plasma membrane [27–29].

Much insight into the synthesis, regulation, and function of lipids has been garnered through studies using the yeast *Saccharomyces cerevisiae* [26,30–34]. Lipid synthesis in the model eukaryote is mostly typical of that found in more complex higher eukaryotes [30,31]. Yeast are easy to culture in large quantities for biochemical studies and their tractable genetics facilitates a molecular characterization of lipid synthesis and its regulation [30,31]. The wealth of genomic, proteomic, and metabolic information is available in the *Saccharomyces* Genome Database (https://www.yeastgenome.org/), providing knowledge of genetic and biochemical interactions of lipid synthesis with other metabolic pathways that impact on cell physiology.

All membrane phospholipids, as well as the storage lipid triacylglycerol (TAG), are derived from phosphatidate (PA) [1,33]. When yeast cells grow under nutrient-rich conditions, the PA that is normally synthesized from glycolysis-derived glycerol-3-P, is channeled into membrane phospholipids required for cell proliferation [35,36]. During nutrient limitation, the growth of yeast cells is reduced, and PA is channeled more into TAG [35,36]. PA is not only an intermediate in lipid synthesis, but also a regulator in the expression of phospholipid synthesis genes [37]. It is also implicated as an activator in cell growth and proliferation, vesicular trafficking, secretion, and endocytosis [38–44]. Of the enzymes that produce PA (e.g., 1-acylglycerol-3-P (lysoPA) acyltransferase, diacylglycerol (DAG) kinase, phospholipase D) and use it as a substrate (e.g., CDP-diacylglycerol (CDP-DAG) synthase, PA phosphatase), DAG kinase and PA phosphatase have emerged as key regulators in PA homeostasis [32,33,45]. The two enzymes also control whether PA is utilized for the synthesis of membrane phospholipids or TAG [32,33,45]. Here, we review the basic steps of lipid synthesis from PA that occurs at the nuclear/ER membrane and summarize the PA-mediated transcriptional regulation for the expression of phospholipid synthesis genes. The genetic and biochemical evidence for the roles of Pah1 PA phosphatase and DAG kinase in regulating PA and lipid homeostasis, as well as the modes and regulation of these enzymes are also discussed.

2. PA-derived synthesis of lipids at the nuclear/ER membrane

The major steps of lipid synthesis occurring at the nuclear/ER membrane are summarized in Fig. 1. Details on the synthesis of lipid precursors such as fatty acids, inositol, and nucleotides, the remodelling of the acyl groups on lipids, and the degradation of lipids may be found elsewhere [2,33,46,47]. In the *de novo* pathway, glycerol-3-P is acylated at the 1-position to produce lysoPA by the Gpt2 and Sct1 glycerol-3-P acyltransferase enzymes [48,49]. These enzymes also acylate dihydroxyacetone-P to produce 1-acyl dihydroxyacetone phosphate, which is converted to lysoPA by Ayr1 reductase (not shown in Fig. 1) [50]. The lysoPA is then acylated at the 2-position to produce PA by the Ale1, Loa1, and Slc1 lysoPA/lysophospholipid acyltransferase enzymes [51–54]. The PA produced by the acyltransferase reactions is then partitioned into CDP-DAG and DAG as catalyzed by Cds1 CDP-DAG synthase [55] and Pah1 PA phosphatase [56], respectively. Although both enzymes function at the nuclear/ER membrane, Cds1 is an integral membrane protein whereas Pah1 is a peripheral membrane protein (see below). In wild type cells growing in the absence of choline and ethanolamine supplementation, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are produced from CDP-DAG in the *de novo* biosynthetic pathway (referred to as the CDP-DAG pathway). CDP-DAG is first converted by Cho1 PS synthase to phosphatidylserine (PS) [57–61], which is then decarboxylated to produce PE by Psd1 PS decarboxylase [62,63]. The most abundant membrane phospholipid PC is produced from PE via the three sequential methylations using AdoMet as a methyl donor [64–66] by Cho2 PE methyltransferase [64,65] (first methylation step to produce phosphatidylmonomethylethanolamine) and Opi3 phospholipid methyltransferase [65,66] (second and third methylation steps to produce phosphatidyldimethylethanolamine and then PC). Whereas the reactions catalyzed by Cho1, Cho2, and Opi3 take place at the nuclear/ER membrane, PS decarboxylation by Psd1 occurs at the mitochondrial membrane [33]. Thus, the PS synthesized in the nuclear/ER membrane is transferred to the mitochondrial membrane for its decarboxylation to PE, which is then transferred to the nuclear/ER membrane for its conversion to PC (Fig. 1) [33]. The ER and mitochondria are tethered to each other by the ER-mitochondria encounter structure (ERMES) [67–69]. The synthesis of PS occurring at the ER-mitochondrial contact site facilitates its transfer to the mitochondria [70], which is mediated by the Ups2-Mdm35 complex [71]. Psd2 is a second PS decarboxylase enzyme [72] associated with endosomes/vacuoles, but its contribution to PC synthesis via the CDP-DAG pathway is unclear.

