Phospholipidase Dδ Negatively Regulates the Function of Resistance to Pseudomonas syringae pv. Maculicola 1 (RPM1)

Xin Yuan, Zhangying Wang, Jianzhong Huang, Hua Xuan and Zhiyong Gao*

State Key Laboratory of Hybrid Rice, Key Laboratory for Research and Utilization of Heterosis in Indica Rice of Ministry of Agriculture, College of Life Sciences, Wuhan University, Wuhan, China

RPM1 is a plant immune receptor that specially recognizes pathogen-released effectors to activate effector-triggered immunity (ETI) in Arabidopsis thaliana. RPM1 triggers ETI and hypersensitive response (HR) for disease resistance. Previous reports indicated that Phospholipase D (PLD) positively regulated RPM1-mediated HR. However, single, double, and triple pld knock-out mutants of 12 members of the PLD family in A. thaliana did not show suppressed RPM1-mediated HR, indicating the functional redundancy among PLD members. In this study, we revealed that PLD could negatively regulate the function of RPM1. We found that RPM1 interacted with PLDδ, but did not interact with PLDβ1, PLDβ2, and PLDγ3. Overexpression of PLDδ conducted to a reduction of protein level and corresponding activity of RPM1. We found that abscisic acid (ABA) reduced the protein level of RPM1, and the ABA-induced RPM1 reduction required PLD activity and PLD-derived phosphatidic acid (PA). Our study shows that PLD plays both negative and positive roles regulating the protein level and activity of RPM1 during stress responses in plants. PLD proteins are regulating points to integrate the abiotic and biotic responses of plants.

Keywords: phospholipase D, RPM1, ABA, HR, plant immunity

INTRODUCTION

Plants develop innate immunity systems to confront pathogenic invasions (Jones and Dangl, 2006). Plants have immunity receptors to recognize pathogen-associated molecular patterns (PAMPs), and stimulate PAMP triggered immunity (PTI) (Couto and Zipfel, 2016). Pathogens evolve effectors to suppress plant PTI, and plants evolve corresponding receptors to recognize effectors, and stimulate effector triggered immunity (ETI) (Cui et al., 2015). Most of the ETI receptors are characterized with a nucleotide-binding domain (NB) and a leucine-rich-repeat domain (LRR), and these NLRs stimulate strong and rapid defense responses, named hypersensitive responses (HR), and results in cell deaths at the infection sites (Baggs et al., 2017).

RPM1 is an NLR receptor in Arabidopsis thaliana (Grant et al., 1995). It does not directly bind its corresponding effector AvrRpm1 or AvrB, but is activated by perceiving the effector-induced phosphorylation of the guarded protein RIN4 (Mackey et al., 2002; Chung et al., 2011; Liu et al., 2011). RPM1 is a plasma-membrane associated protein, and it does not have any transmembrane domain (Boyés et al., 1998; Gao et al., 2011). RIN4, a plasma-membrane localized protein, can stabilize RPM1, and only trace amounts of RPM1 are detected in the rin4 knockout plant.
Yuan et al.

PLD Negatively Regulates the Function of RPM1

Materials and Methods

Plant Growth and Mutants

Arabidopsis thaliana and Nicotiana benthamiana plants were grown in pots with autoclaved vermiculite and watered with Hoagland solution. The growth condition is at 24°C under a 16 h light/8 h dark cycle. A. thaliana plants for HR and disease resistance assays were grown under 8 h light/16 h dark cycle condition. All the lines of A. thaliana are in Columbia-0 background. The pldδ mutant 12B (SALK_023247C) were obtained from ABRC. The mutant line rpm1-3 and the transgenic line pRPM1::RPM1-Myc/rpm1-3 (AT5) were gifts from Dr. Jeff. Dangl (University of North Carolina at Chapel Hill, Chapel Hill, NC, United States).

Vector Construction

The gateway system was used to construct vectors. For transient expression, The CDSs of AtPLD genes were cloned into the expression vector pEarleyGate 101 containing the constitutive high-expression CaMV 35S promoter, and YFP-HA tag (Karimi et al., 2007). HA tag was used for protein detection. RPM1-Myc was cloned into the pGW72 vector to obtain the 35S::RPM1-Myc expression construct (Nakagawa et al., 2007). RPM1(D505V)-Myc was cloned into the pMDC7 vector under the control of the estradiol-inducible promoter to obtain the Est::RPM1(D505V)-Myc expression construct (Karimi et al., 2007). For the bimolecular fluorescence complementation (BiFC) assay, we modified the pEarleyGate 101 vector into vectors that could express the two complementary parts of YFP, the N terminus (nYFP) and the C terminus (cYFP) (Walter et al., 2004; Citovsky et al., 2006). The expression constructs of 35S:: RPM1 -nYFP-HA and 35S:: pLDδ-cYFP-HA were made respectively.

