Discoveries of the phosphatidate phosphatase genes in yeast published in the *Journal of Biological Chemistry*

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This JBC Review on the discoveries of yeast phosphatidate (PA) phosphatase genes is dedicated to Dr. Herbert Tabor, Editor-in-Chief of the *Journal of Biological Chemistry* (JBC) for 40 years, on the occasion of his 100th birthday. Here, I reflect on the discoveries of the *APP1*, *DPP1*, *LPP1*, and *PAH1* genes encoding all the PA phosphatase enzymes in yeast. PA phosphatase catalyzes PA dephosphorylation to generate diacylglycerol; both substrate and product are key intermediates in the synthesis of membrane phospholipids and triacylglycerol. App1 and Pah1 are peripheral membrane proteins catalyzing an Mg^{2+}-dependent reaction governed by the D*X*D*X*(T/V) phosphatase motif. Dpp1 and Lpp1 are integral membrane proteins that catalyze an Mg^{2+}-independent reaction governed by the KX_6RP–PSGH–SRX_5HX_3D phosphatase motif. Pah1 is PA-specific and is the only PA phosphatase responsible for lipid synthesis at the nuclear/endoplasmic reticulum membrane. App1, Dpp1, and Lpp1, respectively, are localized to cortical actin patches and the vacuole and Golgi membranes; they utilize several lipid phosphate substrates, including PA, lyso-PA, and diacylglycerol pyrophosphate. App1 is postulated to be involved in endocytosis, whereas Dpp1 and Lpp1 may be involved in lipid signaling. Pah1 is the yeast lipin homolog of mice and humans. A host of cellular defects and lipid-based diseases associated with loss or overexpression of PA phosphatase in yeast, mice, and humans, highlights its importance to cell physiology.

Herbert Tabor and my association with the *Journal of Biological Chemistry* (JBC)

This JBC Review is a reflection on my association with Dr. Herbert Tabor and the JBC, and the discoveries of the PA[2] phosphatase genes in yeast. I dedicate this JBC Review to Herb on the occasion of his 100th birthday. I also express my esteem and gratitude to Herb for the support he has given me as an author, reviewer, board editor, and associate editor of the JBC.

The JBC is the venue for the seminal work on the biochemistry and molecular biology of membrane lipids. William Dowhan (postdoc advisor) and Eugene P. Kennedy (Dowhan's postdoc advisor) published their best work in the JBC, and emulating them, my best work is also published here. My first JBC paper (postdoctoral studies) was on the interfacial kinetics of the phosphatidylserine synthase of *Escherichia coli* (1), and as an independent investigator, my first JBC paper was on the purification and characterization of the yeast phosphatidylserine synthase (2). The reviewing editors of these papers were thorough indeed, and I learned that the conclusions of our work had to be rigorously supported by sound experimental approaches. This has become a guiding principle of my research and mentoring.

As my career progressed, I aspired to become associated with the JBC, just like the icons in my field (*e.g.* Eugene P. Kennedy, William Dowhan, Susan A. Henry, Edward A. Dennis, Christian R. H. Raetz, Dennis E. Vance, and Robert M. Bell) who were either members of the Editorial Board or Associate Editors. It was during the late 1980s when I received an invitation from Herb to review a lipid enzymology paper. Of course, I accepted the invitation, and this began my association with the journal as a reviewer. I had continued to receive invitations to review papers for the journal, primarily from Associate Editor Robert M. Bell. After "paying my dues" as a reviewer, I was appointed to the editorial board for a 5-year term in 1992 and then again in 1998. The associate editors that I worked closely with were Robert M. Bell, Stephen M. Prescott, Claudia Kent, and William L. Smith. I reviewed ~6 papers per month and submitted my reviews within a few days. I was rewarded for this service by being appointed associate editor in 2006. Then the real work began; Herb was assigning me 30–40 papers a month. Fortunately, the JBC has an amazing editorial board whose members are dedicated to the journal. Making an editorial decision on the good and the bad manuscripts is relatively easy, but it is those papers that fall in the "gray zone" that make decisions difficult. I have tried to follow Herb's guiding principle to give the benefit of doubt to the authors with the opportunity to make their science better and thus worthy of publication in the JBC. A few years ago, Herb stepped down as Editor-in-Chief, but he still assigns manuscripts as an emeritus editor of the journal. I am pleased to work closely with Herb on the "Classics and Reflections" committee.

