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Fat-regulating phosphatidic acid phosphatase: a review of its roles and regulation in lipid homeostasis

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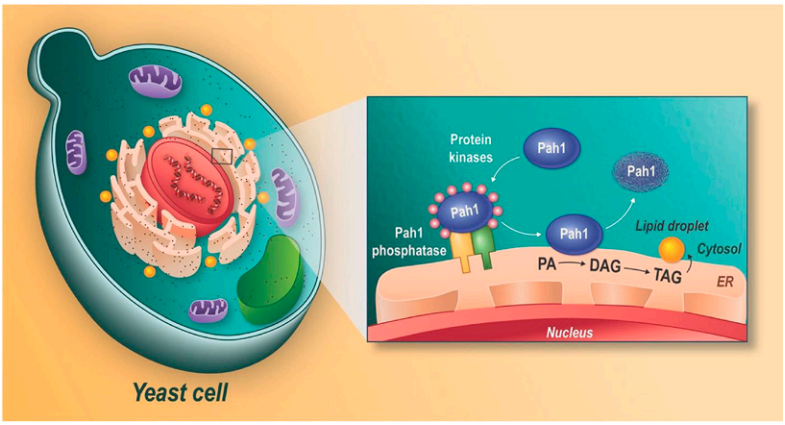
Abstract **Phosphatidic acid (PA) phosphatase is an evolutionarily conserved enzyme that plays a major role in lipid homeostasis by controlling the cellular levels of its substrate, PA, and its product, diacylglycerol. These lipids are essential intermediates for the synthesis of triacylglycerol and membrane phospholipids; they also function in lipid signaling, vesicular trafficking, lipid droplet formation, and phospholipid synthesis gene expression. The importance of PA phosphatase to lipid homeostasis and cell physiology is exemplified in yeast, mice, and humans by a host of cellular defects and lipid-based diseases associated with loss or overexpression of the enzyme activity. In this review, we focus on the mode of action and regulation of PA phosphatase in the yeast *Saccharomyces cerevisiae*. The enzyme Pah1 translocates from the cytosol to the nuclear/endoplasmic reticulum membrane through phosphorylation and dephosphorylation. Pah1 phosphorylation is mediated in the cytosol by multiple protein kinases, whereas dephosphorylation is catalyzed on the membrane surface by an integral membrane protein phosphatase. Posttranslational modifications of Pah1 also affect its catalytic activity and susceptibility to degradation by the proteasome. Additional mechanistic understanding of Pah1 regulation should be instrumental for the identification of small-molecule inhibitors or activators that can fine-tune PA phosphatase function and thereby restore lipid homeostasis.**—Carman, G. M., and G-S. Han. **Fat-regulating phosphatidic acid phosphatase: a review of its roles and regulation in lipid homeostasis.** ***J. Lipid Res.*** **2019.** 60: **2–6**.



Supplementary key words diacylglycerol • triacylglycerol • Nem1-Spo7 protein phosphatase complex • obesity • lipodystrophy

ROLES OF PA PHOSPHATASE IN LIPID METABOLISM AND ITS IMPORTANCE TO CELL PHYSIOLOGY

Phosphatidic acid (PA) phosphatase,[2] the enzyme that catalyzes the Mg^{2+}-dependent dephosphorylation of PA to produce diacylglycerol (DAG) (**Fig. 1**), has emerged as a vital regulator of lipid homeostasis in eukaryotic organisms (1). The PA phosphatase reaction was first characterized in 1957 from chicken liver extracts by Smith et al. (2). Yet, the existence of the enzyme had been implicated two years earlier by Kates (3) from a study on the hydrolysis of phosphatidylcholine with spinach chloroplasts. Among many attempts to purify PA phosphatase from diverse organisms, Lin and Carman (4) in 1989 could prepare the enzyme to near homogeneity from the yeast *Saccharomyces cerevisiae*. The enzyme-encoding gene *PAH1* was identified from *S. cerevisiae* in 2006 by Han et al. (5), revealing that it is evolutionarily conserved in higher eukaryotes including human.