Transient Expression in the Leaves of N. benthamiana

The expression constructs were electro-transformed into Agrobacterium tumefaciens GV3101. Agrobacteria were cultured overnight at 28°C with suitable antibiotics. Overnight-grown Agrobacteria were centrifuged and re-suspended in the induction buffer (10 mM MES pH 5.6, 10 mM MgCl2, 150 μM acetosyringone). Samples were infiltrated into the leaves of 5-week-old N. benthamiana with a 1 mL needless syringe at desired OD600 nm values. Agrobacteria containing the P19 plasmid was always co-infiltrated with the samples at an OD600 nm of 0.2. The transiently expressed proteins were extracted and analyzed at 48 h after infiltration (Pitino et al., 2016).

Protein Extraction and Co-immunoprecipitation (Co-IP) Assay

For protein expression, three leaf disks (7 mm diameter) were ground with 110 μL of the protein extraction buffer (20 mM Tris-HCl PH 8.0, 5 mM EDTA, 10 mM DTT, 1% SDS). The
supernatants were collected with centrifugation at 8,000 × g for 3 min and mixed with fourfold protein loading buffer. Actin was used as an internal protein loading control. For the fractioning experiments, leaf tissues were ground in a mortar with the extraction buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl, 20% glycerol, 1 mM DTT, 10 mM EDTA, 1 mM PMSF, protease inhibitor cocktail). Samples were centrifuged at 6,000 × g for 10 min at 4°C to remove the debris. The supernatant containing total proteins (T) was separated into the cytosolic fraction (S) and the microsomal fraction (M) with centrifugation at 20,000 × g for 60 min at 4°C. The plasma-membrane localized H⁺-ATPase was used as the microsomal marker. The big subunit of Rubisco was stained with Ponceau S as the cytosolic marker.

For Co-IP experiment, because PLDδ and RPM1 are membrane associated proteins, the microsomal fractions were used for Co-IP. The protein content of RPM1-Myc in the microsomal fractions were determined with Western blots, and the microsomal fractions were re-suspended with the suspension buffer (40 mM Tris-HCl PH 7.5, 150 mM NaCl, 1 mM DTT, 5 mM EDTA, protease inhibitor cocktail, 1% Triton X-100) to equalize RPM1-Myc protein levels between the control and test samples. The samples were rotated at 4°C for 1 h and centrifuged at 20,000 × g for 1 h to remove the insoluble proteins. 30 μL of the anti-GFP conjugated agarose beads (MBL, #D153-8) were added into 1 mL of the supernatants and incubated at 4°C for 3 h. Beads were washed three times with the suspension buffer. Immunoprecipitates were eluted with 50 μL of the elution buffer (2% SDS) at 37°C for 5 min.

The primary antibodies used for Western blots were the anti-Myc (GeneScript, #A00704), the anti-HA (Roche, #11867423001), the anti-HA (GeneScript, #A00704), the anti-HA (Roche, #11867423001), the anti-HA (Roche, #11867423001), and the anti-T7 (Novagen, #69522).

Confocal Microscopy

The nYFP-tagged proteins and the cYFP-tagged proteins were co-expressed in the leaves of N. benthamiana. The fluorescent images were observed using confocal microscopy according to the BiFC protocol (Walter et al., 2004). YFP was excited at 488 nm and fluorescent emissions were observed at 518–540 nm.

### TABLE 1 | Phospholipase D (PLD) genes and their protein expressions in N. benthamiana.

| PLD genes | Detectable expression in N. benthamiana | Detectable interaction with RPM1 |
|-----------|----------------------------------------|----------------------------------|
| PLDδ (AT4G35790) | Yes | Yes |
| PLDδ2 (AT1G52570) | Yes | Yes |
| PLDδ1 (AT2G42010) | Yes | No |
| PLDδ2 (AT4G00240) | Yes | No |
| PLDδ3 (AT4G11840) | Yes | No |
| PLDδ1 (AT3G15730) | No | N/A |
| PLDδ3 (AT5G25870) | No | N/A |
| PLDδ (AT1G55180) | No | N/A |
| PLDδ1 (AT3G16785) | No | N/A |
| PLDδ2 (AT4G06630) | No | N/A |
| PLDδ1 (AT4G11850) | No | N/A |
| PLDδ2 (AT4G11830) | No | N/A |