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[2] The abbreviations used are: PA, phosphatidate; DAG, diacylglycerol; TAG, triacylglycerol; DGPP, diacylglycerol pyrophosphate; HAD, haloacid dehalogenase; ER, endoplasmic reticulum.

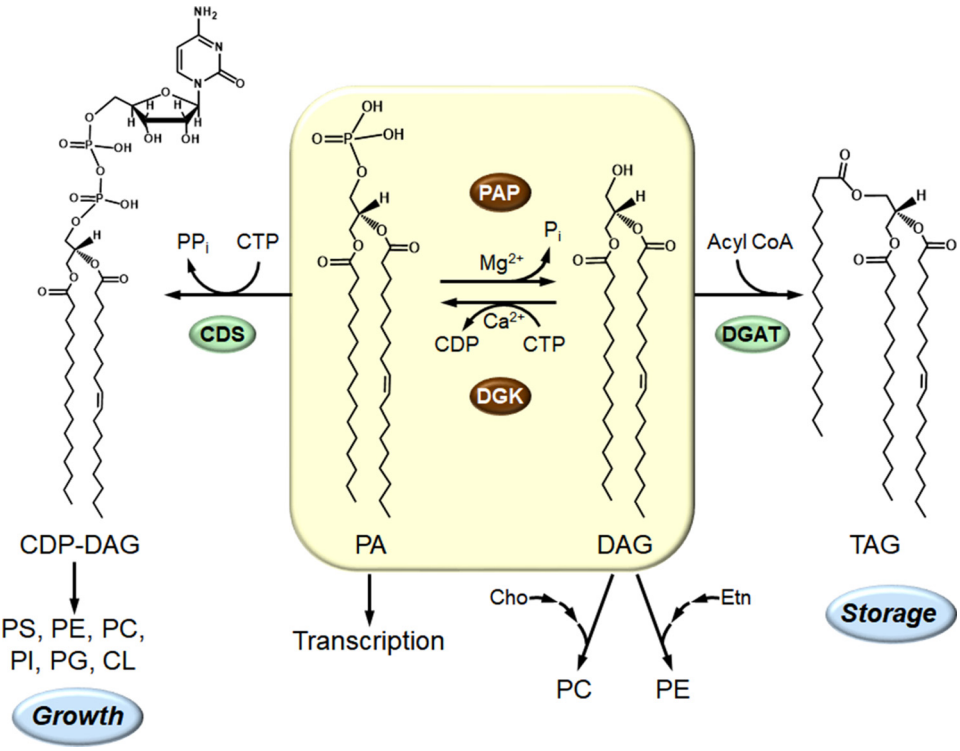

Figure 1. Central role of the PA phosphatase in the synthesis of TAG and membrane phospholipids in yeast. The structures of CDP–DAG, PA, DAG, and TAG are shown with fatty acyl groups of 16 and 18 carbons with and without a single double-bond where indicated. The PA phosphatase (*PAP*) plays a major role in governing whether cells utilize PA for the synthesis of TAG via DAG or whether they utilize PA for the synthesis of membrane phospholipids via CDP–DAG. The PA phosphatase reaction is counterbalanced by the CTP-dependent DAG kinase (*DGK*) reaction. When the CDP–DAG pathway for phospholipid synthesis is blocked, phosphatidylcholine and phosphatidylethanolamine may be synthesized from the DAG derived from the PA phosphatase reaction when cells are supplemented with choline and ethanolamine, respectively, via the CDP-choline and CDP-ethanolamine branches of the Kennedy pathway. In addition to its role in lipid synthesis, PA signals the transcriptional regulation of phospholipid synthesis genes via the Henry (Opi1/Ino2/Ino4) regulatory circuit. More comprehensive pathways of lipid synthesis, along with details of the Henry regulatory circuit, may be found in Refs. 5, 6. *CDS*, CDP–DAG synthase; *DGAT*, DAG acyltransferase; *PS*, phosphatidylserine; *PE*, phosphatidylethanolamine; *PC*, phosphatidylcholine; *PI*, phosphatidylinositol; *PG*, phosphatidylglycerol; *CL*, cardiolipin; *Cho*, choline; *Etn*, ethanolamine.

Why we study PA phosphatase

Research in my laboratory utilizes biochemical and molecular genetics approaches to study the regulation of lipid synthesis in the yeast *Saccharomyces cerevisiae* (3–6). The purification and characterization of lipid synthesis enzymes and the molecular characterization of lipid synthesis regulation are facilitated in yeast because of their genetic tractability and ease of growth. Importantly, our work with yeast has proven to be relevant to the mechanisms that regulate lipid synthesis in humans (5–7).

