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.

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

Phosphatidic acid (PA) phosphatase,2 the enzyme that catalyzes the Mg2+-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.

Abbreviations: CKII, casein kinase II; DAG, diacylglycerol; PA, phosphatidic acid; PPA, 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 Mg2+ ions (1).

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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 conserved and nonconserved regions (Fig. 2). The conserved N-LIP and C-LIP (HAD-like) domains (5, 26) are
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 DXDX(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, 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 crystalllography. 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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