### FIGURE 1 | PLDδ interacts with RPM1. (A) The interaction of PLDδ and RPM1 in Nicotiana benthamiana. 3SS::PLDδ-YFP-HA and 3SS::RPM1-Myc were co-expressed in the leaves of N. benthamiana. Total membrane proteins were extracted from samples collected at 2 days after infiltration. PLDδ-YFP-HA was immunoprecipitated with agarose beads conjugated with anti-GFP antibody, and RPM1-Myc was detected for Co-IP. Co-expression of 3SS::YFP-HA and 3SS::RPM1-Myc was used as the negative control. (B) Representative confocal images of BiFC. BiFC analysis was performed in N. benthamiana. PLDδ was fused with the C-terminal portion of YFP (cYFP), and RPM1 was fused to the N-terminal portion of YFP (nYFP). Different plasmids were co-expressed in N. benthamiana. Images were pictured at 2.5 days after infiltration. All the experiments were repeated three times with similar results. (C) The interaction of PLDδ and RPM1 in Arabidopsis thaliana. Co-IP assay was performed with stable transgenic plants containing 3SS::PLDδ-YFP-HA and pRPM1::RPM1-Myc. The transgenic plants containing pRPM1::RPM1-Myc was used as the negative control.
RESULTS

RPM1 Interacts With PLDδ in planta

Phospholipase D mediates the signal transduction of RPM1 (Andersson et al., 2006). We cloned and transiently expressed all the members of AtPLD family in the leaves of N. benthamiana to determine their interactions with RPM1. Of the 12 PLD members, PLDδ, PLDα2, PLDβ1, PLDβ2, and PLDγ3 had detectable protein expression, and were used for Co-IP assay. We co-expressed 35S::PLD-YFP-HA and 35S::RPM1-Myc in the leaves of N. benthamiana, and immunoprecipitated PLD-YFP-HA with the anti-GFP antibody to determine the Co-IP of RPM1-Myc. Co-expression of 35S::YFP-HA and 35S::RPM1-Myc was used as the negative control. The Co-IP assay showed that PLDα2 and PLDδ interacted with RPM1, while PLDβ1, PLDβ2, and PLDγ3 did not (Table 1). PLDδ was used for subsequent experiments due to stronger interaction with RPM1 (Figure 1A). We validated the interaction with the BiFC assay. The Yellow fluorescence protein (YFP) was split into two complementary parts, the N terminus (nYFP) and the C terminus (cYFP) (Walter et al., 2004; Citovsky et al., 2006). The expression constructs 35S:: RPM-nYFP-HA and 35S:: PLDδ-cYFP-HA were co-expressed in the leaves of N. benthamiana. The results showed clear yellow fluorescence in the samples co-expressing RPM1-nYFP-HA and PLDδ-cYFP-HA, but no fluorescence was detected in the negative controls (Figure 1B). We transformed 35S::PLDδ-YFP-HA into the pRPM1::RPM1-Myc/rpm1-3 plant (AT5) to determine the interaction of RPM1 and PLDδ in A. thaliana. The Co-IP of RPM1-Myc with PLDδ-YFP-HA in the transgenic plants indicated the interaction of RPM1 and PLDδ in A. thaliana (Figure 1C).
PLDδ Suppresses RPM1(D505V)-Induced Cell Death in N. benthamiana

Since PLDδ interacted with RPM1, we tested whether PLDδ affected the function of RPM1. We firstly determined whether PLDδ could affect RPM1-induced HR. RPM1(D505V), which mimics the active state of RPM1, is sufficient to induce the cell death (Gao et al., 2011). The estradiol inducible Est::RPM1(D505V)-Myc construct was co-expressed with 35S::PLDδ-YFP-HA or 35S::YFP-HA in the leaves of N. benthamiana to compare the occurrences of the cell death induced by RPM1(D505V). The cell death could be stained with trypan blue or be quantified with ion-leakage assay. Results showed that PLDδ-YFP-HA could obviously suppress RPM1(D505V) induced cell death (Figures 2A,B).

We hypothesized that PLDδ either suppressed the activation of RPM1, or reduced the protein level of RPM1 to affect its function.

