Plant Physiology and Biochemistry 49 (2011) 357–362

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Short communication

Arabidopsis thaliana lipid phosphate phosphatase 2 is involved in abscisic acid signalling in leaves

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ARTICLE INFO

Article history:
Received 25 October 2010
Accepted 4 January 2011
Available online 13 January 2011

Keywords:
Abscisic acid
Arabidopsis
Diacylglycerol kinase
Lipid phosphate phosphatase
Phosphatidic acid
Phosphatidic acid kinase
Stomatal movements

ABSTRACT

Lipid phosphate phosphatases (LPPs, E.C. 3.1.3.4) catalyse the dephosphorylation of diacylglycerol pyrophosphate (DGPP) and phosphatidic acid (PA), which are secondary messengers in abscisic acid (ABA) signalling. In this study, we investigated the effect of ABA on the expression of *AtLPP* genes as they encode putative ABA-signalling partners. We observed that *AtLPP2* expression was down-regulated by ABA and we performed experiments on *Atlpp2-2*, an *AtLPP2* knockout mutant, to determine whether *AtLPP2* was involved in ABA signalling. We observed that *Atlpp2-2* plantlets contained about twice as much PA as the wild-type Col-0 and exhibited higher PA kinase (PAK) activity than Col-0 plants. In addition, we showed that ABA stimulated diacylglycerol kinase (DGK) activity independently of AtLPP2 activity but that the ABA-stimulation of PAK activity recorded in Col-0 was dependent on *AtLPP2*. In order to evaluate the involvement of AtLPP2 activity in guard cell function, we measured the ABA sensitivity of *Atlpp2-2* stomata. The inhibition of stomatal opening was less sensitive to ABA in *Atlpp2-2* than in Col-0. Watered and water-stressed plants of the two genotypes accumulated ABA to the same extent, thus leading us to consider *Atlpp2-2* an ABA-signalling mutant. Taken together our observations show that *AtLPP2* is a part of ABA signalling and participate to the regulation of stomatal movements.

1. Introduction

The phytohormone abscisic acid (ABA) participates in the whole plant life cycle from seed development to flowering [1]. In addition, under unfavourable environmental conditions, ABA accumulation induces stomatal closure in the leaf epidermis and modifies the expression of numerous genes in different tissues [2]; [3]. ABA-signalling pathways are complex. ABA, perceived by several receptors, induces the phosphorylation and dephosphorylation of many target proteins, relaying the signal via multiple second messengers [1,4–7]. Among these ABA response messengers are lipid molecules including phosphatidic acid (PA) and diacylglycerol pyrophosphate (DGPP). For example, ABA treatment induces a transient increase in PA and DGPP in guard cells, cells in suspension, aleurone layer cells and seeds [8–11]. In *Arabidopsis thaliana* cells in suspension, addition of DGPP triggers the expression of ABA-specific genes [12], and application of PA or DGPP counteracts the GA-stimulated α−amylase secretion in barley seeds [11]. In Arabidopsis guard cells, PA can bind to ABI1 thus inhibiting its protein phosphatase activity [13]. In addition, genetic studies have strengthened the tenet that PA is involved in ABA signalling. Antisense phospholipase Dα (*PLDα*) and knockout *PLDα1* constructs were used to show that the activity of PLDα participates in ABA-promoted stomatal movements [13–15]. In barley seeds, recent data have indicated that after ABA application the rise in PA and DGPP levels depends on diacylglycerol kinase (DGK) and phosphatidic acid kinase (PAK) activities, respectively [11]. Besides enzymes that synthesise PA and DGPP, enzymes that degrade them are also important because they can potentially attenuate the biological effects of these lipid mediators on signalling pathways. Phosphatidate phosphatases (PAPs, E.C. 3.1.3.4) dephosphorylate PA producing DAG and inorganic

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Abbreviations: ABA, abscisic acid; DAG, diacylglycerol; DGK, diacylglycerol kinase; DGPP, diacylglycerol pyrophosphate; DMSO, dimethyl sulfoxyde; P_i, inorganic phosphate; LPP, lipid phosphate phosphatase; MS, Murashige and Skoog; PA, phosphatidic acid; PAK, phosphatidic acid kinase; PAP, phosphatidate phosphatase; TFA, trifluoroacetic acid; TLC, thin layer chromatography.

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doi:10.1016/j.plaphy.2011.01.010

phosphate (P_i). Two types of PAP enzymes are distinguished according to the Mg^{2+} requirement for catalytic activity. Conventional PAPs, named PAP1, are Mg^{2+} dependent whereas PAP2, also generally named lipid phosphate phosphatases (LPPs), are Mg^{2+} independent [16]. PAP2/LPPs regulate not only the level of PA, but also the level of DGPP because they can remove the β phosphate from DGPP to form PA and P_i [17,18]. In *A. thaliana*, five PAP1 were identified from cyanobacterial orthologs, among them, three were shown to be located in the chloroplast where they may be essential for galactolipid synthesis [19]. Based on the deduced protein sequence similarities with yeast *LPPs*, four genes encoding PAP2/LPPs were identified in *A. thaliana* [10,20]. AtLPP2 was shown to act in ABA inhibition of seed germination thus confirming the role of PA and DGPP in ABA signalling [10]. However, little is known about the role of AtLPP2 in phospholipids formation under ABA treatment.

In this study, we observed that the expression of *AtLPP2* is down-regulated by ABA. We also show that *Atlpp2-2* mutant, which is deficient in *AtLPP2* mRNA, has a higher level of PA and a higher level in PAK activity than the wild-type Col-0. Stomata of *Atlpp2-2* mutant are less sensitive to the ABA-induced inhibition of their opening than Col-0. Consequently, we suggest that AtLPP2 is part of the ABA-signalling network regulating some aspects of leaf physiology.

