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The discovery of the fat-regulating phosphatidic acid phosphatase gene

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Abstract

Phosphatidic acid phosphatase is a fat-regulating enzyme that plays a major role in controlling the balance of phosphatidic acid (substrate) and diacylglycerol (product), which are lipid precursors used for the synthesis of membrane phospholipids and triacylglycerol. Phosphatidic acid is also a signaling molecule that triggers phospholipid synthesis gene expression, membrane expansion, secretion, and endocytosis. While this important enzyme has been known for several decades, its gene was only identified recently from yeast. This discovery showed the importance of phosphatidic acid phosphatase in lipid metabolism in yeast as well as in higher eukaryotes including humans.

Keywords

phosphatidic acid phosphatase; yeast; lipin; phospholipid; triacylglycerol

The enzyme phosphatidic acid (PA) phosphatase was first discovered in animal tissues (Smith et al., 1957). It catalyzes the Mg^{2+}-dependent dephosphorylation of PA to yield diacylglycerol (DAG) and inorganic phosphate (Fig. 1). The PA phosphatase product DAG is acylated with fatty acyl CoA molecules to form triacylglycerol (TAG), or it is utilized for the synthesis of the major membrane phospholipids phosphatidylethanolamine and phosphatidylcholine (Carman and Han, 2009). The enzyme substrate PA is the precursor of other membrane phospholipids including phosphatidylinositol and cardiolipin (Carman and Han, 2006; Carman and Henry, 2007; Carman and Han, 2009). Thus, the regulation of PA phosphatase governs the synthesis of TAG and the pathways by which phospholipids are made.

PA phosphatase activity is crucial to controlling lipid metabolism and cell physiology. In the simple eukaryote yeast *Saccharomyces cerevisiae*, the loss of PA phosphatase causes a 90% loss in TAG content and lipid droplets (due to a decrease in DAG), and the aberrant transcriptional regulation of phospholipid synthesis genes and expansion of the nuclear/ ER membrane (due to an increase in PA) (Santos-Rosa et al., 2005; Han et al., 2006, 2007; Carman and Han, 2009). The importance of PA phosphatase in lipid metabolism and cell physiology is further emphasized by the fact that the overexpression of PA phosphatase in mice leads to obesity and insulin sensitivity, whereas loss of the enzyme prevents normal adipose tissue development, resulting in lipodystrophy and insulin resistance (Péterfy et al., 2001; Phan and Reue, 2005). Moreover, mice lacking PA phosphatase activity exhibit peripheral neuropathy (Langner et al., 1989; Langner et al., 1991; Nadra et al., 2008) caused

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by degradation of myelin through the MEK/ERK signaling pathway that is activated by elevated levels of PA (Nadra et al., 2008). In humans, mutations in PA phosphatase are associated with metabolic syndrome, type 2 diabetes, recurrent acute myoglobinuria in children, and inflammatory disorders associated with the Majeed syndrome (Reue and Donkor, 2007; Reue and Brindley, 2008; Donkor et al., 2009; Reue and Dwyer, 2009). Although PA phosphatase had been known for many years, a gene encoding the enzyme had not been discovered until recently (Han et al., 2006). The story behind its discovery is an interesting one that will be discussed in this review to emphasize the serendipitous nature of scientific investigations.