Several papers published in the JBC by Eugene P. Kennedy (8–18), William Dowhan (19–24), Christian R. H. Raetz (25–28), Edward A. Dennis (29–33), and Robert M. Bell (34–39) have had a major influence on the way I think about science. Their work has provided me with a framework and high standard for performing well-designed experiments to address basic questions on the enzymology and regulation of lipid metabolism.

Studies from several laboratories, primarily that of Susan A. Henry, have indicated that a key step in the regulation in lipid synthesis occurs at the point where PA is bifurcated to CDP–DAG and DAG (Fig. 1). CDP–DAG is utilized for the synthesis of all membrane phospholipids, whereas DAG is primarily used for the synthesis of TAG (Fig. 1) (reviewed in Refs. 4, 6, 40, 41). The DAG is also used to synthesize phosphatidylcholine or phosphatidylethanolamine via the Kennedy pathway when cells are supplemented with choline or ethanolamine (Fig. 1). Moreover, the PA molecule itself regulates the expression of phospholipid synthesis genes by controlling the cellular location of the transcriptional repressor protein Opi1 (42), a key component of the Henry regulatory circuit (4, 6). The microsomal CDP–DAG synthase, which utilizes PA as a substrate to form CDP–DAG (43), is not a highly-regulated enzyme, and thus, the important regulator of PA utilization is the other branch point enzyme PA phosphatase (Fig. 1). By the nature of its reaction, PA phosphatase controls the synthesis of TAG and membrane phospholipids and the abundance of the important signaling molecule PA (4, 44). The reaction of the enzyme, namely the dephosphorylation of PA to generate DAG (Fig. 1), was first characterized by Kennedy and co-workers in 1957 (10). In 1987, when we initiated our studies on PA phosphatase, the enzyme had not been purified; the identity of the gene encoding the enzyme was unknown; and no mutants were available. How the PA phosphatase genes in yeast were discovered is discussed in the remainder of this JBC Review.

Identification of the *DPP1* and *LPP1* genes

In 1989, my graduate student Yi-Ping Lin successfully purified PA phosphatase from a total membrane fraction of yeast (45). The eight-step procedure developed by Yi-Ping yielded a 91-kDa protein that was used to carry out enzymological and

kinetic studies of the enzyme (45, 46). Another graduate student, Wen-I Wu, joined the laboratory; her project was to purify a sufficient quantity of the 91-kDa enzyme to obtain amino acid sequence information to be used in a reverse-genetics approach to isolate the PA phosphatase gene. To improve the purification, Wen-I substituted the detergent Triton X-100 for sodium cholate and made changes to the column resins and chromatography conditions. The result was a purified protein with a molecular mass of 34 kDa, not the 91-kDa protein that Yi-Ping had purified. Moreover, PA phosphatase activity in this preparation was not dependent on Mg^{2+} ions as it was for the PA phosphatase purified by Yi-Ping (45). The pH optima of the two PA phosphatase preparations also differed. I suggested to Wen-I that she abandon her modified scheme to purify PA phosphatase and use the established procedure developed by Yi-Ping. Wen-I complied with my suggestion and used the 91-kDa enzyme to study the regulation of PA phosphatase activity by phospholipids (47), sphingolipids (48), and nucleotides (49). Unfortunately, we did not obtain unambiguous sequence information for the 91-kDa protein to synthesize a well-defined oligonucleotide probe to "fish out" the PA phosphatase gene from a genomic library.

In the meantime, Wissing and Behrbohm (50, 51) had just discovered a novel PA kinase in plants that converts PA to DGPP. This novel phospholipid, which contains a pyrophosphate group attached to DAG, is a metabolite of PA that is synthesized during G-protein activation in response to stress (52). In a collaboration with Wissing, we discovered that a PA phosphatase activity in plants, yeast, bacteria, and mammals could dephosphorylate DGPP (53). This activity has an acidic pH optimum and does not require Mg^{2+} ions like the PA phosphatase activity associated with the 34-kDa protein that Wen-I Wu had purified from yeast. Wen-I showed that the 34-kDa enzyme utilized DGPP as a substrate with a specificity constant 10-fold greater when compared with that of PA (54). Thus, we called the 34-kDa enzyme DGPP phosphatase (54). Through a kinetic analysis, we found that the enzyme first dephosphorylates DGPP to form PA, and then it dephosphorylates PA to form DAG (54). In fact, in the presence of DGPP, the enzyme does not dephosphorylate PA (54). We also found that the enzyme utilizes a variety of lipid phosphate substrates that include DGPP, PA, lyso-PA, ceramide phosphate, sphingosine phosphate, farnesyl pyrophosphate, geranylgeranyl pyrophosphate, dolichyl pyrophosphate, and dolichyl phosphate (54–56). In contrast, the 91-kDa PA phosphatase is specific for PA (45, 54, 57).