PLDδ Reduces the Protein Level of RPM1(D505V) in N. benthamiana

We tested whether PLDδ could affect the protein level of RPM1(D505V) in the leaves of N. benthamiana. RPM1(D505V) is unstable due to the cell death induced by the protein, and it is hard to accumulate detectable protein level. Lanthanum chloride (LaCl₃) can inhibit RPM-mediated HR, and prevent the degradation of the activated RPM1 or RPM1(D505V) (Grant et al., 2000; El Kasmi et al., 2017). We co-expressed Est::RPM1(D505V)-Myc and 35S::PLDδ-YFP-HA or 35S::YFP-HA in the leaves of N. benthamiana, and induced the expression of RPM1(D505V) with estradiol at 2 days after infiltration. The leaves were treated with 4 mM of LaCl₃ at the same time of the induction. The protein levels of RPM1(D505V) were detected at 3, 5, and 8 h after induction. Our results showed that PLDδ-YFP-HA obviously reduced the protein level of RPM1(D505V) compared with the negative control YFP-HA (Figure 3A).

RPM1(D505V) is a plasma-membrane associated protein. We collected the samples at 5 h after induction, and separated total proteins into the soluble and the microsomal membrane fractions.
using ultracentrifugation. Certain amount of RPM1(D505V) participated in the soluble fraction of the sample co-expressed with PLDδ, while less amount of RPM1(D505V) participated in the soluble fraction of the sample co-expressed with the negative control YFP-HA (Figure 3B).

PLDδ Activity Is Required to Negatively Regulate the Function of RPM1(D505V) in N. benthamiana

PLDδ is an oleate and phosphatidylinositol 4,5-bisphosphate (PIP2) activated enzyme. PLDδ(R410D) and PLDδ(R622P) are two activity-deficient mutants due to loss oleate binding or PIP2 stimulated activity respectively (Wang and Wang, 2001). We made PLDδ(R410D/R622P) double mutant to determine whether PLD activity was required to affect the function of RPM1(D505V). Contrast to PLDδ, the activity deficient mutant did not affect the autoactivity and the protein level of RPM1(D505V) (Figures 4A,B).

Overexpression of PLDδ Negatively Affects the Function of RPM1 in A. thaliana

We transformed 35S::PLDδ-YFP-HA into AT5 plant to determine the effects of PLDδ on RPM1 in A. thaliana. Three independent transgenic lines PLD-8, PLD-9, and PLD-21 were used to assay the protein level of RPM1. The results showed that the protein levels of RPM1 were lower in the PLDδ transgenic plants (Figure 5A). According to our assay, PLDγ3 did not interact with RPM1 in N. benthamiana. We transformed 35S::PLDγ3-YFP-HA into AT5 plant to determine the protein levels of RPM1 in three independent PLDγ3-OE plants. The results showed that the protein levels of RPM1 in the transgenic plants were the same as that in AT5 (Figure 5B). The results suggested that the interaction of PLD with RPM1 was necessary to reduce the protein level of RPM1.

Since the protein level of RPM1 reduced in the PLDδ-OE plants, we reasoned that the function of RPM1 should be deficient in the PLDδ-OE plants. We compared the functions of RPM1 in PLD-8 and PLD-9 with those in AT5 plant. The loss-of-function mutant rpm1-3 was used as a negative control, and the pldδ-KO mutant (SALK_023247C) was included for the assays. RPM1 can be activated by its corresponding effector AvrB, and thus restrict the growth of the avirulent pathogen Pto DC3000(avrB). We spray-inoculated the leaves of the plants with Pto DC3000(avrB), and counted bacteria numbers at 0 and 3 days after inoculation. The result indicated that PLD-8 and PLD-9 plants displayed obviously less disease resistance than AT5 (Figures 6A,B). The pldδ-KO mutant displayed the same bacteria growth restriction as AT5, which was consistent with previous report. We quantified...
the RPM1-mediated HR by measuring the ion-leakage of the plants. The ion-leakage data showed that the PLD-8 and PLD-9 had weaker HR than AT5 (Figure 6C). Based on above results, we concluded that overexpressing of PLDδ reduced the protein level of RPM1, and thus negatively affected the function of RPM1.