Our laboratory initiated studies to purify the PA phosphatase from yeast during the latter part of the 1980s. At that time, there had been several unsuccessful attempts to purify the enzyme from animal tissues due to the lability of the enzyme from yeast because the organism is easy to grow in large quantities. Moreover, there is a great deal known about the genetic and molecular biology of yeast, and thus, we felt that this system would facilitate the molecular characterization of the PA phosphatase gene. In 1989, my graduate student Yi Ping Lin successfully purified the PA phosphatase from *S. cerevisiae* by an 8-step procedure that included column chromatography with DEAE-cellulose, Affi-Gel, hydroxylapatite, Mono Q, and Superose 12 (Lin and Carman, 1989). The purification procedure, which took over a week to complete, yielded only microgram quantities of purified enzyme (Lin and Carman, 1989). While this amount of enzyme was sufficient to carry out detailed enzymological and kinetic experiments (Lin and Carman, 1989; Lin and Carman, 1990; Wu et al., 1993; Wu and Carman, 1994; Wu and Carman, 1996), it was insufficient for sequencing by classical Edman degradation analysis. In fact, attempts to obtain unambiguous protein sequence information cost the time and effort of three graduate students. In 1993, Wen-I Wu, one of the graduate students involved in the failing effort to obtain sequence information, placed a few tubes containing partially purified PA phosphatase into the −80°C freezer for storage.

In the summer of 2005, I asked my students to clean out the −80°C freezer in laboratory because we had run out of space. Gil-Soo Han, a postdoctoral fellow in the laboratory, found several tubes of the purified PA phosphatase that were placed there by Wen-I Wu. Gil-Soo inquired whether it would be alright to analyze the PA phosphatase sample for its enzymatic activity and to have it sequenced by mass spectrometry (a much more sensitive method that is now commonly used to obtain protein sequence information). Incredibly, the enzyme preparation retained the same level of PA phosphatase activity described in Wen-I Wu's laboratory notebook from 1993! Although the sample of enzyme was not homogenous, Gil-Soo was able to match up the enrichment of the protein (by SDS-PAGE) with the elution profile of PA phosphatase activity from a Mono Q ion-exchange column. A gel slice containing the protein was subjected to trypsin digestion followed by amino acid sequence analysis of peptide fragments by matrix-assisted laser desorption ionization tandem time-of-flight mass spectrometry. Unambiguous amino acid sequence information obtained from 23 peptides matched perfectly with the deduced amino acid sequence of the *SMP2* gene in the *Saccharomyces* Genome Database (Han et al., 2006). Subsequently, we cloned the yeast *SMP2* gene, expressed and purified it from *Escherichia coli*, and showed that the *SMP2* gene product has the same PA phosphatase activity and enzymological properties as the enzyme originally purified by Yi-Ping Lin in 1989 (Han et al., 2006). Because the name *SMP2* has no meaning in a functional sense, we renamed the gene *PAH1* (for phosphatidic acid phosphohydrolase) because its protein product has the molecular function of PA phosphatase (phosphohydrolase). The computer analysis of the enzyme revealed that it contains a conserved haloacid dehalogenase (HAD)-like domain (Koonin and Tatusov, 1994; Madera et al., 2004) in the middle of the protein sequence (Fig. 2), which contains a D*X*D*X*T/V (residues 398–402) motif that is found in a superfamily of Mg^{2+}-dependent

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phosphatase enzymes with diverse substrate specificity (Koonin and Tatusov, 1994; Madera et al., 2004).

Interestingly, it was 1993 (the year Wen-I Wu placed the PA phosphatase sample in the freezer) when Irie et al. (1993) identified *SMP2* (*PAH1*) as a gene involved in plasmid maintenance and respiration in *S. cerevisiae*. However, the molecular function of the *SMP2* (*PAH1*) gene had not been established at that time. No further work on *SMP2* (*PAH1*) was reported until 2005 (the year we discovered Wen-I Wu's sample of PA phosphatase in the freezer). Santos-Rosa et al. (2005) reported the identification of *SMP2* (*PAH1*) as a gene whose overexpression complements the aberrant nuclear membrane expansion phenotype of *nem1*Δ and *spo7*Δ mutants. *NEM1* (catalytic subunit) and *SPO7* (regulatory subunit) code for an ER-associated protein phosphatase complex that dephosphorylates PA phosphatase and activates the *in vivo* function of the enzyme (Siniossoglou et al., 1998; Santos-Rosa et al., 2005). Moreover, this work showed that the *smp2*Δ (*pah1*Δ) mutation caused the same phenotype as the protein phosphatase mutations, as well as the derepression of the phospholipid synthesis genes *INO1* (involved in phosphatidylinositol synthesis), *OPI3* (involved in phosphatidylcholine synthesis), and *INO2* (a positive phospholipid synthesis transcription factor) (Santos-Rosa et al., 2005). Santos-Rosa et al. (2005) went on to show through chromatin immunoprecipitation experiments that PA phosphatase interacts with the promoters of the *INO1*, *OPI3*, and *INO2* genes, suggesting its role as a transcription factor in the regulation of phospholipid synthesis.