DAG produced by PA phosphatase is acylated to produce the storage lipid triacylglycerol (TAG) at the ER

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Abbreviations: CKII, casein kinase II; DAG, diacylglycerol; PA, phosphatidic acid; PKA, protein kinase A; PKC, protein kinase C; TAG, triacylglycerol.

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[2] The PA phosphatase that is involved in de novo lipid synthesis differs in catalytic activity from the lipid phosphate phosphatases that are involved in lipid signaling and dephosphorylate a broad spectrum of substrates (e.g., PA, lysoPA, and DAG pyrophosphate) by a mechanism that does not require Mg^{2+} ions (1).

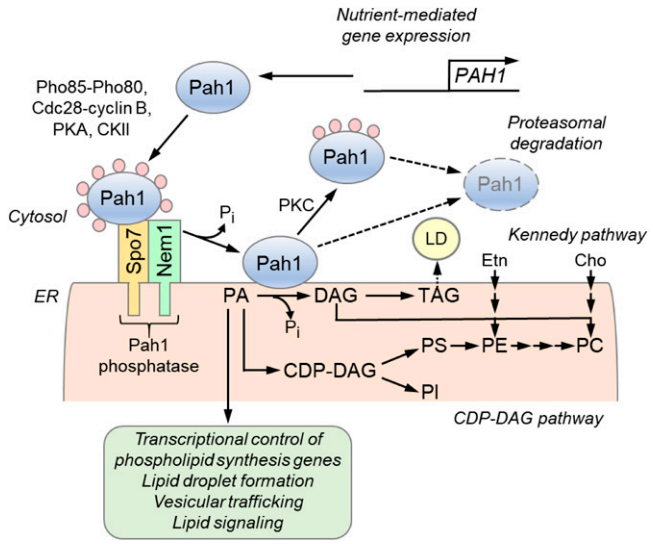

Fig. 1. Roles and regulation of *PAH1*-encoded PA phosphatase in lipid synthesis. The expression of the *PAH1* gene that encodes the PA phosphatase protein Pah1 is regulated throughout growth by nutrient status. Pah1 in the cytosol is phosphorylated by multiple protein kinases during vegetative growth when the synthesis of phospholipids occurs at the expense of TAG. As cells progress into stasis, the phosphorylated Pah1 (pink circles) translocates to the ER membrane through its dephosphorylation by the Pah1 phosphatase, which is composed of Nem1 (catalytic subunit) and Spo7 (regulatory subunit). Dephosphorylated Pah1 that is associated with the ER membrane catalyzes the conversion of PA to DAG, which is then acylated to form TAG that is stored in lipid droplets (LD). Dephosphorylated Pah1 or PKC-phosphorylated Pah1 that is not phosphorylated at the seven target sites for Pho85-Pho80 protein kinase is degraded by the proteasome (dashed line arrows). PA is also utilized for the synthesis of membrane phospholipids via the CDP-DAG pathway, and it has signaling functions (green). When the CDP-DAG pathway for phospholipid synthesis is blocked, phosphatidylcholine (PC) or phosphatidylethanolamine (PE) may be synthesized from the DAG derived from the PA phosphatase reaction if cells are supplemented with choline (Cho) or ethanolamine (Etn) via the Kennedy pathway. PI, phosphatidylinositol; PS, phosphatidylserine.

membrane, which is then incorporated into lipid droplets (6) (Fig. 1). The DAG is also used to synthesize the membrane phospholipids phosphatidylcholine or phosphatidylethanolamine via the Kennedy pathway (7). The substrate PA is a precursor for membrane phospholipids that are synthesized via the liponucleotide intermediate CDP-DAG (7) (Fig. 1). In addition to their uses for lipid synthesis, PA/DAG play roles as regulatory molecules (e.g., lipid signaling, lipid droplet formation, vesicular trafficking, phospholipid synthesis gene expression) (8, 9). Thus, by the nature of its reaction, PA phosphatase controls the synthesis of TAG and membrane phospholipids, and the abundance of lipid signaling molecules.