**ABA-Induced PLD Activity Reduces the Protein Level of RPM1**

Because PLD is enzyme, we want to determine whether PLD activity is required to affect the protein level of RPM1. ABA was able to stimulate the activity of PLD (Jacob et al., 1999), so we treated the leaves of AT5 with or without 100 μM of ABA, and compared the protein levels of RPM1 between the samples. The protein levels of RPM1 assayed at 3 h after treatments, and the results showed that ABA treatment obviously reduced the protein level of RPM1 (Figure 7A). Since PLD activity leads to the synthesis of PA, we treated the plants with n-butanol to block the synthesis of PA and thus the PLD enzymatic function. When the plants were treated with both n-butanol and ABA at the same time, ABA-induced RPM1 reduction was abolished (Figure 7B). The results indicated that PLD activity and its derived PA were required to mediate the ABA-induced RPM1 reduction.

**DISCUSSION**

We revealed that PLD could negatively regulate the function of RPM1 (Figure 6), but this did not conflict with previous finding that PLD positively regulated RPM1-mediated HR. As an immune receptor, RPM1 has pro-signaling inactive state and active state. RPM1 is activated by its cognate effectors. We detected the protein levels of RPM1 in PLDδ-OE plants and ABA-treated plants without the inoculation of pathogenic bacteria. The results reflect the protein levels of the inactive RPM1. Since the gene expression of RPM1 is not induced by pathogens, the protein level of the inactive pro-signaling RPM1 determines the output of the RPM1-initiated signaling (Adams-Phillips et al., 2008). PLD negatively regulates the function of RPM1 by reducing the protein level of the pro-signaling inactive RPM1. Previous report indicated that PLD played a positive role on the function of RPM1 (Andersson et al., 2006). PLD activity and its derived PA are downstream components in the RPM1 signal transduction pathway, and their positive roles are effective after the activation of RPM1. PLD activity induced by RPM1 activation may also negatively regulate the protein level and function of RPM1, but active RPM1 is quickly degraded accompanying with the HR response (Boyes et al., 1998), indicating the potential negative regulation of active RPM1 by PLD activity is not necessary.

The exact mechanism on how PLD reduces the protein level of RPM1 is not clear. Overexpression of PLDδ but not PLDγ3, which did not interact with RPM1, reduced the protein level of RPM1 in A. thaliana, suggesting the PLD-RPM1 interaction is required (Figure 5). Our study indicates that PLD activity is also required to reduce RPM1 level. We found that the protein level of RPM1 obviously reduced at 3 hours after ABA treatment, and PLD activity is required for ABA-induced RPM1 reduction in Arabidopsis (Figure 7). In addition, PLDδ(R410D/R622P), an activity-deficient mutant, could not reduce the protein level of RPM1(D505V) in N. benthamiana further demonstrated the necessity of PLD activity to reduce the protein level of RPM1 (Figure 4).

Transiently expressed PLD resulted in the partial distribution of RPM1(D505V) to the soluble fraction of plant cells, suggesting that PLD could interfere the plasma-membrane location of RPM1 (Figure 3B). RPM1 does not have transmembrane domains. The isoelectric point of RPM1 is 8.51, and it should be positively charged at physiological conditions. The potential interaction of the positively charged RPM1 with the negatively charged phospholipids may facilitate the membrane location of RPM1. PLD can hydrolyze PC and PE to PA which still is negative charged. It seemed that PLD activity would not affect RPM1 location. However, PA can be further dephosphorylated by phosphatidic acid phosphohydrolase (PAP) to diacylglycerol (DAG) (Becker and Hannun, 2005). Therefore, PLD activity should result in the net increase of DAG and decrease of PC and PE. Because DAG does not contain negatively charged phosphate group, RPM1 is released from the membrane and degraded in the cytosol of the cells. It would be interested to determine the binding of RPM1 with phospholipids such as PE and PC, and compare the lipid components between PLDδ OE plants and the wild type or plants treated with and without ABA treatment (Wang and Wang, 2001; Andersson et al., 2006). PLDδ reduced the protein level of RPM1(D505V), a mimic mutant of the active RPM1, in N. benthamiana, but PLDδ did not obviously reduce the protein level of RPM1 in N. benthamiana. The different effects of PLDδ on RPM1(D505V) and RPM1 reflect the conclusion that PLDδ activity is required to reduce the protein level of RPM1. RPM1-Myc itself is not active in N. benthamiana. Overexpression...
of PLDδ does not definitely lead to PLD activity, especially the transiently expressed PLDδ only accumulated two days. RPM1(D505V) is autoactive, and can stimulate PLD activity. Therefore, PLDδ obviously reduces the protein level of RPM1(D505V) in N. benthamiana. The reason that overexpression of PLDδ reduced the protein level of RPM1 in Arabidopsis (Figure 5A) is possibly due to the long-term effects of PLDδ during the time its activity can be stimulated by environmental and physiological factors (Li et al., 2009).

