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Distinct phosphorylation optimizes pathogen-induced PA and ROS bursts

Phospholipids hold a multifaceted function in cellular biology. The primary function of phospholipids is to serve as essential building blocks of cell membranes, which gives cells crucial structural support and facilitates cellular organization. Beyond that, phospholipids also act as key signaling molecules that coordinate vital cellular processes such as organismal development, homeostasis, and adaptation to environmental changes. Despite being the smallest and simplest phospholipid, phosphatidic acid (PA) plays a crucial role as a metabolic intermediate and second messenger. It regulates various cellular and physiological processes in a wide range of species, from microbes and plants to mammals (Zhou et al., 2023). PA has been extensively studied in plants, where it is primarily produced through enzymatic reactions (Yao and Xue, 2018). For instance, phospholipase D (PLD) catalyzes the hydrolysis of the P–O bond in structural lipids like phosphatidylcholine or phosphatidylethanolamine, yielding PA along with the remaining head group such as choline or ethanolamine. Additionally, PA can be generated through the phosphorylation of diacylglycerol by diacylglycerol kinase (DGK). In *Arabidopsis thaliana* plants, a total of 12 PLD and 7 DGK enzymes have been identified.

PA's versatility as a second messenger stems from its ability to regulate many proteins involved in various cellular processes, plant development, hormone signaling, and stress responses (Yao and Xue, 2018). For example, both PA and DGK5 have been shown to bind the abscisic acid (ABA) biosynthesis enzyme ABA2, leading to the suppression of its enzymatic activity and consequent reduction in ABA levels (Li et al., 2024). Additionally, PA interacts with the heterodimeric capping protein, which attaches to the barbed end of actin filaments and controls their polymerization. This binding inhibits the protein's ability to function, thereby facilitating actin reorganization and cytoskeletal dynamics necessary for plant adaptation to diverse environmental conditions (Huang et al., 2006).

In plants, PA is rapidly induced by various abiotic stresses such as submergence and osmotic stresses. Additionally, when plants detect microbe-associated molecular patterns (MAMPs) or pathogen effectors, there is a transient increase in PA levels (Kong et al., 2024). However, the mechanisms underlying this pathogen-induced burst of PA generation and its role in mediating plant immunity remain obscure. Two groundbreaking papers have recently revealed their exciting findings about DGK5, which is responsible for synthesizing PA from diacylglycerol (Kong et al., 2024; Qi et al., 2024). These studies demonstrate that DGK5 is both positively and negatively regulated by two distinct kinases through unique phosphorylation events leading to the transient spike in PA levels.

To protect themselves from pathogen infections, plants developed plasma-membrane-localized pattern-recognition receptors (PRRs) to sense the presence of pathogens by recognizing conserved molecules in microbes called MAMPs, triggering pattern-triggered immunity (PTI). On the other hand, plant intracellular nucleotide-binding leucine-rich repeat proteins detect pathogen effectors to turn on effector-triggered immunity (ETI) (Jones and Dangl, 2006). Recently, a group of scientists have demonstrated that these two branches of plant immunity rely on overlapping pathways and mutually potentiate each other (Figure 1) (Chang et al., 2022).

To gain insight into the PRR protein complex and how the PRR protein complex activates plant defense, Kong et al. first focused on identifying novel interactors of BOTRYTIS-INDUCED KINASE 1 (BIK1) (Kong et al., 2024). As a plasma-membrane-localized receptor-like cytoplasmic kinase, BIK1 not only associates with multiple PRRs but also phosphorylates RESPIRATORY BURST OXIDASE HOMOLOG D (RBOHD) (Kong et al., 2024). DGK5, which can phosphorylate diacylglycerol to produce PA, was identified as an interactor of BIK1 through several classical assays. Significantly, DGK5 was subsequently shown to play a pivotal role in regulating both PTI and ETI.

Intriguingly, upon immune activation, DGK5 was found to be phosphorylated by two different kinases at distinct residues, leading to opposite roles of DGK5 in PA production (Figure 1) (Kong et al., 2024). Specifically, BIK1 phosphorylates DGK5 at serine 506, leading to a rapid increase in PA levels and subsequent immune activation. This process is then suppressed by the PRR-associated cytoplasmic kinase MPK4 through its phosphorylation of DGK5 at threonine 446.

In line with the essential role of DGK5 in both PTI and ETI, PA generated by DGK5 is pivotal in orchestrating plant defense mechanisms associated with both pathways. Subsequently, the focus shifts to elucidating the mechanisms by which PA contributes to plant defense. Notably, previous studies have elucidated that PA derived from PLD binds to the NADPH oxidase RBOHD, thereby modulating ABA-induced reactive oxygen species (ROS) production (Zhang et al., 2009). This finding prompted Kong et al. to direct their focus toward RBOHD and ROS. To investigate the impact of DGK5-derived PA on RBOHD, Kong et al. speculated that PA contributes to the stabilization of RBOHD. This hypothesis was supported by the observation that *dgk5* mutants displayed impairments in flg22-induced ROS production (Kong et al., 2024).