The *cho1* [58], *psd1 psd2* [72], and *cho2 opi3* [66,73] mutants defective in the CDP-DAG pathway are auxotrophic for choline or ethanolamine. The choline and ethanolamine supplemented to the cells are channeled into PC and PE, respectively, by way of the CDP-choline and CDP-ethanolamine branches of the Kennedy pathway (Fig. 1). In yeast, the Kennedy pathway is a salvage or auxiliary pathway that permits the synthesis of PC and PE when the primary CDP-DAG pathway is blocked [30]. In the Kennedy pathway, choline or ethanolamine in growth medium is transported into the cell by the Hnm1 choline/ethanolamine transporter [74]. The water-soluble lipid precursors are then phosphorylated in the cytoplasm to form phosphocholine and phosphoethanolamine by Cki1 choline kinase [75] and Eki1 ethanolamine kinase [76], respectively. The lipid intermediates are then

activated with CTP to form CDP-choline and CDP-ethanolamine by Pct1 phosphocholine cytidylyltransferase[77] and Ect1 phosphoethanolamine cytidylyltransferase [78], respectively. The locations of the enzyme reactions are ambiguous; they are reported in the cytoplasm, nuclear periphery, or within the nucleus [79–81]. The last steps in the Kennedy pathway, namely the conversions of CDP-choline and CDP-ethanolamine to PC and PE, respectively, are catalyzed by the Cpt1 choline phosphotransferase [82,83] and Ept1 ethanolamine phosphotransferase, respectively [84,85]. The locations of Cpt1 and Ept1 are also unclear; they are reported to localize in the nuclear/ER membrane or the mitochondrial membrane [79,80]. The DAG required in the reactions is derived from the Pah1 PA phosphatase reaction [56] that takes place at the nuclear/ER membrane [36,86]. Whereas the CDP-DAG pathway is the predominant one used by wild type cells in the absence of choline and ethanolamine supplementation, they utilize the Kennedy pathway when supplemented with choline or ethanolamine [30,32,33]. The choline required for the CDP-choline branch of the pathway may also be provided from the Spo14 phospholipase D-mediated turnover of the PC that is synthesized via the CDP-DAG pathway [76,87,88]. Yeast mutants (e.g., *cki1 eki1* and *cpt1 ept1*) defective in both branches of the Kennedy pathway can synthesize PC only via the CDP-DAG pathway [76,89–92]. These mutants, unlike those defective in the CDP-DAG pathway, do not exhibit any auxotrophic requirements [76,92].

It should be noted that mutants defective in PC or PE synthesis via the CDP-DAG pathway are not strict choline/ethanolamine auxotrophs. Their growth is also supported by supplementation of lysoPE, lysoPC, or PC with short acyl chains. Inside the cell, lysoPC and lysoPE are acylated by the Ale1 lysophospholipid acyltransferase [51,52,93,94], whereas the short chain PC is remodeled for its membrane incorporation with long fatty acyl chains (16 and 18 carbons) by phospholipase B and lysophospholipid acyltransferase enzymes [95–98].

The CDP-DAG used for the synthesis of PS is also converted to PI by the nuclear/ER membrane-associated Pis1 PI synthase [99,100] (Fig. 1). The PI derived from CDP-DAG also serves as a precursor of the D-3, D-4, and D-5 phosphoinositides [101–105], glycosyl PI anchors [106,107], and sphingolipids {1478, 2318}, which are synthesized in other cellular compartments {2805, 2975} (not shown in Fig. 1). The headgroup of these inositol-containing lipids is synthesized in the cytoplasm from glucose-6-P via the reactions catalyzed by Ino1 inositol-3-P synthase [111] and Inm1 inositol-3-P phosphatase [112].

In addition to being the source of PS and PI, the CDP-DAG molecule donates its phosphatidyl moiety to glycerol-3-P and phosphatidylglycerol (PG) to form phosphatidylglycerophosphate (PGP) and cardiolipin (CL), respectively. These two reactions occurring in the mitochondria are catalyzed by the membrane-associated Pgs1 PGP synthase [113,114] and Crd1 CL synthase [115–117] (Fig. 1). The PG used for the CL synthesis is derived from PGP by Gep4 PGP phosphatase [118]. After its synthesis, CL is remodeled with respect to its fatty acyl composition by the Cld1 CL-specific phospholipase A [119] and the Taz1 monolysoCL-specific acyltransferase [120,121] (not shown in Fig. 1). The CDP-DAG used for the synthesis of the mitochondrial phospholipids differs in its origin from that used for the synthesis PS and PI. The mitochondrial CDP-DAG is produced in the organelle by Tam41 CDP-DAG synthase [122] using PA that is translocated from the nuclear/ER membrane by the Ups1 lipid transfer protein [123].

As yeast cells progress from the exponential to the stationary phase of growth due to the depletion of nutrients in the medium, the utilization PA shifts from phospholipid synthesis to TAG synthesis [35,124,125]. The DAG produced by Pah1 PA phosphatase is converted to TAG by the ER-associated Dga1 acyl-CoA dependent DAG acyltransferase [126] and/or the Lro1 phospholipid-dependent DAG acyltransferase [127]. The Are1 and Are2 sterol acyltransferase enzymes also acylate DAG to produce TAG, but their contributions are minor when compared with those of Dga1 and Lro1 [128]. The TAG synthesized at the nuclear/ER membrane is then packaged into lipid droplets [129,130], an organelle that emerges from the nuclear/ER membrane [131,132] (Fig. 1). Indeed, nuclear/ER membrane-associated enzymes as well as Pah1 PA phosphatase, which are responsible for TAG synthesis, may be found at the ER-lipid droplet contact sites [131]. While lipid droplets are known to be produced from the outer nuclear/ER membrane, a recent study has shown that they are also produced at a low level from the inner nuclear membrane [133].