PLD mediates the cross-talking between ABA signaling and RPM1-mediated disease resistance. We found that ABA treatment could reduce the protein level of RPM1 in A. thaliana. ABA is a phytohormone responding to the drought stress, while RPM1 is an immune receptor for disease resistance. ABA signaling activates PLD, and PLD activity regulates the protein level of RPM1 and its function. Therefore, PLD could be an integrating point to balance the plant responses to the complex environmental stimuli. Other abiotic stresses such as cold, heat, and ROS can also affect PLD activity (Li et al., 2009; Guo et al., 2012; Zhang et al., 2017). It has been reported that temperature affects RPM1 function (Wang et al., 2009). PLD activity may mediate this correlation. Although we clearly determined that PLDδ could negatively regulate the protein level of RPM1, we haven’t tested all the members of the PLD family yet. Since each PLD member has specific and overlapping functions, further research about the effects of PLD members with RPM1 and other immune receptors will help to understand how PLD proteins integrate environmental and physiological responses with disease resistances.

AUTHOR CONTRIBUTIONS

XY and ZG designed the experiments, analyzed the data, and wrote the manuscript. XY, ZW, JH, and HX conducted the experiments.

FUNDING

This study was supported by the National Key Research and Development Program of China (Grant No. 2016YFD0100600) and the National Natural Science Foundation of China (Grant No. 31270315) to ZG.

ACKNOWLEDGMENTS

We thank Dr. Jeff Dangl (University of North Carolina) for providing A. thaliana seeds, Dr. Yingtang Lu (Wuhan University) for providing pathogen bacteria. We also thank Dr. Yunkuan Liang (Wuhan University) for critical reading.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2018.01991/full#supplementary-material

REFERENCES

Adams-Phillips, L., Wan, J., Tan, X., Dunning, F. M., Meyers, B. C., Michelmore, R. W., et al. (2008). Discovery of ADP-ribosylation and other plant defense pathway elements through expression profiling of four different Arabidopsis-Pseudomonas R-avr interactions. Mol. Plant Microbe Interact. 21, 646–657. doi: 10.1094/mpmi-21-5-0646

Andersson, M. X., Kourchtenko, O., Dangl, J. L., Mackey, D., and Ellerstrom, M. (2006). Phospholipase-dependent signalling during the AvrRpm1- and AvrRpt2-induced disease resistance responses in Arabidopsis thaliana. Plant J. 47, 947–959. doi: 10.1111/j.1365-313X.2006.02844.x

Baggs, E., Dagdas, G., and Krasileva, K. V. (2017). NLR diversity, helpers and integrated domains: making sense of the NLR IDentity. Curr. Opin. Plant Biol. 38, 59–67. doi: 10.1016/j.pbi.2017.04.012

Bargmann, B. O., and Munnik, T. (2006). The role of phospholipase D in plant stress responses. Curr. Opin. Plant Biol. 9, 515–522. doi: 10.1016/j.pbi.2006.07.011

Becker, K. P., and Hannun, Y. A. (2005). Protein kinase C and phospholipase D: intimate interactions in intracellular signalling. Cell. Mol. Life Sci. 62, 1448–1461. doi: 10.1007/s00018-005-4531-7

Ben Othman, A., Ellouzi, H., Planchais, S., De Vos, D., Faiyue, B., Carol, P., et al. (2017). Phospholipases Dzeta1 and Dzeta2 have distinct roles in growth and antioxidant systems in Arabidopsis thaliana responding to salt stress. Planta 246, 721–735. doi: 10.1007/s00425-017-2728-2

Boyés, D. C., Nam, J., and Dangl, J. L. (1998). The Arabidopsis thaliana RPM1 disease resistance gene product is a peripheral plasma membrane protein that is degraded coincident with the hypersensitive response. Proc. Natl. Acad. Sci. U.S.A. 95, 15849–15854. doi: 10.1073/pnas.95.26.15849

Chung, E. H., da Cunha, L., Wu, A. J., Gao, Z., Cherkis, K., Afzal, A. J., et al. (2011). Specific threonine phosphorylation of a host target by two unrelated type III effectors activates a host innate immune receptor in plants. Cell Host Microbe 9, 125–136. doi: 10.1016/j.chom.2011.01.009

Citoovsky, V., Lee, I. Y., Vyas, S., Glick, E., Chen, M. H., Vainstein, A., et al. (2006). Subcellular localization of interacting proteins by bimolecular fluorescence complementation in planta. J. Mol. Biol. 362, 1120–1131. doi: 10.1016/j.jmb.2006.08.017