The importance of PA phosphatase to lipid homeostasis and cell physiology is highlighted in yeast cells lacking the enzyme (e.g., the *pah1*Δ mutant) and in mice and humans containing the mutations of the enzyme genes (e.g., *Lpin* and *LPIN*, respectively). The *pah1*Δ mutant exhibits abnormal expansion of the nuclear/ER membrane (10), which is attributed to increases of the PA level and phospholipid synthesis that occur at the expense of TAG synthesis (5, 11). The increased phospholipid synthesis correlates with the derepression of phospholipid synthesis gene expression (10, 12), whereas the decreased TAG synthesis correlates with a decrease in lipid droplet formation (13–15). The *pah1*Δ mutant is susceptible to fatty acid-induced toxicity (13) and hypersensitive to oxidative stress (16) and exhibits a decrease in chronological life span (16). It also shows a defect in diverse cellular processes including vacuole fusion and acidification (17, 18), autophagy (19), cell wall integrity (20, 21), and growth on nonfermentable carbon sources (i.e., respiratory deficiency) (5, 22) and at elevated temperatures (5, 10, 22). Some of the *pah1*Δ mutant phenotypes (e.g., nuclear/ER membrane expansion, increased phospholipid synthesis gene expression and phospholipid content, and reduced lipid droplet formation) are suppressed by the loss of the *DGK1* gene, which encodes CTP-dependent DAG kinase (1, 13, 14, 23), indicating that a proper balance of PA/ DAG is important to lipid metabolism and cell physiology. In mammals, lipin 1 influences lipid metabolism in multiple tissues. Lipin 1 deficiency in human and mice causes rhabdomyolysis (24, 25), and deficiency in mice is also characterized by hepatic steatosis during the neonatal period, lipodystrophy, insulin resistance, and peripheral neuropathy (26, 27). *Lpin1* overexpression results in increased lipogenesis and obesity (28). Polymorphisms in the human *LPIN1* gene are associated with insulin resistance and the metabolic syndrome (29). Human lipin 2 deficiency causes chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anemia (30, 31), whereas genetic variations in the human *LPIN2* gene are associated with type 2 diabetes (32).

The identification of the PA phosphatase gene has been an exciting discovery with the anticipation that the enzyme could be a target for inhibition of TAG synthesis to ameliorate obesity. However, as indicated above, abolishing PA phosphatase activity may lead to other disease conditions, and thus the enzyme inhibition needs to be fine-tuned at various levels. The known disease conditions also indicate that a fine-tuning of enzyme activation is equally important. The roles and regulation of PA phosphatase are largely conserved from yeast to human, and because of its experimental advantages, yeast has been utilized to study the enzyme and its role in lipid metabolism (1). In this article, a progress in understanding the mode of action and regulation of yeast PA phosphatase is discussed with a perspective on research that might lead to discovering molecules that inhibit or stimulate activity to modulate lipid homeostasis.[3]

CONSERVED AND NON-CONSERVED REGIONS IN THE YEAST PA PHOSPHATASE PROTEIN Pah1

The yeast PA phosphatase protein Pah1 consists of the conserved and nonconserved regions (**Fig. 2**). The conserved N-LIP and C-LIP (HAD-like) domains (5, 26) are

[3] A perspective review on the mammalian lipin PA phosphatase will also be published as part of the 60th Anniversary issue of the *Journal of Lipid Research*.