3. PA-mediated regulation of lipid synthesis at the nuclear/ER via the Henry regulatory circuit

The synthesis of lipids is regulated by controlling the expression of lipid synthesis genes and by biochemical modulation of the gene products. The gene expression is strongly influenced by the availability of fatty acids, phospholipid precursors (e.g., inositol, serine, choline, ethanolamine), and nucleotides (e.g., CTP) [30–33,45]. Inositol auxotrophy of the *ino2* and *ino4* mutants [134] and inositol excretion of the *opi1* mutant [135] are linked to the misregulation of *INO1* expression through a UAS_{INO} element (also known as inositol response element) {786, 561, 1193}. Moreover, mutations in any step of the CDP-DAG pathway (e.g., *cds1*, *cho1*, *psd1*, *cho2*, or *opi3*) result in the elevation of the PA level and the excretion of inositol because of the derepression of *INO1* expression [30–33,45]. These findings led Henry and Patton-Vogt [138] to propose that the phospholipid PA is a major component in transcriptional regulation of the UAS_{INO}-containing genes. Many of the genes involved in phospholipid synthesis contain the UAS_{INO} element in the promoter, which include those encoding enzymes responsible for fatty acid synthesis (e.g., *ACC1*, *FAS1*, *FAS2*), phospholipid synthesis (e.g., *CDS1*, *CHO1*, *PSD1*, *CHO2*, *OPI3*, *EKI1*, *EPT1*, *CKI1*, *CPT1*), and inositol synthesis (e.g., *INO1*) [37,139]. The UAS_{INO} element is also present in genes encoding the choline/ethanolamine (*HNM1*) and inositol (*ITR1*) permeases that are responsible for the uptake of phospholipid precursors into the cell [74,140].

The transcriptional regulation of the UAS_{INO}-containing genes (Fig. 2), called “Henry regulatory circuit”, includes the Ino2-Ino4 transcriptional activator complex, the Opi1 repressor, and the Scs2 protein [141,142,142–146]. In the nucleus, transcription of the UAS_{INO}-containing genes is activated by the Ino2-Ino4 complex through its binding to the *cis*-acting element {786, 561, 1193}. This transcriptional activation is negatively controlled by the Opi1 repressor through its interaction with Ino2 [37,139]. The nuclear localization of Opi1 is crucial for its repressor function and is controlled by PA and the Scs2 protein. The Opi1 repressor interacts with Scs2 at the nuclear/ER membrane, and its protein interaction is stabilized by its interaction with PA. When the level of PA is high, Opi1 is tethered to the nuclear/ER membrane through its protein and lipid interactions [146,147]. When the PA

level is lowered, Opi1 loses its interaction with the phospholipid and it dissociates from Scs2 and enters the nucleus for its repressor function [33,37,146]. The interaction of Opi1 with PA is mediated by its amphipathic helix [148], and the protein-lipid interaction is favored when the fatty acyl chain of PA is 16 carbons long [149]. The interaction of Opi1 with Scs2 is mediated by its FFAT (two phenylalanines (FF) in an Acidic Tract) motif [145]. As expected, the interaction of Opi1 with Scs2 is essential for the PA-mediated regulation of gene expression in the Henry regulatory circuit [150]. The Opi1-Scs2 interaction also governs the transfer of PA from the nuclear/ER membrane to the mitochondrial membrane where it reacts with CTP to form CDP-DAG for the synthesis of CL [150] (see above). Opi1 repressor function is also controlled by its phosphorylation. For example, the phosphorylations by casein kinase (CK) II [151] and protein kinase A (PKA) [152] stimulate the repressor function, whereas phosphorylation by protein kinase C (PKC) [153] inhibits repressor function. The mechanistic basis for phosphorylation-mediated regulations of Opi1 is unknown.

As indicated above, the PA-mediated regulation of lipid synthesis genes is triggered by growth phase, nutrient availability, and the gene mutations [30,31,33,37,139,154]. For example, the expression of the *CHO1* gene, which encodes PS synthase, is derepressed in the exponential phase of cells grown in the absence of phospholipid precursors such as inositol, choline, ethanolamine, and serine [135,155–158] as well as in the presence of the essential nutrient zinc [159]. Conversely, the *CHO1* expression is repressed in the exponential phase of cells grown with inositol supplementation, and the gene repression is enhanced by the supplementation of choline, ethanolamine, or serine [135,155–158]. The gene expression is also repressed by the depletion of zinc in the growth medium [159] or when cells progress from the exponential to the stationary phase of growth [160,161].

Unlike the partitioning of CDP-DAG to PS, its partitioning to PI is not regulated by controlling the expression of the enzyme responsible for the reaction step, but by controlling the production of its water-soluble substrate inositol. The *INO1* gene encoding inositol-3-P synthase, a key enzyme for the *de novo* synthesis of inositol, is coordinately regulated with *CHO1* and other UAS_{INO}-containing genes in the CDP-DAG pathway [162]. The *PIS1* gene, which encodes PI synthase, is subject to a different transcriptional control, and its expression is induced under zinc-limited conditions by the Zap1 transcriptional activator [163].

Interestingly, the expression of the *PAH1* gene encoding PA phosphatase is regulated by some of the same growth conditions that control gene expression via the Henry regulatory circuit, but with an opposite effect [124,164]. The regulation of *PAH1* expression involves the transcription factors Ino2/Ino4/Opi1, Gis1/Rph1 (for inositol and growth phase regulation), and the transcription factor Zap1 (for zinc-mediated regulation) [124,164]. The induction of *PAH1* expression in the stationary phase or in response to zinc depletion correlates with the elevation of PA phosphatase activity [124,164]. On the one hand, the elevation of PA phosphatase activity in zinc-replete stationary phase cells is responsible for the increase of TAG synthesis at the expense of phospholipid synthesis [124]. On the other hand, the reduction of PA phosphatase activity in zinc-deplete exponential phase cells is responsible for increased synthesis of PC via the CDP-choline branch of the Kennedy pathway [164].