Previous studies have shown that the expression of phospholipid synthesis genes, which contain a UAS_{INO} element, is controlled by the positive transcription factors Ino2p and Ino4p, and by the Opi1p repressor (Carman and Henry, 2007). Maximum expression of these genes is driven by the interaction of an Ino2p-Ino4p complex with the UAS_{INO} element in their promoters, whereas expression of these genes is attenuated by interaction of Opi1p with DNA-bound Ino2p (Carman and Henry, 2007). The repressive effect of Opi1p is most dramatic when cells are supplemented with inositol (Carman and Henry, 2007). The molecular function of the *SMP2* (*PAH1*) product as PA phosphatase indicated that the enzyme might control the expression of phospholipid synthesis genes by controlling the levels of PA. Loewen et al. (2004) have shown that reduced PA concentration, brought about by inositol supplementation, promotes the translocation of Opi1p from the ER into the nucleus where it interacts with Ino2p to repress *INO1* expression. By analogy, the same mechanism should apply to the regulation of other UAS_{INO}-containing genes including *OPI3* and *INO2*. The reduction in PA concentration in response to inositol supplementation has been attributed to increased PI synthesis (Loewen et al., 2004), which draws upon the PA pool in the biosynthetic pathway (Carman and Henry, 1999). The PA-mediated regulation of Opi1p function is also explained by involvement of the PA phosphatase enzyme (Han et al., 2007; Carman and Han, 2009). In fact, catalytic site mutations that abolish PA phosphatase activity cause the derepression of phospholipid synthesis gene expression like that caused by the *pah1*Δ (*smp2*Δ) mutation (Han et al., 2007).

In another twist of scientific fate, the *SMP2* (*PAH1*) product was identified as the yeast homolog of a mouse fat-regulating protein known as lipin 1 (Péterfy et al., 2001). In 2001, Péterfy et al. coined the name lipin 1 as the product of the gene (*Lpin1*) whose mutation was responsible for the fatty liver dystrophy phenotype of *fld* mice at birth (Langner et al., 1989). While it was known that loss of lipin 1 in mice cause lipodystrophy and overexpression of lipin 1 causes obesity (Péterfy et al., 2001; Phan and Reue, 2005), the molecular function of lipin 1 had not been established. Knowing that PA phosphatase was the product of *SMP2* (*PAH1*), and that its protein product shared sequence homology with the fat-regulating lipin 1 (at the N-terminal and within an HAD-like domain) (Fig. 2) led us to the hypothesis that lipin 1 is in fact a PA phosphatase enzyme (Han et al., 2006). To address this hypothesis, we

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purchased a human *LPIN1* cDNA and expressed it as a His-tagged protein in *E. coli* for ease of purification by Ni^{2+}-nitrilotriacetic acid chromatography (Han et al., 2006). We showed that the purified human lipin 1 protein was indeed a PA phosphatase enzyme (Han et al., 2006; Han and Carman, 2010). The function of lipin 1 in mammalian cells appears to be more complex than just serving as a PA phosphatase enzyme. In addition to its PA phosphatase activity, lipin 1 functions as a transcriptional coactivator in the regulation of lipid metabolism gene expression (Péterfy et al., 2005; Finck et al., 2006; Koh et al., 2008; Reue and Zhang, 2008). Moreover, there are three spliced variant forms of lipin 1, as well as lipin 2 and lipin 3, all of which possess PA phosphatase activity (Péterfy et al., 2001; Han et al., 2006; Donkor et al., 2007; Han and Carman, 2010).