We had obtained unambiguous sequences in the 34-kDa protein by Edman degradation (58). At this time, the yeast genome was supposedly sequenced, and the gene encoding the enzyme should have been in the database. However, this was not the case. It was suggested by the curators of the *Saccharomyces* database that perhaps we had not purified a yeast protein. So, to be sure we had not purified DGPP phosphatase from an organism other than *S. cerevisiae*, we purified the enzyme again and obtained the same protein sequences. We had heard through the "grapevine" that several *bona fide* yeast genes were not in the database. Eventually, however, all the genes were deposited in the database, and the gene (*DPP1* for diacylglycerol pyrophosphate phosphatase) was identified (58). Cloning and expression studies, fronted by David Toke (postdoctoral associate), along with a biochemical analysis confirmed the *DPP1*–DGPP phosphatase relationship (58). Shortly after publishing the identification of the *DPP1* gene, it was shown to be one of the most highly expressed genes in response to zinc deprivation (59, 60).

During this time, Joseph Stukey (postdoctoral associate) discovered a novel phosphatase sequence motif through a bioinformatics analysis using the *E. coli pgpB*-encoded phosphatidylglycerophosphate phosphatase as the query (61). The motif consists of three domains with the consensus sequences KX_6RP (domain 1)–PSGH (domain 2)–SRX_5HX_3D (domain 3) (61). Dpp1 was shown to contain this phosphatase motif (Fig. 2) (58), and subsequently, the *pgpB*-encoded protein was shown to exhibit DGPP phosphatase activity (62).

A search of the protein database using Dpp1 as the query identified a closely related protein with a subunit size of 32 kDa that contains the novel phosphatase sequence motif (Fig. 2) (63). We named the gene encoding this protein *LPP1* for lipid phosphate phosphatase because, like Dpp1, Lpp1 utilizes a variety of lipid phosphate substrates (*e.g.* PA, lyso-PA, and DGPP) (63). Orthologs of Dpp1 and Lpp1 in mammalian cells, named lipid phosphate phosphatases, also exhibit a broad substrate specificity and play roles in lipid signaling (64–66). A series of cloning, expression, and biochemical studies confirmed the *LPP1*–lipid phosphate phosphatase relationship (63). We constructed a mutant that lacks both the *DPP1* and *LPP1* genes; the analysis of PA phosphatase activity in the mutant indicated that other PA phosphatase genes exist in yeast, including the gene coding for the 91-kDa enzyme (Fig. 3*A*) (63, 67).

Identification of the *PAH1* gene

In the summer of 2005, we were cleaning the −80 °C freezer in the laboratory. Gil-Soo Han, a postdoctoral associate, found some Mono Q chromatography fractions used for the purification of the 91-kDa PA phosphatase that were placed there in 1993 by Wen-I Wu. According to Wen-I's laboratory notebook, some of the fractions had PA phosphatase activity, whereas others did not have activity. Gil-Soo subjected the fractions to SDS-PAGE and was able to match up the enrichment of the 91-kDa protein with the elution profile of PA phosphatase activity. A gel slice containing the 91-kDa protein was subjected to trypsin digestion followed by amino acid sequence analysis of peptide fragments by MS (a more sensitive method to obtain protein sequence when compared with Edman degradation). Unambiguous sequence information obtained from 23 peptides matched perfectly with the deduced amino acid sequence of the *SMP2* gene (57). *SMP2* was originally identified as a gene involved in plasmid maintenance and respiration (68). Later, Siniossoglou and co-workers (69) identified *SMP2* as a gene whose overexpression complements the abnormal expansion of the nuclear/ER membrane that is caused by mutations (*nem1*Δ or *spo7*Δ) in the subunits of a membrane-associated protein phosphatase (70). The deletion of *SMP2* was shown to cause the same membrane expansion phenotype as the protein phosphatase mutants, as well as the derepression of several phospholipid synthesis genes (69). Collectively, the data raised