4. Pah1 PA phosphatase and Dgk1 DAG kinase are key enzymes that control the PA level and lipid synthesis at the nuclear/ER membrane

Pah1 PA phosphatase [56] and Dgk1 DAG kinase [165] have emerged as key enzymes that control PA content and lipid synthesis regulation at the nuclear/ER membrane. As discussed above, Pah1 PA phosphatase dephosphorylates PA to produce DAG [56], whereas DAG kinase phosphorylates DAG to produce PA [165]. Dgk1, a nuclear/ER integral membrane protein [80], is an unconventional DAG kinase in that it utilizes CTP instead of ATP as a phosphate donor [165]. Pah1 functions as a peripheral membrane enzyme, but it is largely localized in the cytoplasm [80] as a highly phosphorylated form (see below). Fluorescence imaging of Pah1 localization on the nuclear/ER membrane is difficult because of its low abundance on the membrane, transient interaction with the substrate PA, inherent protein instability, and susceptibility to proteasomal degradation [166,167]. However, the membrane localization of the GFP-tagged Pah1 was shown by overexpression of Dgk1 [86] or the Nem1-Spo7 phosphatase complex [36]. The higher level of Dgk1 DAG kinase increases the production of PA [165] that interacts with Pah1 [168], whereas the higher level of Nem1-Spo7 facilitates the dephosphorylation of Pah1 for its localization to the nuclear/ER membrane [36,169,170]. The catalytically inactive form of Pah1 [171], which is stable to proteasomal degradation [166] and binds non-productively to PA, was shown to localize at the nuclear/ER membrane, the nuclear/ER membrane-lipid droplet contact site, or the nuclear-vacuole junction [125]. Notwithstanding the fluorescence imaging of its nuclear/ER membrane localization, Pah1 is known to associate with the membrane for its cellular function through the control of the membrane-associated Nem1-Spo7 phosphatase [172]; its dephosphorylation by the phosphatase complex is essential for its membrane localization and catalytic function [36,169,170].

4.1. Phenotypes of the pah1Δ mutant

The loss of Pah1 PA phosphatase activity in the cell affects many aspects of lipid metabolism and cell physiology (Fig. 3). Due to the lack of DAG production, the *pah1*Δ mutant exhibits a great reduction in the level and synthesis of TAG [56,124]. In addition, the mutant cells exhibit a significantly lower number of lipid droplets, which seems to be related to the effect of DAG limitation on the organelle formation [173]. The lack of DAG production and thereby its acylation defect causes the accumulation of fatty acids [56], which renders the mutant cells susceptible to fatty acid-induced lipotoxicity [56,174]. Indeed, TAG synthesis is important to mitigate the toxic effects of fatty acids [175–177].

The lack of DAG production in the *pah1*Δ mutant causes the accumulation of its precursor PA at the nuclear/ER membrane [178], drastically affecting phospholipid synthesis and related cellular processes. The mutant cells exhibit a high increase in the level and synthesis of phospholipids [56,124,174], the PA-mediated derepression of the UAS_{INO}-containing phospholipid synthesis genes [169,179], and a reduction in the ATP level [180]. The notable phenotype of the *pah1*Δ mutant in relation to the increased level of phospholipids is its aberrant nuclear morphology with the expansion of the nuclear/ER membrane [169]. Data indicate that the membrane expansion phenotype is caused by increased phospholipid synthesis in conjunction with the elevated PA content [165,170].

In addition to its role as a precursor in phospholipid synthesis, PA exhibits a strong stimulatory effect on PS synthase [181], which catalyzes a committed step in PC synthesis via the CDP-DAG pathway (Fig. 1). The accumulation of fatty acids in *pah1*Δ mutant cells, which is caused by a defect in TAG synthesis, may also be attributed to an increase in their synthesis through the derepression of fatty acid synthesis genes (e.g., *FAS1*, *FAS2*, and *ACC1*) that are regulated via the Henry regulatory circuit [182–185] (Fig. 2). The reduced level of ATP in the *pah1*Δ mutant is not due to a defect in its production by mitochondria (i.e., oxidative phosphorylation), but instead attributed to its exhaustive use for the synthesis of fatty acids and phospholipids [180]. Interestingly, the increased synthesis of phospholipids in the *pah1*Δ mutant alleviates growth inhibition caused by the infection of brome mosaic virus, which utilizes host phospholipid resources for its replication [186]. Moreover, the *pah1*Δ mutation confers increased stability to the yeast and heterologous plasmids [187].

The *pah1*Δ mutant, which contains the elevated levels of superoxides and lipid hydroperoxides, is hypersensitive to hydrogen peroxide, [180]; it is defective in growth on non-fermentable carbon sources (e.g., glycerol) and has a shorter chronological life span [180], exhibiting apoptotic cell death in the stationary phase [174]. The elevated levels of superoxides and lipid hydroperoxides in the mutant cells may be attributed to the increased level of phospholipids as well as to the reduced activity of Sod2 superoxide dismutase and catalase [180]. The apoptotic cell death may be attributed to the increased fatty acid content [188]. It is unclear why the mutant cells are defective in growth on non-fermentable carbon sources.

Other phenotypes of the *pah1*Δ mutant include its weakened cell wall [189,190], vacuole fragmentation [191], and defect in autophagy induction after TORC1 inactivation [192]. The loss of cell wall strength might be connected to a defect in the cell wall integrity pathway that is controlled by Pkc1 PKC {3478, 3477}. The enzyme that is stimulated by the lipids DAG and PS [195] would be expected to be less active in the *pah1*Δ mutant. Vacuole fusion is controlled not only by protein factors but also by lipid factors that include PA, DAG, phosphoinositides, and sterols [196]. The increased level of PA in the *pah1*Δ mutant has an inhibitory effect on SNARE priming through its interaction with Sec18 [197]. Conversely, the defect of PA production in the *dgk1*Δ mutant, which lacks Dgk1 DAG kinase catalyzing DAG phosphorylation [165], has a positive effect on SNARE priming and vacuole fusion [198]. The elevated level of PA in *pah1*Δ cells also causes the derepression of the UAS_{INO}-containing V-ATPase genes that affect vacuole acidification [199], and the acidification of the vacuole has a negative effect on membrane fusion [200]. The underpinning of why the *pah1*Δ mutation has a negative impact on autophagy is unclear, but it might be related to a defect in processes involving the membrane fission/fusion events of vesicles.

