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Phosphatidate phosphatase regulates membrane phospholipid synthesis via phosphatidylserine synthase

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ABSTRACT

The yeast *Saccharomyces cerevisiae* serves as a model eukaryote to elucidate the regulation of lipid metabolism. In exponentially growing yeast, a diverse set of membrane lipids are synthesized from the precursor phosphatidate via the liponucleotide intermediate CDP-diacylglycerol. As cells exhaust nutrients and progress into the stationary phase, phosphatidate is channeled via diacylglycerol to the synthesis of triacylglycerol. The *CHO1*-encoded phosphatidylserine synthase, which catalyzes the committed step in membrane phospholipid synthesis via CDP-diacylglycerol, and the *PAH1*-encoded phosphatidate phosphatase, which catalyzes the committed step in triacylglycerol synthesis are regulated throughout cell growth by genetic and biochemical mechanisms to control the balanced synthesis of membrane phospholipids and triacylglycerol. The loss of phosphatidate phosphatase activity (e.g., *pah1*Δ mutation) increases the level of phosphatidate and its conversion to membrane phospholipids by inducing Cho1 expression and phosphatidylserine synthase activity. The regulation of the *CHO1* expression is mediated through the inositol-sensitive upstream activation sequence (UAS_{INO}), a *cis*-acting element for the phosphatidate-controlled Henry (Ino2–Ino4/Opi1) regulatory circuit. Consequently, phosphatidate phosphatase activity regulates phospholipid synthesis through the transcriptional regulation of the phosphatidylserine synthase enzyme.

Contents

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1. Introduction

The yeast *Saccharomyces cerevisiae*[1] is used as a model eukaryotic organism to elucidate the metabolism, cell biology, and regulation of glycerolipids. The strong homology of yeast proteins, pathways, and regulatory networks with those of higher eukaryotes has provided numerous insights into the genetics and biochemistry of lipid-related diseases (Henry et al., 2012; Kohlwein, 2010; Kurat et al., 2006; Natter and Kohlwein, 2013). The synthesis of lipids is a dynamic process that yeast cells engage in throughout their growth (Carman and Han, 2011; Chang and Carman, 2008; Henry et al., 2012). In exponentially growing yeast, a diverse set of membrane phospholipids (e.g., phosphatidylserine and its derivatives phosphatidylethanolamine and phosphatidylcholine, phosphatidylinositol and its derivative phosphoinositides and sphingolipids, and phosphatidylglycerophosphate and its derivatives phosphatidylglycerol and cardiolipin) are synthesized from the precursor phosphatidate via the liponucleotide intermediate CDP-diacylglycerol (Henry et al., 2012). As the cells exhaust nutrients and progress into the stationary phase (e.g., quiescence), phosphatidate is channeled to the synthesis of triacylglycerol via its dephosphorylation to diacylglycerol (Hosaka and Yamashita, 1984; Pascual et al., 2013; Taylor and Parks, 1979). Upon growth resumption with fresh medium, the stored triacylglycerol is mobilized to diacylglycerol and free fatty acid for the synthesis of phosphatidate and its conversion to membrane phospholipids (Carman and Han, 2011; Fakas et al., 2011a; Gaspar et al., 2011; Henry et al., 2012; Kurat et al., 2009; Rajakumari et al., 2008). This review focuses on the *PAH1*-encoded phosphatidate phosphatase (PAP)[2] (EC 3.1.3.4) and the *CHO1*-encoded phosphatidylserine synthase (PSS)[3] (EC 2.7.8.8), which are highly regulated to control the synthesis of triacylglycerol and membrane phospholipids during cell growth.