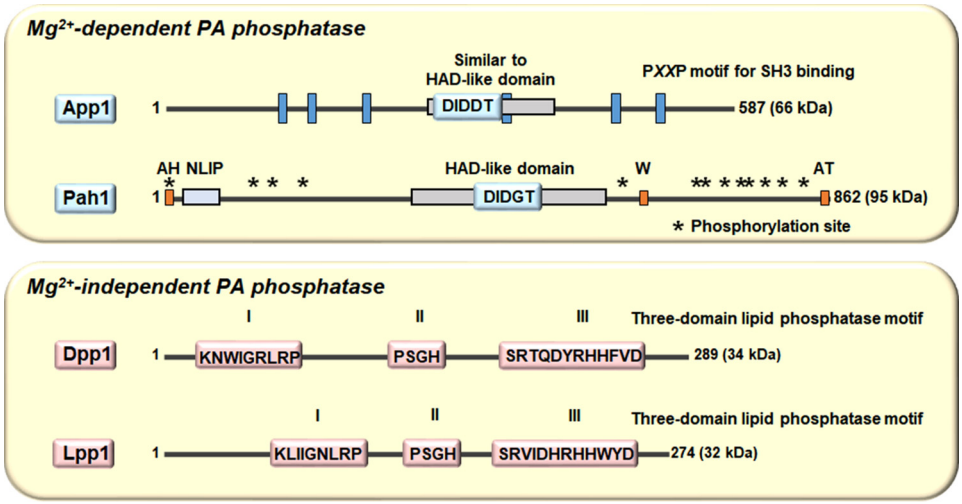

Figure 2. Mg^{2+}-dependent and -independent PA phosphatase enzymes in yeast have distinct catalytic motifs. The diagrams show linear representations of App1 and Pah1 (*upper panel*) and Dpp1 and Lpp1 (*lower panel*). The Mg^{2+}-dependent PA phosphatase activities of App1 and Pah1 are governed by the D*X*D*X*(T/V) motifs in the HAD-like domain, whereas the Mg^{2+}-independent PA phosphatase activities of Dpp1 and Lpp1 are governed by the three-domain phosphatase motif. For Pah1, the approximate positions are indicated for the amphipathic helix (*AH*) required for ER membrane interaction (77), the NLIP and HAD-like domains that are required for PA phosphatase activity (116), the acidic tail (*AT*) required for interaction with Spo7 of the Pah1 phosphatase (70, 77, 80), the tryptophan (*W*) residue within the C-terminal conserved sequence WRDPLVDID required for Pah1 function (117), and the sites phosphorylated by Pho85–Pho80, Cdc28 – cyclin B, protein kinase A, protein kinase C, and casein kinase II that regulate the location, activity, and proteasomal degradation of Pah1 (76, 78, 79, 83, 84, 118).

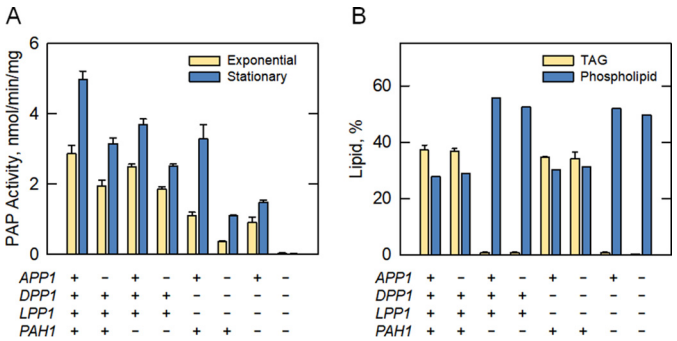

Figure 3. Contributions of the *APP1*, *DPP1*, *LPP1*, and *PAH1* genes to the PA phosphatase activity and lipid content in yeast. Cells expressing or lacking the indicated gene (+ and −, respectively) were examined for PA phosphatase (*PAP*) activity (*A*) or for lipid (TAG and phospholipids) content (*B*). The PA phosphatase in yeast cells, which is elevated in the stationary phase, is contributed by the *APP1*, *DPP1*, *LPP1*, and *PAH1* genes. The quadruple mutant with deletions in all four genes lacks detectable PA phosphatase activity. Cells with the deletion of *PAH1* exhibit a massive reduction in TAG content. The data were taken from Ref. 67.