Finally, the *pah1*Δ mutant exhibits both heat sensitivity [56,165,169,187] and cold sensitivity [201]. Since the lack of growth at the elevated temperature (e.g., 37°C) is characteristic of cell wall mutants, the growth defect of the *pah1*Δ mutant is likely to be caused by its cell wall defect. Although the basis for growth defect at the reduced temperature (e.g., 15°C) is not known, the altered levels and composition of lipids in the *pah1*Δ mutant might have a detrimental effect on its cold-growth capability.

4.2. Role of Dgk1 DAG kinase in pah1Δ phenotypes

The phenotypes of the *pah1*Δ mutant, which include the elevated PA content and phospholipid synthesis along with the aberrant nuclear morphology [124,165,174], are suppressed (or at least partially suppressed) by the deletion of the *DGK1* gene. The *dgk1*Δ effects indicate that DAG kinase activity plays a major role in the elevation of the PA level to elicit the alteration of lipid synthesis and cellular processes [165]. Other *pah1*Δ phenotypes that are suppressed by the *dgk1*Δ mutation and thus thought to be related to the increased PA level rather than the decreased DAG level include its reduced lipid droplet numbers, hypersensitivity to hydrogen peroxide, growth defect on non-fermentable carbon sources, and shortened chronological life span [165,173,180]. As expected, the *dgk1*Δ mutation does not suppress the *pah1*Δ phenotypes that are caused by the lack of DAG production, which include the reduced levels of DAG and TAG [165] and vacuole fragmentation [198].

An important question that deserves attention is the source of DAG utilized by Dgk1 at the nuclear/ER membrane. In *pah1*Δ mutant cells, DAG can be produced from PA by other PA phosphatase enzymes such as App1 [202], Dpp1 [203], and Lpp1 [204]. However, these enzymes, which are localized to other subcellular compartments, have not been shown to be involved in lipid synthesis or its regulation [205]. During growth resumption from stasis, Dgk1 utilizes the DAG derived from TAG [206] by the Tgl3 {2063} and Tgl4 {2834} TAG lipase enzymes. However, this source of DAG is not likely in the *pah1*Δ mutant, which is already defective in the synthesis of TAG [56]. Other potential sources of DAG production include the reactions catalyzed by Plc1 PI-4,5-P_2-specific phospholipase C [209], Pgc1 PG-specific phospholipase C [210], and Aur1 inositol phosphorylceramide synthase {3654}. Plc1 [212] and Pgc1 [80] are localized to the cytoplasm/nucleus and cytoplasm/mitochondria, respectively, and do not appear to be in a right place to provide DAG for Dgk1 activity at the nuclear/ER. The more likely source of DAG is considered to be the reaction catalyzed by Aur1 inositol phosphorylceramide synthase {3654}, an early Golgi-associated enzyme [80] that catalyzes the conversion of PI and phytoceramide to phosphorylceramide and DAG.

Cells carrying the *dgk1*Δ mutation alone do not exhibit any distinct phenotypes under optimal growth conditions [165]. However, the mutational effect becomes evident on quiescent cells in the stationary phase that resume growth in the presence of cerulenin, an inhibitor of *de novo* fatty acid synthesis [206]. Under this condition, the Dgk1-mediated production of PA from DAG, which is generated from TAG hydrolysis [213], is essential for the synthesis of membrane phospholipids (e.g., PC) via the CDP-DAG pathway [206]. Accordingly, the *dgk1*Δ mutant, which cannot produce PA for phospholipid synthesis, fails to resume growth from the stationary phase in the absence of fatty acid synthesis. Yet, the growth defect of the *dgk1*Δ mutant is partially ameliorated by supplementation of choline that drives PC synthesis using DAG via the Kennedy pathway [206].

5. Regulation of Pah1 PA phosphatase and Dgk1 DAG kinase by phosphorylation/dephosphorylation

5.1. Phosphorylation and dephosphorylation of Pah1

Pah1, which functions at a location different from that of its synthesis, is regulated for its translocation by phosphorylation and dephosphorylation (Fig. 1). To date, 38 sites of its phosphorylation have been identified [214], and about half of them have been ascribed to specific protein kinases [214]. We do know that the phosphorylations by Pho85-Pho80 [215], Cdc28-cyclin B [169,216], and PKA [217] prevent Pah1 function by causing its retention in the cytoplasm apart from the substrate PA present at the nuclear/ER membrane (Fig. 1). The phosphorylations of Pah1 by Pho85-Pho80 [215] and PKA [217] also reduce its PA phosphatase activity. Additionally, Pah1 is phosphorylated by CKII [218] and PKC [219], but these phosphorylations do not have direct effects on the localization of Pah1 or its catalytic activity. Instead, the phosphorylation of Pah1 by CKII inhibits its phosphorylation by protein kinase A [218], and the phosphorylation by PKC, which is favored without phosphorylation by Pho85-Pho80, promotes the degradation of Pah1 by the 20S proteasome [167,219] (Fig. 1). Pah1 is also phosphorylated by CKI, whose sites of phosphorylation overlap with some of the sites phosphorylated by Pho85-Pho80, PKA, and PKC.