Enormous progress has been made in understanding the molecular function and regulation of PA phosphatase since the discovery of its gene in 2006. The reader is directed to several recent review articles that summarize our current understanding of the regulation of expression and mode of action of this important enzyme in lipid metabolism (Carman and Han, 2006; Reue and Brindley, 2008; Carman and Han, 2009; Reue and Dwyer, 2009; Siniossoglou, 2009; Csaki and Reue, 2010). In closing, I recommend that you have plenty of freezer space, don't throw away any tubes of purified enzyme, and keep old laboratory notebooks handy.

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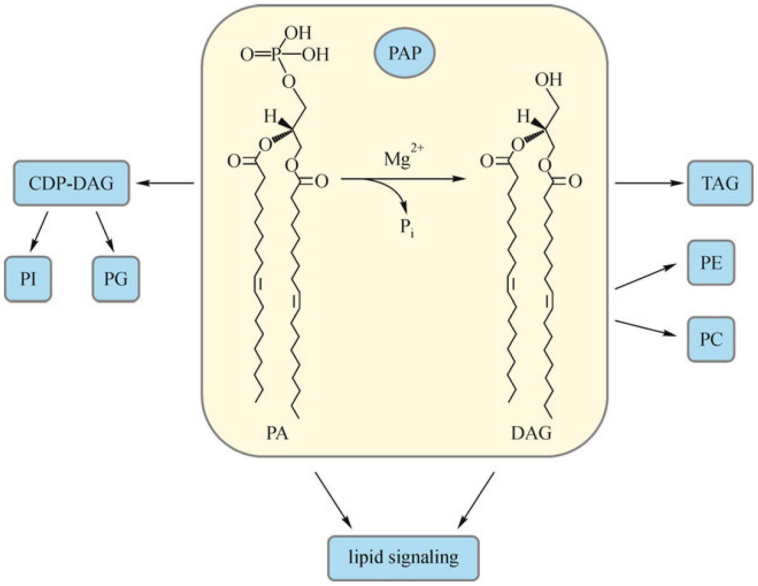

Figure 1.

The PA phosphatase reaction and its roles in lipid metabolism. The reaction catalyzed by PA phosphatase (highlighted in yellow) is shown. The reaction product diacylglycerol (DAG) is used for the synthesis of triacylglycerol (TAG) and the synthesis of phosphatidylethanolamine (PE) or phosphatidylcholine (PC). The reaction substrate PA is used for the synthesis of phosphatidylinositol (PI) or phosphatidylglycerol (PG) via CDP-diacylglycerol (CDP-DAG). The substrate and product of the PA phosphatase reaction also play roles in lipid signaling.

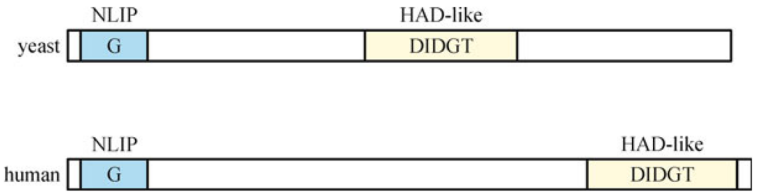

Figure 2.
Domain structures of the yeast and human PAP enzymes. The diagram shows the positions of the conserved NLIP (*blue*) and HAD-like (*yellow*) domains in yeast PAP and human lipin 1. These domains are conserved in lipin 2 and lipin 3 (not shown). A conserved glycine residue within the NLIP domain and conserved aspartate residues in the catalytic sequence DIDGT within the HAD-like domain are essential for PAP activity.

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