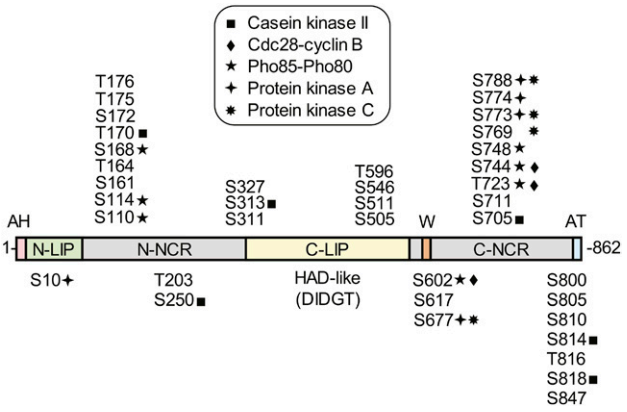

Fig. 2. Domains/regions and phosphorylation sites in Pah1. The diagram shows the positions of the amphipathic helix (AH, pink) required for ER membrane interaction, the N-LIP (green) and C-LIP (contains HAD-like domain with DIDGT catalytic motif) (yellow) domains that are required for PA phosphatase activity, the acidic tail (AT, blue) required for interaction with Spo7 of the Pah1 phosphatase, N-terminal non-conserved region (N-NCR, grey), C-terminal non-conserved region (C-NCR, grey), the serine (S) and threonine (T) residues in their approximate regions that are known phosphorylation sites, and the sites that are phosphorylated by CKII, Cdc28-cyclin B, Pho85-Pho80, PKA, and PKC, and the tryptophan (W) residue within the C-terminal conserved sequence WRDPLVDID (orange) required for Pah1 function in vivo.

essential for catalytic activity and thus for its physiological function (33), and a conserved tryptophan residue within the sequence WRDPLVDID is also required for physiological function (34). The N-terminal sequence of Pah1 forming an amphipathic helix binds to the membrane (35), and its C-terminal acidic tail clustered with negatively charged amino acids is required to interact with the ER-associated Pah1 phosphatase (Nem1-Spo7 complex) (35–37). The non-conserved regions of Pah1, which are located between the conserved domains and after the C-LIP domain (Fig. 2), are unfolded and render the enzyme susceptible to proteasomal degradation (34). These regions contain most of the Pah1 phosphorylation sites and play a major role in the enzyme localization and in the control of its stability (35, 38–43).

PHOSPHORYLATION/DEPHOSPHORYLATION IS A KEY MECHANISM FOR REGULATING PA PHOSPHATASE FUNCTION

Yeast PA phosphatase is regulated by genetic and biochemical mechanisms. On a transcriptional level, the *PAH1* gene is regulated by cell growth and its expression is maximal in the stationary phase when nutrients are depleted (11, 18, 44). As expected, the level of *PAH1* expression correlates with the extent of TAG synthesis (11, 18, 44). Biochemically, the enzyme activity of Pah1 is stimulated by negatively charged phospholipids (e.g., CDP-DAG and phosphatidylinositol) (45), but inhibited by positively charged sphingoid bases (e.g., phytosphingosine) (46) and by nucleotides (e.g., ATP and CTP) (47). In addition, Pah1 undergoes phosphorylation and dephosphorylation, and these posttranslational modifications are crucial to control its membrane localization, catalytic activity, and stability (1, 48) (Fig. 1). Pah1 is phosphorylated by several protein kinases, which include cyclin-dependent protein kinases [e.g., Pho85-Pho80 (40) and Cdc28-cyclin B (39)], protein kinase A (PKA) (41), protein kinase C (PKC) (42), and casein kinase II (CKII) (49) (Figs. 1, 2). The phosphorylation of Pah1 by Pho85- Pho80 (40), Cdc28-cyclin B (10, 39), and PKA (41) causes its localization in the cytosol apart from the substrate PA present in the membrane (Fig. 1). Moreover, Pah1 phosphorylated by Pho85-Pho80 (40) and PKA (41) has reduced catalytic activity. Its phosphorylation by CKII has little effect on catalytic activity but inhibits its subsequent phosphorylation by PKA (49). The PKC phosphorylation of Pah1 does not affect its localization or catalytic activity, but instead regulates its stability (42). This phosphorylation, which is favored without phosphorylation by Pho85-Pho80, promotes proteolysis by the 20S proteasome (42, 50) (Fig. 1).

