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Phospholipases play multiple cellular roles including growth, stress tolerance, sexual development, and virulence in fungi

Ananya Barman, Dibakar Gohain, Utpal Bora, Ranjan Tamuli*

Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati 781 039, Assam, India

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ABSTRACT

Phospholipases are ubiquitous enzymes that hydrolyze phospholipids. Based on the cleavage site of the ester linkage in the substrate phospholipids, phospholipases are classified into four major types, phospholipase A (PLA), phospholipase B (PLB), phospholipase C (PLC), and phospholipase D (PLD), which are further classified into various subtypes. Phospholipases hydrolyze phospholipids into various signaling products including phosphatidic acid (PA), diacylglycerol (DAG), free fatty acids (FFAs), and lyso-phospholipids (LPLs). These signaling products regulate numerous processes such as cytoskeletal dynamics, growth, homeostasis, membrane remodeling, nutrient acquisition, secretion, signal transduction, stress tolerance, sexual development, and virulence in various organisms including fungi. Due to these key cellular roles, phospholipases are also promising targets in diagnostic and therapeutic applications. In this review, we discuss current knowledge about the cellular roles of different classes of phospholipases in fungi.

1. Introduction

Lipids are essential constituents of living cells, and a class of complex lipids called phospholipids serves as the building blocks of almost all cellular membranes by forming the lipid bilayer and maintains cell shape and integrity (Bollag, 2016). Phospholipids are also important cellular intermediates that play multiple roles in cell development, metabolism, and signaling (Hong et al., 2016). The composition and content of phospholipids vary among different membranes, and typically changes during development and due to different stress cues (Hong et al., 2016). Similar to most eukaryotes, fungi also possess phospholipid based plasma membrane and intracellular organelles. The most abundant classes of phospholipids found in fungi includes phosphatidylcholine (PC) and phosphatidylethanolamine (PE), while phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), diphosphatidylglycerol (cardiolipin), and lyso-derivatives of PC and PE are found only in minor amounts (Dembitskii and Pechenkina, 1991). The hydrolysis of the phospholipids by the enzyme phospholipase yields fatty acids and a number of lipophilic molecules such as diacylglycerol (DAG), free fatty acids (FFAs), phosphatidic acid (PA), and lyso-phospholipids (LPLs) that are involved in signaling (Köhler et al., 2006; Hong et al., 2016). Phospholipases are classified into four major types phospholipase A (PLA_1 and PLA_2), phospholipase B (PLB), phospholipase C (PLC), and phospholipase D (PLD) on the basis of cleavage of ester linkage within a phospholipid molecule (Fig. 1). The PLA and PLB belongs to acyl hydrolase class of phospholipids, while PLC and PLD are members of the phosphodiesterase class of phospholipids (Richmond and Smith, 2011). In this review, we discuss about various cellular roles of the phospholipase superfamily members in fungi.

2. Phospholipase A (PLA)

The PLA superfamily of enzymes catalyze the hydrolysis of membrane phospholipids into FFAs and other lipid soluble molecules. The majority of the eukaryotic PLAs consists of the Gly-Xaa-Ser-Xaa-Gly (Xaa represents any amino acid) motif in its active site with catalytic Ser at the center, a catalytic triad of Ser-Asp-His residues, and a number of disulfide bonded Cys residues necessary for the stability of these enzymes. Based on their site of cleavage, PLA enzyme family can be classified into two types, phospholipase A_1 (PLA_1) cleaves the ester bond of glycerophospholipids at the *sn-1* position and produces FFAs and 2-acyl-LPL, while phospholipase A_2 (PLA_2) cleaves at the *sn-2* ester linkage and liberates FFAs and 1-acyl-LPL (Fig. 2 a, b; Ghannoum, 2000; Arioka et al., 2005; Köhler et al., 2006). FFAs and LPLs in turn regulate various biological functions in different organisms (Cavazzini et al., 2013).

2.1. Phospholipase A_1 (PLA_1)

PLA_1 activity has been studied in a wide range of tissues and cells of

* Corresponding author.
E-mail address: ranjantamuli@iitg.ernet.in (R. Tamuli).