the suggestion that Smp2 might be a transcription factor (69). Gil-Soo went on to express and purify His_6-tagged Smp2 from *E. coli* and showed that it possessed PA phosphatase activity; it had the same enzymological properties as the 91-kDa enzyme originally purified by Yi-Ping in 1989 (45, 57). Because the name *SMP2* has no meaning in a functional sense, we renamed the gene *PAH1* for phosphatidic acid phosphohydrolase. (We could not use the acronym PAP for the PA phosphatase gene because it was already being used for the poly(A) polymerase gene.) A bioinformatics analysis of Pah1 indicated that the protein contains a conserved haloacid dehalogenase (HAD)–like domain with the D*X*D*X*(T/V) motif found in a superfamily of Mg^{2+}-dependent phosphatase enzymes with diverse substrate specificity (Fig. 2) (71, 72). To determine whether we had identified all the PA phosphatase genes in yeast, a mutant lacking *DPP1*, *LPP1*, and *PAH1* was constructed and analyzed for PA phosphatase activity (57, 67). This analysis showed that there was still another PA phosphatase gene to be identified (Fig. 3*A*) (57, 67).

Identification of the *APP1* gene

Attempts to identify the remaining PA phosphatase gene through bioinformatics and by genetic screens were unsuccessful. Accordingly, we set forth to identify the gene through the reverse genetics approach. A new graduate student, Minjung Chae, joined the laboratory and was convinced to take on the project. Minjung was told that all she had to do was purify PA phosphatase from the *dpp1*Δ *lpp1*Δ *pah1*Δ triple mutant, get a protein sequence, and the rest would be easy. Minjung spent about 2 years developing an eight-step procedure that did not result in the complete purification of the enzyme (67). The PA phosphatase activity in the triple mutant was relatively low (*e.g.* 5-fold lower when compared WT cells), and the purification was hampered by the lability of the PA phosphatase activity (67). Nonetheless, a peak of activity from the last column of the procedure correlated with the enrichment of a minor protein band upon SDS-PAGE (67). The band was excised from the SDS-polyacrylamide gel and digested with trypsin, and the resulting peptides were analyzed by LC–MS. The analysis yielded protein sequences of several proteins of unknown function, one of which we had hoped would be PA phosphatase. Based on the genetic information in the yeast database, we expressed and purified His_6-tagged versions of these proteins from *E. coli*, but none of them exhibited PA phosphatase activity. This suggested that the enzyme in the preparation had to be very low in abundance. Accordingly, we used more sensitive LC–MS/MS to detect low abundance proteins. To our dismay, the analysis showed that the excised protein band contained 112 proteins (or proteolytic fragments thereof)! We were devastated by this result and Minjung returned home for a break.