Couto, D., and Zipfel, C. (2016). Regulation of pattern recognition receptor signalling in plants. Nat. Rev. Immunol. 16, 537–552. doi: 10.1038/nri.2016.77

Cui, H., Tsuda, K., and Parker, J. E. (2015). Effector-triggered immunity: from pathogen perception to robust defense. Annu. Rev. Plant Biol. 66, 487–511. doi: 10.1146/annurev-arplant-052113-040012

El Kasmi, F., Chung, E. H., Anderson, R. G., Li, J., Wan, L., Eitas, T. K., et al. (2017). Signaling from the plasma-membrane localized plant immune receptor RPM1 requires self-association of the full-length protein. Proc. Natl. Acad. Sci. U.S.A. 114, E7385–E7394. doi: 10.1073/pnas.1708288114

Ella, K. M., Meier, K. E., Kumar, A., Zhang, Y., and Meier, G. P. (1997). Utilization of alcohols by plant and mammalian phospholipase D. Biochem. Mol. Biol. Int. 41, 715–724. doi: 10.1080/15216549702001761

Gao, Z., Chung, E. H., Eitas, T. K., and Dangl, J. L. (2011). Plant intracellular innate immune receptor Resistance to Pseudomonas syringae pv. maculicola 1 (RPM1) is activated at, and functions on, the plasma membrane. Proc. Natl. Acad. Sci. U.S.A. 108, 7619–7624. doi: 10.1073/pnas.1104410108

Grant, M., Brown, L., Adams, S., Knight, M., Ainslie, A., and Mansfield, J. (2000). The RPM1 plant disease resistance gene facilitates a rapid and sustained increase in cytosolic calcium that is necessary for the oxidative burst and hypersensitive cell death. Plant J. 23, 441–450. doi: 10.1046/j.1365-313x.2000.00804.x

Grant, M. R., Godiard, L., Straube, E., Ashfield, T., Lewald, J., Sattler, A., et al. (1995). Structure of the Arabidopsis RPM1 gene enabling dual specificity disease resistance. Science 269, 843–846. doi: 10.1126/science.7638602
Yuan et al.

PLD Negatively Regulates the Function of RPM1

Guo, L., Devaiah, S. P., Narasimhan, R., Pan, X., Zhang, Y., Zhang, W., et al. (2012). Cytosolic glycerolaldehyde-3-phosphate dehydrogenases interact with phospholipase Dβ to transduce hydrogen peroxide signals in the Arabidopsis root under stress. Plant Cell 24, 2200–2212. doi: 10.1105/tpc.111.094946

Hong, Y., Devaiah, S. P., Bahn, S. C., Thamassandra, B. N., Li, M., Welti, R., et al. (2009). Phospholipase D epsilon and phosphatidic acid enhance Arabidopsis nitrogen signaling and growth. Plant J. 58, 376–387. doi: 10.1111/j.1365-313X.0.03788.x

Hong, Y., Pan, X., Welti, R., and Wang, X. (2008). Phospholipase Dalpha3 is involved in the hypersensitive response in Arabidopsis. Plant Cell 20, 803–816. doi: 10.1105/tpc.107.056390

Hubert, D. A., He, Y., McNulty, B. C., Tornero, P., and Dangl, J. L. (2009). Specific Arabidopsis HSP90.2 alleles recapitulate RAR1 co-chaperone function in plant NB-LRR disease resistance protein regulation. Proc. Natl. Acad. Sci. U.S.A. 106, 9556–9563. doi: 10.1073/pnas.0904877106

Jacob, T., Ritchie, S., Assmann, S. M., and Gilroy, S. (1999). Abscisic acid signal transduction in guard cells is mediated by phospholipase D activity. Proc. Natl. Acad. Sci. U.S.A. 96, 12192–12197. doi: 10.1073/pnas.96.21.12192

Johansson, O. N., Fahlberg, P., Karimi, E., Nilsson, A. K., Ellerstrom, M., and Andersson, M. X. (2009). Redundancy among phospholipid D isozymes in resistance triggered by recognition of the Pseudomonas syringae effector AvrRpm1 in Arabidopsis thaliana. Front. Plant Sci. 5:639. doi: 10.3389/fpls.2014.00639

Jones, J. D., and Dangl, J. L. (2006). The plant immune system. Nature 444, 323–329. doi: 10.1038/nature05286