The dephosphorylation of Pah1, which is catalyzed by the nuclear/ER membrane-associated Nem1 (catalytic subunit)-Spo7 (regulatory subunit) phosphatase complex [169,172], facilitates its association with the membrane and the substrate PA [86], and at the same time has the effect of stimulating its catalytic activity [170,215,216,220]. The Nem1-Spo7 protein phosphatase removes all phosphates on Pah1 [169], and its dephosphorylation efficiency on the kinase-specific phosphorylation sites is in the order Pho85-Pho80 > PKA = CKII > Cdc28-cyclin B > PKC [218,220]. The highest affinity of Nem1-spo7 for the sites of Pah1 phosphorylated by Pho85-Pho80 is consistent with the strongest effects of the non-phosphorylatable alanine mutations on bypassing the requirement of the phosphatase complex for its function on the membrane (e.g., increase in nuclear/ER membrane association and TAG synthesis) [170,215,216]. Nem1-Spo7 phosphatase activity has the pH optimum of 5 [220], which is like the intracellular pH of yeast cells in the stationary phase when Pah1 activity and TAG level are maximal and the conversion of PA to the lipid storage lipid is favored over membrane phospholipid synthesis [124,125,220,221].

Cells carrying the *nem1*Δ or *spo7*Δ mutation lack a functional phosphatase complex and exhibit the abnormal expansion of the nuclear/ER membrane, a phenotype shown by the lack of Pah1 PA phosphatase. Likewise, the mutants lacking the phosphatase complex exhibit a defect in balancing the coordinated synthesis of TAG and membrane phospholipids [124], and exhibit defects in phospholipid synthesis gene regulation [169], lipid droplet formation [222], vacuole morphology [192], and autophagy [192,222]. Given the requirement of dephosphorylation for the function of Pah1, it is not surprising that the *nem1*Δ and/or *spo7*Δ mutant exhibits the same phenotypes as the *pah1*Δ mutant.

5.2. Phosphorylation of the Nem1-Spo7 protein phosphatase

Like its substrate Pah1, the Nem1-Spo7 phosphatase is a phosphoprotein [223,224] that is subject to phosphorylation by PKA [225] and PKC [195]. Wild type cells expressing the Nem1-Spo7 complex deficient its phosphorylation by PKA show reduced phospholipid synthesis but increased TAG synthesis [225]. This finding suggests that the PKA phosphorylation of Nem1-Spo7 is inhibitory on its function to dephosphorylate and activate Pah1 PA phosphatase. The effect of the PKC-mediated phosphorylation of the phosphatase complex is yet unknown. The catalytic subunit Nem1 is also phosphorylated in wild type cells when supplemented with rapamycin [226], an inhibitor of the TORC1 protein kinase complex [227]. The rapamycin-induced Nem1 phosphorylation correlates with the dephosphorylation of Pah1 and an increase in TAG content [226], indicating that the phosphorylation of Nem1 occurring under nutrient limitation is stimulatory on its catalytic function to activate Pah1 PA phosphatase. The protein kinase involved in this regulation has yet to be identified [226].

5.3. Phosphorylation of Dgk1

Dgk1 treated with alkaline phosphatase exhibits a great reduction in DAG kinase activity, indicating that its phosphorylation is stimulatory on catalytic activity. The N-terminus of Dgk1 contains at least 5 phosphorylation sites [223,224], two of which are target sites for CKII [228]. The CKII-mediated phosphorylation of the dephosphorylated Dgk1 restores most of its enzyme activity that is lost by treatment with alkaline phosphatase [228]. The site-specific mutational analysis of the phosphorylation sites indicates that the CKII-mediated phosphorylation of Dgk1 is required for its function in eliciting the *pah1*Δ mutant phenotypes such as PA accumulation, nuclear/ER membrane expansion, and reduced lipid droplet formation [228].

6. Summary and perspectives

In this review, we have discussed the PA-mediated regulation of lipid synthesis that occurs at the nuclear/ER membrane (Fig. 1). The importance of Pah1 PA phosphatase in the production of DAG and simultaneous control of the PA level is manifested by a multitude of defects in cells lacking the enzyme activity (Fig. 3). Dgk1 DAG kinase and Pah1 PA phosphatase, which counteract each other in the production of PA and DAG, play a major role in the control of the lipid levels. The PA-mediated regulation of phospholipid synthesis is largely governed by Pah1 (Fig. 1) and Opi1 (Fig. 2), which translocate to the nuclear/ER membrane with opposite effects on their function. Whereas Pah1 is active on the membrane, Opi1 becomes inactive. In addition, Pah1 PA phosphatase activity, which reduces the level of PA by converting it to DAG, has a positive regulatory effect in dissociating Opi1 from the membrane for its repressor function. The membrane localization of Pah1 is controlled by its phosphorylation and dephosphorylation; its phosphorylated form is cytoplasmic, whereas its unphosphorylated form (by dephosphorylation) is associated with the membrane. The dephosphorylation of Pah1 by the Nem1-Spo7 protein phosphatase complex is required for its localization and function at the nuclear/ER membrane. However, it is unknown how the enzyme and substrate interact with each other, and what effects the phosphorylations of the Nem1 and Spo7 subunits have on the formation of the phosphatase complex and its activity

on Pah1. The phosphorylation and dephosphorylation of Pah1 also govern its susceptibility to degradation by the 20S proteasome. However, it is unknown whether Pah1 is also subject to the ubiquitin-mediated proteasomal degradation. Like Pah1, Opi1 is subject to multiple phosphorylations, but the effect of phosphorylation on its localization is unclear.

Key enzymes in phospholipid synthesis (Ura7 CTP synthetase, Cho1 PS synthase, and Cki1 choline kinase) are also subject to multiple phosphorylations [32]. One of the protein kinases common to the phosphorylation of the enzymes is PKA [152,229–231] (Fig. 4). In yeast, PKA is a principal protein kinase that transmits signals through the *RAS*/cAMP pathway; the stimulation of PKA is coupled with enhanced metabolic activity and active cell growth [232,233]. The PKA-mediated phosphorylation of the Nem1-Spo7 complex, Pah1, and Opi1 has an inhibitory effect on their function, whereas the phosphorylation of Ura7, Cho1, and Cki1 stimulate their function (Fig. 4). The net effect of the phosphorylation-mediated regulation is to channel PA for the synthesis of phospholipids rather than TAG.