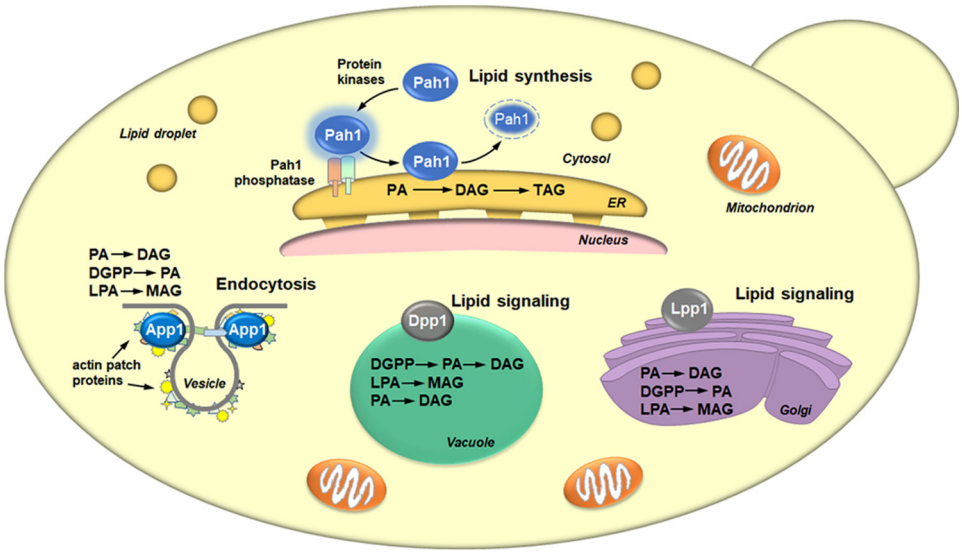

Figure 4. Cellular locations and roles of yeast PA phosphatases. The figure depicts a yeast cell with the cellular locations of the App1, Dpp1, Lpp1, and Pah1 PA phosphatase enzymes and their physiological roles. The lipid phosphate phosphatase reaction catalyzed by each enzyme is indicated in the order of substrate preference. For Pah1, the enzyme in the cytosol is phosphorylated by multiple protein kinases. The phosphorylated Pah1 (indicated by the *blue halo*) translocates to the ER membrane through its dephosphorylation by the Pah1 phosphatase, which is composed of Nem1 (catalytic subunit, *green*) and Spo7 (regulatory subunit, *peach*). Dephosphorylated Pah1 that is associated with the ER membrane catalyzes the conversion of PA to DAG, which is then acylated to form TAG. Dephosphorylated Pah1 is degraded by the proteasome (indicated by the *dashed lines*). App1 associates with actin patch proteins at cortical actin patches. The App1 enzyme is postulated to be involved in the formation of endocytic vesicles. The vesicle diagram is adapted from Ref. 74. Dpp1 and Lpp1, respectively, are integral membrane proteins in the vacuole and Golgi apparatus. They are postulated to be involved with lipid signaling by controlling the amounts of bioactive lipids.

Upon Minjung's return to the laboratory, we devised a plan to focus on the proteins with unknown function that had a phosphatase motif and proteins predicted to be involved in lipid synthesis. Cell extracts from 20 mutants in the yeast gene-knockout collection were assayed for loss in PA phosphatase activity. Of the mutants, one lacking the *APP1* gene showed a small (30%) but reproducible decrease in PA phosphatase activity (67). *APP1* stands for actin patch protein because App1 is a component of cortical actin patches and interacts with endocytic proteins. The molecular function of App1 had been unknown. To provide evidence that *APP1* encodes a PA phosphatase, His_6-tagged and protein A-tagged, respectively, versions of the App1 protein were expressed and purified from *E. coli* and yeast and assayed for PA phosphatase activity (67, 73). Both purified preparations of App1 exhibited PA phosphatase activity (67, 73). App1 has a subunit size of 66 kDa, and it contains the D*X*D*X*(T/V) catalytic motif in a region that is similar to the HAD-like domain in Pah1. App1 lacks the NLIP domain found in Pah1 but contains six copies of the PX_2P motif for interaction with proteins with Src homology 3 domains (Fig. 2) (67). App1 is a fungus-specific PA phosphatase as no orthologs exist in mammalian cells. The analysis of the *app1*Δ *dpp1*Δ *lpp1*Δ *pah1*Δ quadruple mutant showed no PA phosphatase activity, and thus, all PA phosphatase activity in yeast is encoded by *APP1*, *DPP1*, *LPP1*, and *PAH1* (Fig. 3*A*) (67).

Cellular locations and roles of the PA phosphatases

Fig. 4 depicts the cellular locations and roles of the four PA phosphatases in yeast. All PA phosphatase enzymes must associate with a membrane, the location of their phospholipid substrates. App1 and Pah1 associate with the membrane in a peripheral manner. App1 associates with actin patch proteins at sites of endocytic vesicle formation (74), whereas Pah1 associates with the nuclear/ER membrane upon its dephosphorylation by the Nem1–Spo7 protein phosphatase (*e.g.* Pah1 phosphatase) (69, 70, 75–82). Pah1 in the cytosol is a phosphoprotein whose phosphorylation is carried out by multiple protein kinases that include Pho85–Pho80 (78), Cdc28–cyclin B (76), protein kinase A (79), protein kinase C (83), and casein kinase II (84). Dpp1 and Lpp1, respectively, are integral membrane proteins that reside in the vacuole (85–87) and Golgi apparatus (87). The contribution of each PA phosphatase to lipid metabolism has been assessed by the analysis of mutants lacking the *APP1*, *DPP1*, *LPP1*, and *PAH1* genes (Fig. 3*B*) (67). This analysis clearly shows that Pah1 is the major regulator of TAG content; the enzymes encoded by *APP1*, *DPP1*, and *LPP1* have little effect on the relative amounts of TAG and membrane phospholipids. The association of App1 with cortical actin patches (74) suggests that it may regulate the local concentrations of PA and DAG through its PA phosphatase activity (67). These lipids are known to facilitate membrane fission/fusion events in model systems, and they are also known to interact with and regulate enzymes (*e.g.* phosphatidylinositol 4-phosphate kinase, protein kinase C, and protein kinase D) that play important roles in vesicular trafficking (88–92). Dpp1 and Lpp1, respectively, are thought to control the signaling function of PA, DAG, DGPP, and lyso-PA in vacuole and Golgi membranes (54, 58, 63, 86, 87).