Karimi, M., Depicker, A., and Hilson, P. (2007). Recombinatory cloning with plant gateway vectors. Plant Physiol. 145, 1144–1145. doi: 10.1104/pp.107.106989

Li, M., Hong, Y., and Wang, X. (2009). Phospholipase D- and phosphatidic acid-mediated signaling in plants. Biochim. Biophys. Acta 1791, 927–935. doi: 10.1016/j.bbabio.2009.02.017

Li, M., Qin, C., Welti, R., and Wang, X. (2006). Double knockouts of phospholipase Dα1 and Dα2 in Arabidopsis affect root elongation during phosphate-limited growth but do not affect root hair patterning. Plant Physiol. 140, 761–770. doi: 10.1104/pp.105.070995

Liu, J., Elmore, J. M., Lin, Z. J., and Coaker, G. (2011). A receptor-like cytoplasmic kinase phosphorylates the host target RIN4, leading to the activation of a plant innate immune receptor. Cell Host Microbe 9, 137–146. doi: 10.1016/j.chom.2011.01.010

Mackey, D., Holt, B. F. III, Wiig, A., and Dangl, J. L. (2002). RIN4 interacts with Pseudomonas syringae type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. Cell 108, 743–754. doi: 10.1016/S0092-8674(02)01061-X

Mishra, G., Zhang, W., Deng, F., Zhao, J., and Wang, X. (2006). A bifurcating pathway directs abscisic acid effects on stomatal closure and opening in Arabidopsis. Science 312, 264–266. doi: 10.1126/science.1123769

Nakagawa, T., Kurose, T., Hino, T., Tanaka, K., Kawamukai, M., Niwa, Y., et al. (2007). Development of series of gateway binary vectors, pGWBS, for realizing efficient construction of fusion genes for plant transformation. J. Biosci. Bioeng. 104, 34–41. doi: 10.1263/jb.104.34

Pinosa, F., Buiot, N., Kwaiataal, M., Fahlberg, P., Thordal-Christensen, H., Ellerstrom, M., et al. (2013). Arabidopsis phospholipase D delta is involved in basal defense and nonhost resistance to powdery mildew fungi. Plant Physiol. 163, 896–906. doi: 10.1104/pp.112.233503

Pitino, M., Armstrong, C. M., Cano, L. M., and Duan, Y. (2016). Transient expression of Candidatus liberibacter asiaticus effector induces cell death in Nicotiana benthamiana. Front. Plant Sci. 7:982. doi: 10.3389/fpls.2016.00982

Sang, Y., Zheng, S., Li, W., Huang, B., and Wang, X. (2001). Regulation of plant water loss by manipulating the expression of phospholipase Dalpha. Plant J. 28, 135–144. doi: 10.1046/j.1365-313X.2001.01138.x

Walter, M., Chaban, C., Schütze, K., Batistic, O., Weckermann, K., Nake, C., et al. (2004). Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation. Plant J. 40, 428–438. doi: 10.1111/j.1365-313X.2004.02219.x

Wang, C., and Wang, X. (2001). A novel phospholipase D of Arabidopsis that is activated by oleic acid and associated with the plasma membrane. Plant Physiol. 127, 1102–1112. doi: 10.1104/pp.010444

Wang, Y., Bao, Z., Zhu, Y., and Hua, J. (2009). Analysis of temperature modulation of plant defense against biotrophic microbes. Mol. Plant Microbe Interact. 22, 498–506. doi: 10.1094/mpmi-22-5-0498

Zhang, Q., Song, P., Qu, Y., Wang, P., Jia, Q., Guo, L., et al. (2017). Phospholipase D delta negatively regulates plant thermotolerance by destabilizing cortical microtubules in Arabidopsis. Plant Cell Environ. 40, 2220–2235. doi: 10.1111/pce.13023

Zhao, J., Devaiah, S. P., Wang, C., Li, M., Welti, R., and Wang, X. (2013). Arabidopsis phospholipase Dβ1 modulates defense responses to bacterial and fungal pathogens. New Phytol. 199, 228–240. doi: 10.1111/nph.12256

Zhao, J., Wang, C., Bedair, M., Welti, R., Sumner, L. W., Baxter, I., et al. (2011). Suppression of phospholipase Dγmases confered increased aluminum resistance in Arabidopsis thaliana. PLoS One 6:e28086. doi: 10.1371/journal.pone.0028086

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Yuan, Wang, Huang, Xuan and Gao. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.