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Abbreviations:

ER	endoplasmic reticulum
PC	phosphatidylcholine
PI	phosphatidylinositol
PS	phosphatidylserine
PE	phosphatidylethanolamine
TAG	triacylglycerol
PA	phosphatidate
lysoPA	1-acylglycerol-3-P
DAG	diacylglycerol
CDP-DAG	CDP-diacylglycerol
PG	phosphatidylglycerol
CL	cardiolipin
PGP	phosphatidylglycerophosphate
PKA	protein kinase A
PKC	protein kinase C
CK	casein kinase

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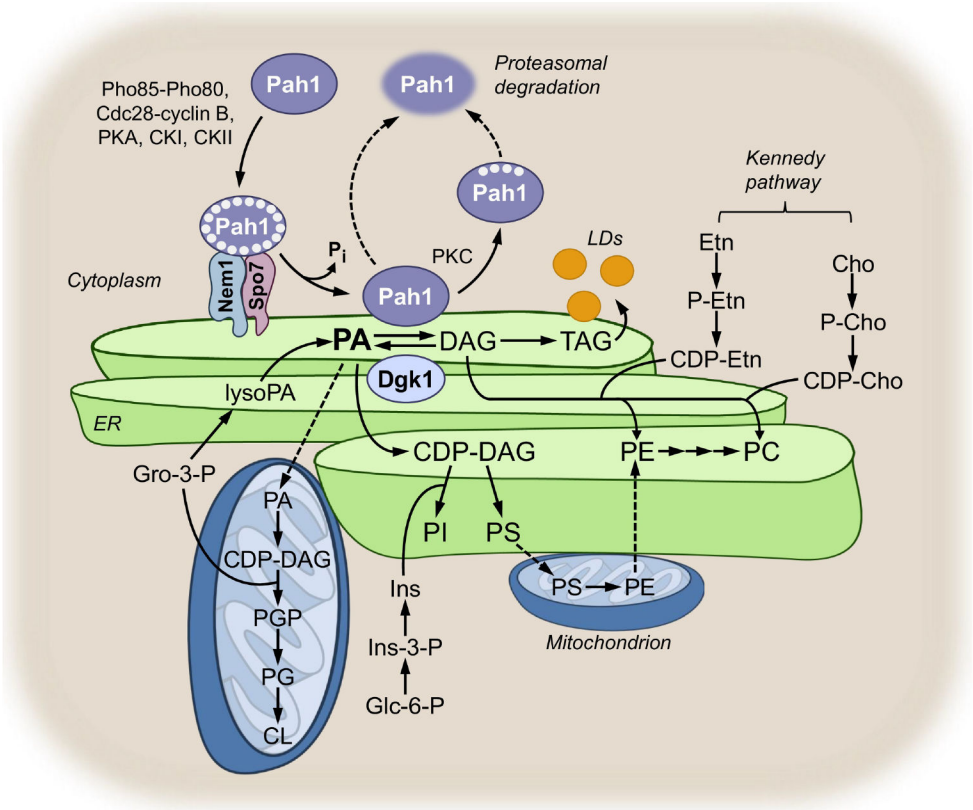

Fig. 1.

Synthesis of phospholipids and TAG and model for the regulation of Pah1 PA phosphatase. The figure depicts the pathways for the synthesis of lipids and their precursors that occur in the nuclear/ER membrane (*green*), in the mitochondria (*blue*), and in the cytoplasm (*tan*). A greater detail of lipid synthetic pathways may be found elsewhere [32,33,176,234]. The phosphorylated form of Pah1 is indicated by the *white circles* within the *ellipse* surrounding Pah1 and lipid droplets are indicated by the *yellow circles* emanating from the nuclear/ER. Gro, glycerol; Ins, inositol; Glc, glucose; Etn, ethanolamine; P-Etn, phosphoethanolamine; Cho, choline; P-Cho, phosphocholine; LDs, lipid droplets.

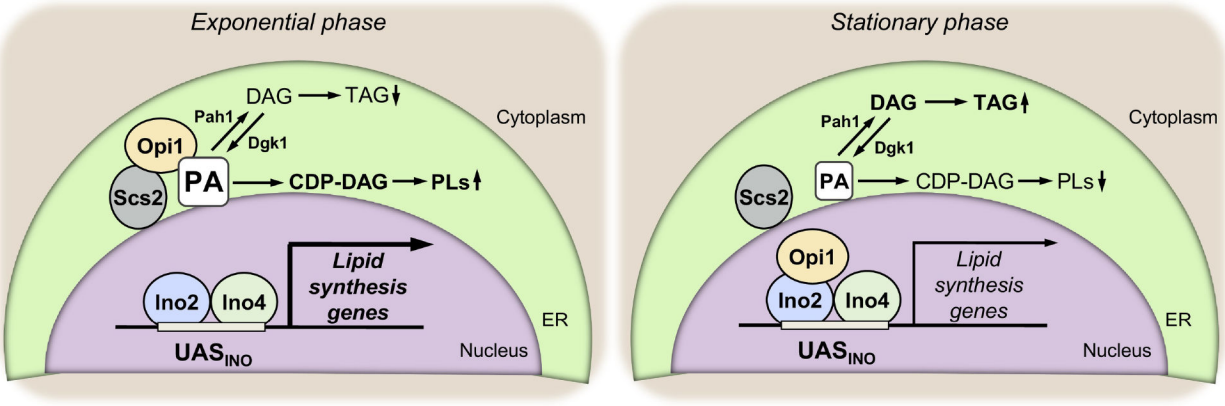

Fig. 2.