PAP catalyzes the Mg^{2+}-dependent dephosphorylation of phosphatidate to produce diacylglycerol (Han et al., 2006; Lin and Carman, 1989) (Fig. 1A), whereas PSS catalyzes the Mn^{2+}-dependent formation of phosphatidylserine by displacing the CMP moiety from CDP-diacylglycerol with serine (Bae-Lee and Carman, 1984; Kiyono et al., 1987; Letts et al., 1983; Nikawa et al., 1987) (Fig. 2A). PAP activity is governed by a conserved DXDX(T/V) catalytic motif within its haloacid dehalogenase-like domain (Han et al., 2006, 2007; Koonin and Tatusov, 1994; Madera et al., 2004; Péterfy et al., 2001) (Fig. 1B), whereas PSS activity is governed by a conserved CDP-alcohol phosphotransferase motif $DGX_2ARX_{7,8}GX_3DX_3D$ within a larger domain common to other phospholipid biosynthetic enzymes that catalyze similar types of reactions (Williams and McMaster, 1998) (Fig. 2B). For catalytic function *in vivo*, both PSS and PAP associate with the membrane to access their phospholipid substrates. PSS is an integral membrane enzyme in the endoplasmic reticulum (Habeler et al., 2002; Huh et al., 2003; Kumar et al., 2002; Natter et al., 2005), whereas PAP as a peripheral membrane enzyme that translocates from the cytosol to the nuclear/endoplasmic reticulum membrane (Barbosa et al., 2015; Karanasios et al., 2010, 2013).

The PAP reaction is the committed step for the synthesis of the neutral lipid triacylglycerol, whereas the PSS reaction is the committed step in the CDP-diacylglycerol pathway for the *de novo* synthesis of the major membrane phospholipids phosphatidylcholine and phosphatidylethanolamine (Carman and Han, 2011; Henry et al., 2012) (Fig. 3). The diacylglycerol produced from the PAP reaction can also be used for the synthesis of phosphatidylcholine and phosphatidylethanolamine, respectively, in the CDP-choline and CDP-ethanolamine branches of the Kennedy pathway when supplemented with choline or ethanolamine, and this biosynthetic pathway becomes essential for the cells defective in PSS and other enzymes in the CDP-diacylglycerol pathway of phospholipid synthesis (Carman and Han, 2011; Henry et al., 2012) (Fig. 3). The PAP and PSS reactions have a close relationship with phosphatidate in that it is the substrate of PAP and the precursor of the CDP-diacylglycerol used as the substrate of PSS (Fig. 3). The partitioning of phosphatidate between diacylglycerol and CDP-diacylglycerol is a major regulatory step that bifurcates lipid synthesis into branches that lead to triacylglycerol and membrane phospholipids (Fakas et al., 2011b; Han et al., 2006; Pascual et al., 2013) (Fig. 3). The PAP enzyme plays a major role in this metabolism by exerting a negative regulatory effect on the level of phosphatidate used for the *de novo* synthesis of membrane phospholipids. The regulations of the PAP and PSS enzymes and their connections in controlling lipid synthesis are discussed below.

2. Importance of PAP and PSS in lipid metabolism and cell physiology

The analyses of yeast mutants lacking PAP and PSS have shed light on the importance of the enzymes in lipid metabolism and cell physiology. The *pah1*Δ mutant exhibits increased levels of the PAP substrate phosphatidate, but decreased levels of the enzyme product diacylglycerol and its derivative triacylglycerol (Fakas et al., 2011b; Han et al., 2006; Han et al., 2007). The *pah1*Δ mutation results in a variety of phenotypes that include the induction of phospholipid synthesis genes, the increase of phospholipid synthesis, the expansion of the nuclear/endoplasmic reticulum membrane, the susceptibility to fatty acid-

[1] In this review, *Saccharomyces cerevisiae* is used interchangeably with yeast.

[2] The PAP orthologs in various organisms are known by different acronyms that are based on the names of genes that encode the enzyme. For example, in *S. cerevisiae*, the protein product of the *PAH1* gene is known as Pah1 (Han et al., 2006), whereas in human and mouse, the protein products of the *LPIN1* and *Lpin1* genes, respectively, are known as lipin 1 (Péterfy et al., 2001). The PAP encoded by *PAH1* differs from the lipid phosphate phosphatase enzymes encoded by *APP1* (Chae et al., 2012; Chae and Carman, 2013), *DPP1* (Toke et al., 1998) and *LPP1* (Toke et al., 1999), which dephosphorylate a broad spectrum of substrates (e.g., phosphatidate, lysophosphatidate, diacylglycerol pyrophosphate) and are not involved in *de novo* lipid synthesis.

[3] The *S. cerevisiae* PSS should differs from the PSS from Gram-negative bacteria (e.g., *Escherichia coli*), which catalyzes its CDP-DAG-dependent reaction via a metal cofactor-independent ping-pong reaction mechanism (Larson and Dowhan, 1976) or the PSS enzyme from mammalian cells, which catalyzes an exchange reaction between phosphatidylcholine or phosphatidylethanolamine with serine (Vance, 1998).

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