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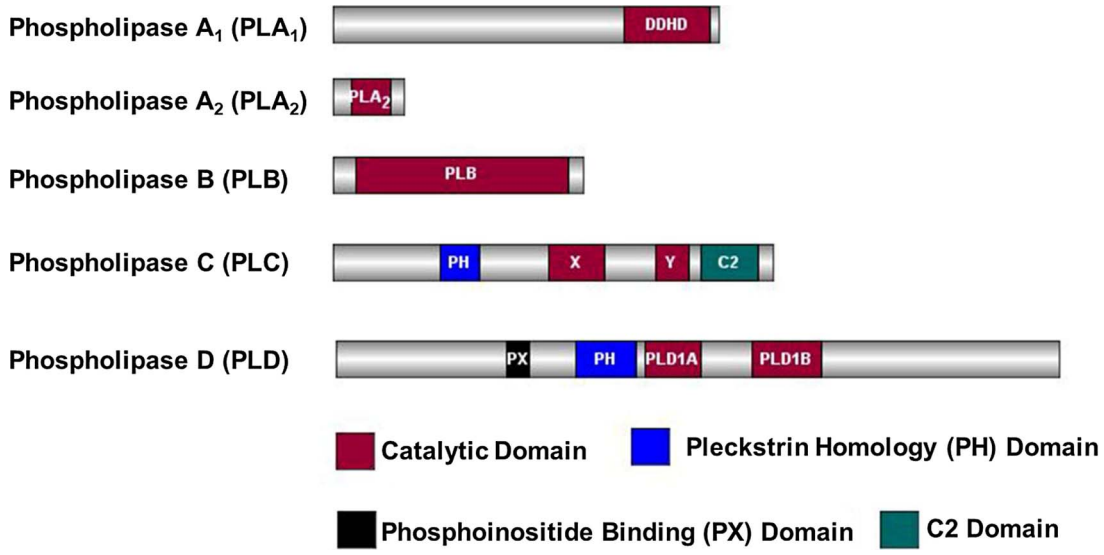

Fig. 1. General domain organization of the fungal phospholipase enzymes PLA_1, PLA_2, PLB, PLC, and PLD. The domains are indicated using the software IBS Illustrator (Liu et al., 2015), and described in the text. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

different organisms, particularly in plants and mammals, where they are known to play an important role in various cellular functions (Richmond and Smith, 2011). However, in fungi, PLA_1 activity is reported only in the filamentous fungus *Aspergillus oryzae* that is used widely in fermentation industry for the production of bean paste, miso, soy sauce, alcoholic beverages such as sake, vinegar, and for the production of industrially important lipase enzyme (Machida et al., 2005). The *A. oryzae* PLA_1 is comprised of 295 amino acid residues, consisting of a signal sequence of 26 hydrophobic residues at the N-terminus, two potential N-glycosylation sites, four Cys residues at positions 22, 35, 40, and 268 for at least two potential disulfide bonds, and a consensus pentapeptide sequence Gly-His-Ser-Xaa-Gly, typically present in the catalytic center of lipases (Watanabe et al., 1999). The *A. oryzae* PLA_1 shows 47% sequence homology to mono-and diacylglycerol lipases of commercially important fungus *Penicillium camembertii* (Watanabe et al., 1999). The *A. oryzae* PLA_1 enzyme catalyses acyl group removal from position 1 of lecithin and forms lyso-lecithin, an important surfactant used in food industries, animal feeds, cosmetics, and pharmaceuticals (Watanabe et al., 1999; Shiba et al., 2001). Recombinant expression of PLA_1 in *A. oryzae* and *Saccharomyces cerevisiae* has also been optimized on an industrial scale for the increased enzyme production (Shiba et al., 2001).