2. Results

2.1. Regulation of AtLPP gene expression by ABA

Several data indicate that among ABA-signalling proteins, some of them are encoded by genes that are transcriptionally regulated by ABA [1]. Therefore, we investigated the effect of ABA on the expression of *AtLPP* genes as they encode putative ABA-signalling partners. Semi-quantitative analysis of *AtLPP* genes showed that their level of expression in leaves of wild-type plants treated for 3 h with ABA was differentially modified according to the gene considered. Indeed, the level of expression of *AtLPP1* was not modified in Col-0, but the expression of *AtLPP2* decreased and the expression of *AtLPP3* increased in leaves (Fig. 1). *AtLPP4* mRNA was not detected in leaves (Fig. 1). These results suggest that AtLPP2 and AtLPP3 could play a role in ABA response. Here, we choose to study the involvement of AtLPP2 by the means of the mutant *Atlpp2-2* that contains a T-DNA insertion in the *AtLPP2* gene.

2.2. Atlpp2-2 accumulates more PA than Col-0 and has a higher PAK activity

First, we verified by RT-PCR that the mutant *Atlpp2-2* did not express *AtLPP2* mRNA so it can be considered as an RNA-null or knockout (Supplementary Fig. S1). Then, we measured the endogenous level of PA in *Atlpp2-2* in comparison to Col-0 plants. Leaves of *Atlpp2-2* mutant had twice more PA (8.6 ± 1.4 nmol g^{-1} FW) than Col-0 (4.7 ± 1.1 nmol g^{-1} FW; Fig. 2A). Thus, the disruption of the *AtLPP2* gene modifies the level of PA accumulation in leaf. In the mutant lacking PAP2 activity, the level of PA is the result of the equilibrium between PLD and DGK activity that produces it and the PAK activity that uses it. Here, we focus our study on the measurement of the activity of these lipid kinases. In microsomal extracts, DGK and PAK activities were almost twice higher in *Atlpp2-2* than in Col-0 plants (Fig. 2B). The activities of DGK and PAK were 15 ± 9.5 pmol min^{-1} mg^{-1} of protein and 18 ± 0.3 pmol min^{-1} mg^{-1} of protein in Col-0 and 33 ± 7.5 pmol min^{-1} mg^{-1} of protein and 45 ± 9.3 pmol min^{-1} mg^{-1} of protein in *Atlpp2-2*, respectively. This suggests that PAP2 activity originating from *AtLPP2* participates to the regulation of the activity of DGK and PAK.

2.3. Effect of ABA on DGK and PAK activities

We measured the microsomal lipid kinase activities in Col-0 and *Atlpp2-2* plants submitted to a 5–60 min ABA treatment. In

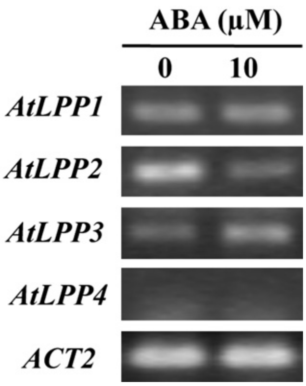

Fig. 1. The expression of *AtLPP2* and *AtLPP3* is regulated by ABA. Semi-quantitative RT-PCR analysis of *AtLPP1*, *AtLPP2* and *AtLPP3* expression in Col-0 leaves treated for 3 h with 10 µM ABA. *ACT2* was used as a control. Data are representative of 3 independent experiments.

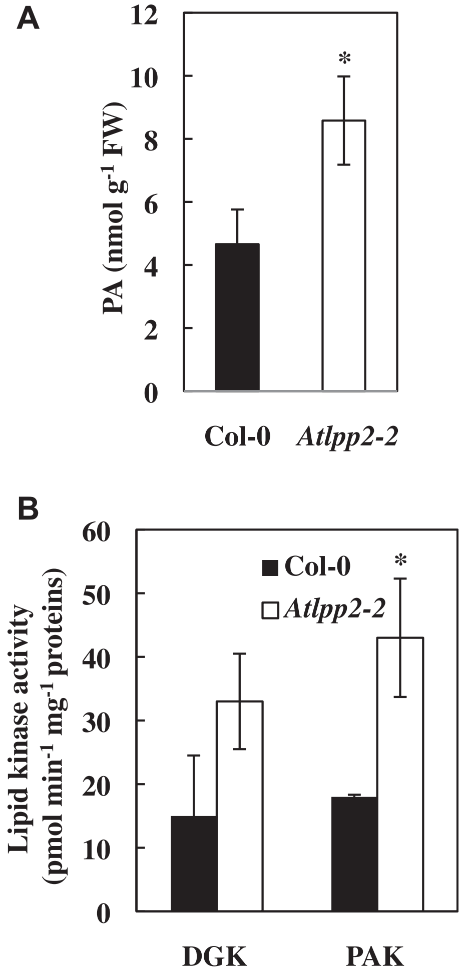

Fig. 2. PA content and lipid kinase activities in *Atlpp2-2*. (A) PA content in Col-0 and *Atlpp2-2* leaves. (B) DGK and PAK activities in Col-0 and *Atlpp2-2* plants. Lipid kinase activities were determined from membrane fractions extracted from 15-d-old plants. Membrane aliquots were incubated for 4 min at 30 °C with 370 MBq ^{32}P-ATP, then lipids were extracted, separated by TLC, scraped off from the plates and quantified by scintillation counting. In (A) and (B): black bars, Col-0; white bars, *Atlpp2-2*. Data are means ± SD, $n = 3$ and were compared using Student's *t test*. Asterisks indicate that the mean value is significantly different from that of the control ($* = P < 0.05$).