Henry regulatory circuit for the PA-mediated regulation of UASINO-containing lipid synthesis genes during growth. The model depicts the regulation that occurs in the exponential and stationary phases of growth. Details are described in the text and elsewhere [32,33,37].

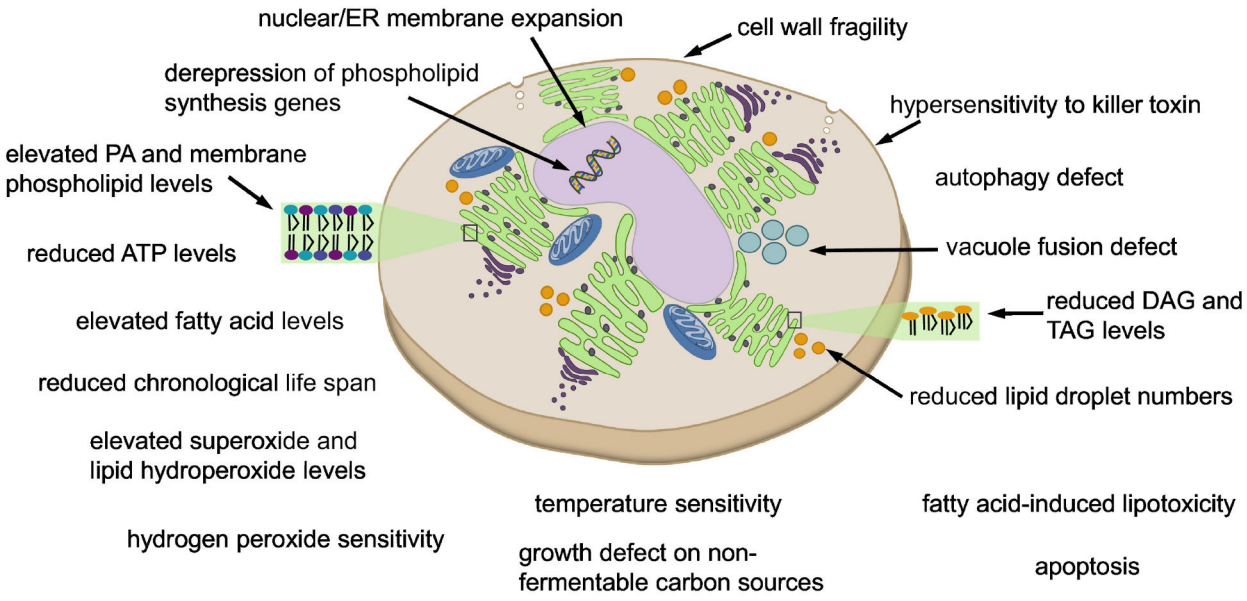

Fig. 3.

Phenotypes and cellular defects caused by the *pah1*Δ mutation.

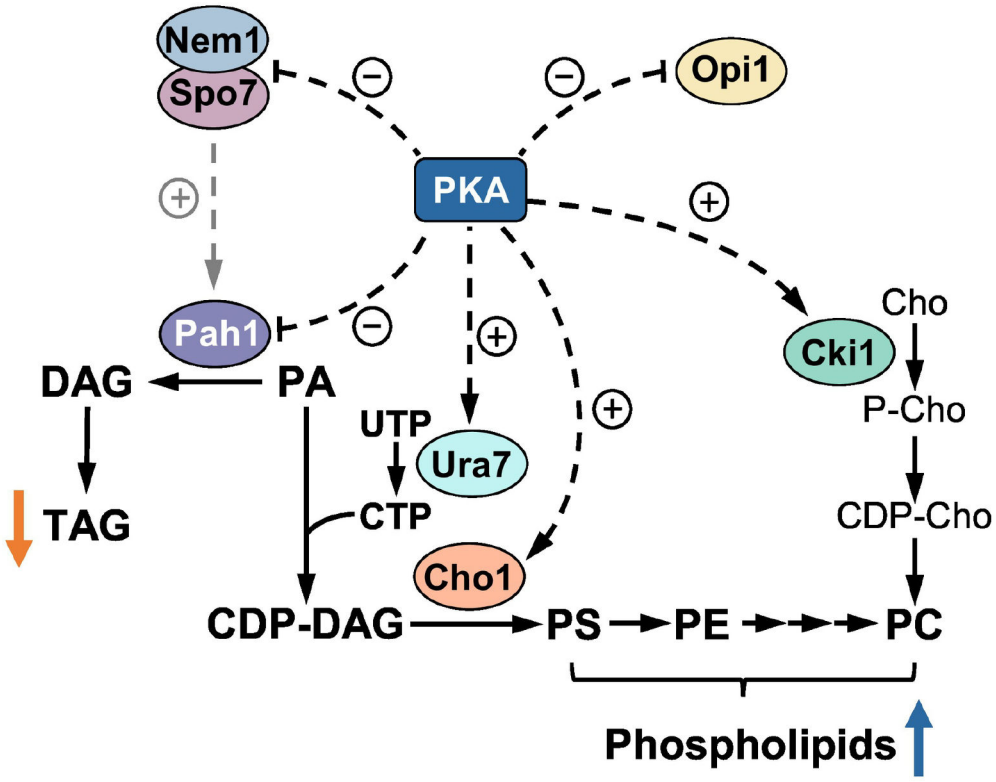

Fig. 4.

Model for the regulation of lipid synthesis by PKA. The phosphorylations of the Nem1-Spo7 protein phosphatase complex, Pah1, and Opi1 by PKA have negative effects on their functions. The positive effect Nem1-Spo7 has on Pah1 function is attenuated (indicated by the *dashed grey arrow*) by its phosphorylation. The phosphorylations of Ura7, Cho1, and Cki1 have positive effects on their functions. The net effect of these phosphorylations is an increase in phospholipids (*blue arrow*) and a decrease in TAG (*orange arrow*).