2.2. Phospholipase A_2 (PLA_2)

PLA_2 enzyme, first identified and purified from cobra and rattlesnake venom and later from the mammalian pancreas, hydrolyzes membrane phospholipid on the *sn-2* position releasing FFFs and LPLs (Dennis et al., 2011; Balsinde et al., 2002; Diaz and Arm, 2003). Based on the primary structure, enzymatic and subcellular localization properties, the PLA_2 superfamily can be broadly divided into calcium (Ca^{2+}) dependent cytosolic PLA_2 ($cPLA_2$), Ca^{2+} dependent low molecular weight secretory PLA_2 ($sPLA_2$), and Ca^{2+} independent intracellular PLA_2 ($iPLA_2$) subfamilies (Diaz and Arm, 2003). The subfamily of PLA_2 enzymes play significant roles in membrane homeostasis, signal transduction, and virulence (Valentín-Berríos et al., 2009).

2.2.1. Cytosolic phospholipase A_2 ($cPLA_2$)

The Ca^{2+} dependent $cPLA_2$ catalyzes the hydrolysis of the ester linkage at the *sn-2* position of membrane glycerophospholipids resulting in production of FFAs and 1-acyl-LPL (Ghannoum, 2000). Besides hydrolysis, $cPLA_2$ also exhibits high lyso-phospholipase and weak transacylase activities (Diaz and Arm, 2003). An increase in intracellular concentrations of Ca^{2+} ($[Ca^{2+}]_i$ or $[Ca^{2+}]c$) facilitates specific subcellular targeting of mammalian PLA_2 (Glover et al., 1995; Schievella et al., 1995; Hirabayashi et al., 2004; Evans et al., 2001). The $cPLA_2$ is activated in response to an increase in intracellular Ca^{2+} or phosphorylation by mitogen-activated kinase (MAPK), and translocate from cytosol to membranes to release linoleic acid (LA) or arachidonic acid (AA) in plant and mammalian cells, respectively (Lin et al., 1993; Lautens et al., 1998; Schievella et al., 1995; Senda et al., 1996). Mammalian and fungal $cPLA_2$ enzymes possess a novel catalytic center comprising of essential elements like Ser, Arg, and Asp that form a catalytic triad essential for the enzyme activity but without the characteristic multiple Cys residues (Pickard et al., 1996; Diaz and Arm, 2003). The $cPLA_2$ proteins have been mainly studied in mammals, however, their microbial homologues have been far less characterized and reported only in a few fungal species as summarized below.

2.2.1.1. $cPLA_2$ is crucial for morphogenetic transitions and membrane function. *A. nidulans*, one of the lesser known pathogen of the *Aspergilli* group and a model filamentous fungus widely used for studying eukaryotic cell biology, possesses a phospholipid-hydrolyzing novel $cPLA_2$ protein PlaA (Galagan et al., 2005; Hong et al., 2005). The *A. nidulans* PlaA shows maximum similarity to mammalian-type $cPLA_2$ proteins (α, β, and γ) (Hong et al., 2005). Similar to the human $cPLA_2$ isoforms, the *A. nidulans* PlaA also consists of two separate catalytic domains A and B, and possesses conserved residues (Gly-Gly-Gly-Xaa-Arg and Gly-Xaa-Ser-Gly-Ser motifs) in domain A that is essential for enzymatic activity (Hong et al., 2005). In addition, amino acid sequence of PlaA comprises of a putative N-terminal myristoylation site (Gly-Ser-Phe-His-Ser-Ser) similar to human $cPLA_2\gamma$ protein with possible regulatory functions, and a Ser residue corresponding to the regulatory Ser in human $cPLA_2\alpha$ protein that is essential for phosphorylation by MAPK (Hong et al., 2005; Köhler et al., 2006). Although PlaA requires Ca^{2+} for its catalytic activity and displays Ca^{2+} dependent hydrolytic activity towards PE and PC, however, it lacks the Ca^{2+} dependent lipid-binding domain (CaLB) like the human $cPLA_2$ (α, β) type-protein (Hong et al., 2005; Köhler et al., 2006). PlaA is expressed maximally in the presence of glucose and lactose as the carbon source, and constitutively in the developmental period of *A. nidulans* (Hong et al., 2005). The *A. nidulans* genome possesses two isoforms of *PlaA*, $PlaA_1$ and $PlaA_2$, the null mutant of $PlaA_1$ generated by deletion of the specific lipase motif