Epilogue

Of the four PA phosphatases, our laboratory has focused more on Pah1 because of its importance to lipid metabolism and lipid-based disease. The discovery that yeast *PAH1* encodes PA phosphatase has led to the identification of genes encoding

the enzyme in mice (93, 94) and humans (57, 95). The protein sequence analysis of Pah1 has revealed that it is the yeast homolog of the mouse fat-regulating protein known as lipin 1 (93). In 2001, Reue and co-workers (93) coined the name lipin 1 as the product of the *Lpin1* gene whose mutation was responsible for fatty liver dystrophy (*fld*) mice at birth (96). It has been known that loss of lipin 1 in mice causes lipodystrophy and that overexpression of lipin 1 causes obesity (93, 97). However, the molecular function of lipin 1 had not been established. Knowing that PA phosphatase was the product of yeast *PAH1* and that Pah1 shares sequence homology with lipin 1 at the N-terminal NLIP and within the HAD-like (CLIP) domains (Fig. 2) led Gil-Soo Han to the hypothesis that lipin 1 is in fact a PA phosphatase (57). The expression, purification, and biochemical analysis of human lipin 1 confirmed this hypothesis (57, 95). There are three spliced variant forms of *Lpin1*, as well as *Lpin2* and *Lpin3*, all of which that encode proteins with PA phosphatase activity (57, 93–95).

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 yeast, loss of *PAH1* (*SMP2*, see above) results in a massive expansion of the nuclear/ER membrane (69); this is ascribed to increases in PA content and phospholipid synthesis that occur at the expense of TAG synthesis (57, 98). The increase in phospholipid synthesis is associated with the induction of phospholipid synthesis gene expression (69, 99), whereas the reduction in the synthesis of TAG is associated with a decrease in lipid droplet formation (100–102). The imbalance of lipid homeostasis in cells lacking *PAH1* results in a multitude of other phenotypes that include fatty acid-induced toxicity (100), hypersensitivity to oxidative stress (103), loss in cell wall strength (104, 105), reduction in chronological life span (103), the inability to fuse vacuoles (106), the inability to degrade cellular components (*e.g.* autophagy) (107), and the inability to grow on nonfermentable carbon sources (57, 68) and at elevated temperatures (57, 68, 69). Some of these phenotypes are suppressed by the loss of the *DGK1* gene that encodes the CTP-dependent DAG kinase (100, 101, 108). These observations emphasize the importance of maintaining a proper balance of PA and DAG to lipid metabolism and cell physiology. Lipin 1 deficiency in human and mouse causes rhabdomyolysis (109, 110), and deficiency in the mouse is also characterized by hepatic steatosis during the neonatal period causing lipodystrophy, insulin resistance, and peripheral neuropathy (93, 111). *Lpin1* overexpression results in increased lipogenesis and obesity (97). Polymorphisms in the human *LPIN1* gene are associated with insulin resistance and the metabolic syndrome (112). Human lipin 2 deficiency causes chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anemia (113, 114), whereas genetic variations in the human *LPIN2* gene are associated with type 2 diabetes (115).

What has become clear from our work on yeast Pah1 is that too little or too much PA phosphatase activity is detrimental to cell physiology. Thus, the current focus of our work is to more fully understand the mode of action and regulation of activity with the goal of identifying effector molecules that will "fine-tune" the enzyme function.

Acknowledgments—I am grateful to the graduate students and postdoctoral associates who have worked on the PA phosphatase genes and enzymes in my laboratory. Michael Homann, a graduate student who worked on the regulation of CDP–DAG synthase, is acknowledged for the recommendation to study PA phosphatase, and Robert M. Bell is acknowledged for the suggestion to enzymatically synthesize the ^{32}P-labeled PA substrate using the E. coli DAG kinase. I acknowledge Symeon Siniossoglou for making seminal contributions to the understanding of Pah1 and its regulation by phosphorylation and dephosphorylation and for being a great collaborator. I acknowledge Gil-Soo Han for discovering the identities of Pah1 and Dgk1 as PA phosphatase and DAG kinase enzymes, respectively, and for helping me move our research forward. Finally, I thank Herb Tabor for being the quintessential editorial mentor. Happy 100th birthday Herb!

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