Unlike its phosphorylation by multiple protein kinases, Pah1 is dephosphorylated by a single protein phosphatase (e.g., Pah1 phosphatase) localized in the nuclear/ER membrane that is composed of the Nem1 catalytic subunit and the Spo7 regulatory subunit (36) (Fig. 1). Both Nem1 and Spo7 contain two transmembrane-spanning regions that are responsible for their association with the nuclear/ER membrane (36). Nem1 binds to Spo7 through its conserved C-terminal region, and this association is responsible for the formation of the complex in the membrane bilayer (36). Like Pah1 (5, 29), Nem1 is a member of the HAD (haloacid dehalogenase) superfamily (51) and its phosphatase activity is dependent on the D*X*D*X*(TV) catalytic motif. The catalytic function of Nem1 on the Pah1 substrate requires its association with Spo7 (10, 36), and the specificity of the dephosphorylations is in the order of the sites phosphorylated by Pho85-Pho80 > PKA = CKII > Cdc28-cyclin B > PKC (43, 49). The dephosphorylation of Pah1 results in its translocation to the membrane (10, 35, 37–41, 52, 53) (Fig. 1). Moreover, dephosphorylated Pah1 is catalytically more active (38, 43). Given the requirement of the protein phosphatase complex on Pah1 function, cells lacking Nem1 and/or Spo7 exhibit phenotypes shown by cells lacking Pah1 (10, 11, 36). Overall, the modifications of Pah1 by phosphorylation and dephosphorylation ensure a precise control of its catalytic function on the target membrane.

Interestingly, while the Nem1-Spo7 complex functions to dephosphorylate Pah1, the complex itself has been shown to be phosphorylated (54, 55). However, the protein kinases involved and the specific effects of the phosphorylations on the complex function have yet to be elucidated.

HOW DO WE DISCOVER MOLECULES TO CONTROL PA PHOSPHATASE FUNCTION WITHOUT DISTURBING LIPID HOMEOSTASIS?

Because of its role in the synthesis of TAG, PA phosphatase can be considered a drug target to ameliorate obesity and/or lipodystrophy. However, effector molecules

specific for the enzyme have yet to be identified. The discovery of PA phosphatase regulators requires a systematic process that involves the library screening of natural products or synthetic compounds and/or the synthesis of substrate mimics serving as a specific inhibitor/activator. Rational drug design, which is commonly used by the pharmaceutical industry, requires the structural information of target proteins. For PA phosphatase, its structural determination has been a challenge because the enzyme is intrinsically unstable due to the unfolded regions. While the phosphorylation stabilizes the protein to some degree (50), it has been difficult to prepare it as a fully phosphorylated form that is suitable for crystallography. A genetically engineered protein that is functional in vivo but lacks the nonconserved regions (34) might be amenable to crystallization. However, it is the disordered regions that are so critical to enzyme regulation. Thus, at this point, rational drug design based on structure is dubious.

As discussed above, a potent inhibitory molecule that abolishes PA phosphatase activity would not be suitable as an obesity drug because it would disrupt lipid homeostasis and lead to other lipid-based diseases or even cancer. Accordingly, molecules that moderately affect PA phosphatase activity seem to be better in the control of TAG synthesis. The fine-tuning of PA phosphatase can also be achieved by the control of its cellular location as mediated by phosphorylation/dephosphorylation. Thus, understanding the phosphorylation and dephosphorylation as well as control of the posttranslational modifications could lead to the discovery of molecules to control PA phosphatase function. In addition, PA phosphatase activity can be indirectly controlled by molecules that affect the function of the Nem1-Spo7 complex through the control of its complex formation as well as the control of the phosphatase interaction with its substrate PA phosphatase. Additional studies are needed to gain a better understanding of these interactions and whether phosphorylation affects the protein-protein interactions. Yet another approach of regulating PA phosphatase function is to control its programmed degradation by the proteasome, which itself is influenced by phosphorylation/dephosphorylation. Clearly, more work is needed to fully understand the structure-function and control of PA phosphatase with the goal of using the enzyme as a target to control TAG-related diseases.

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