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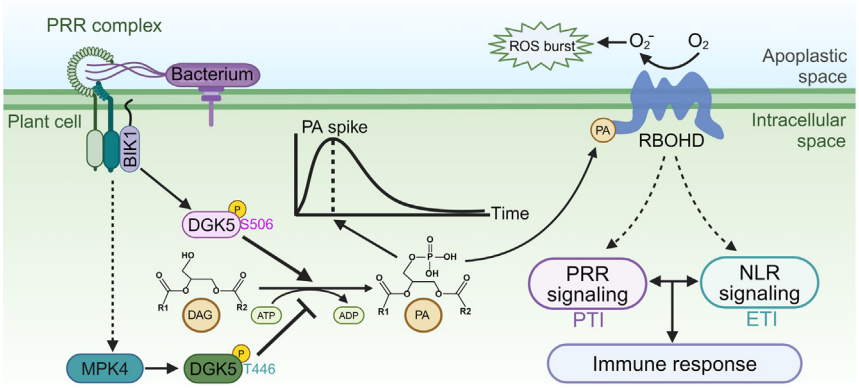

Figure 1. A schematic model illustrating BIK1- and MPK4-mediated distinct phosphorylation of DGK5 in the modulation of PA burst and plant immune response.

Upon the perception of pathogenic bacterial microbe-associated molecular patterns (MAMPs) by pattern-recognition receptors (PRRs), a signal transduction cascade is initiated, leading to the activation of BIK1. This event facilitates the subsequent phosphorylation on diacylglycerol kinase 5 (DGK5) at serine 506, effectively enhancing its enzymatic function essential for the phosphatidic acid (PA) burst. The resultant PA interacts with and stabilizes the NADPH oxidase RESPIRATORY BURST OXIDASE HOMOLOG D (RBOHD), boosting reactive oxygen species (ROS) production. Later, the MPK4 kinase specifically phosphorylates DGK5 at threonine 446. This post-translational modification serves to dampen the DGK activity of DGK5, which consequently decreases the synthesis of PA. Therefore, the distinct phosphorylation of DGK5 by PRR-activated BIK1 and MPK4 balances cellular PA burst, which plays a pivotal role in regulating ROS generation. This balanced modulation of PA and ROS bursts is crucial for coordinating PRR-mediated pattern-triggered immunity (PTI) and nucleotide-binding domain and leucine-rich repeat receptor (NLR)-promoted effector-triggered immunity (ETI), which synergistically reinforce each other to provide robust protection against pathogens. The figure was created with the software BioRender (BioRender.com).

Additionally, previous studies have reported an increase in protein levels of RBOHD following flg22 treatment, further supporting their hypothesis (Ngou et al., 2021; Yuan et al., 2021). Indeed, Kong et al. observed that flg22-induced RBOHD protein abundance was suppressed in *dgk5-1* mutants, while treatment with PA reinstated RBOHD abundance in *dgk5-1* mutants (Kong et al., 2024). Mechanistically, Kong et al. subsequently demonstrated that flg22-induced PA inhibits the ubiquitination of RBOHD, resulting in its stabilization (Figure 1).

The elicitor flg22 triggers the phosphorylation of DGK5 at serine 506 by BIK1, precipitating a surge in PA that fortifies the plant defense mechanisms against bacterial pathogens (Kong et al., 2024). In a parallel response, chitin activates RIPK, which similarly phosphorylates DGK5 at serine 506 and activates DGK5, catalyzing a PA burst that strengthens plant defenses against fungal pathogens (Qi et al., 2024). This concurrent phosphorylation raises a compelling question regarding the regulatory mechanisms by which plants modulate DGK5 phosphorylation to optimize and escalate their immune responses when faced with simultaneous bacterial and fungal infections.

DGK5, which contains a calmodulin-binding domain essential for its interaction with RBOHD and RIPK, catalyzes the synthesis of MAMP-induced PA (Qi et al., 2024), suggesting that calcium ion signaling may play a role in regulating immune responses mediated by PA in plants. Consequently, additional research is needed to determine the precise involvement of calcium ion and calmodulin in this process.

A key role of PA is to stabilize RBOHD by inhibiting its ubiquitination process (Figure 1). Previous studies have revealed that the receptor-like cytoplasmic kinase PBL13 phosphorylates the C terminus of RBOHD, facilitating its ubiquitination mediated by PBL13 interacting RING domain E3 ligase (PIRE) (Lee et al., 2020). It is plausible that PA binding to RBOHD impedes its interaction with PBL13 and/or PIRE. Conversely, PA binding to RBOHD may also enhance its interaction with a deubiquitinase enzyme, which catalyzes the removal of ubiquitin from target proteins, thereby decreasing the ubiquitination and subsequent degradation of RBOHD.

As an early plant defense signal, ROS act as a double-edged sword. While ROS keep plant pathogens under control, prolonged ROS production due to mis-regulations can also be harmful to plant growth. The dual phosphorylation allows plants to effectively induce a ROS burst to turn on plant defense while limiting the detrimental effect of ROS on plants themselves. Due to their inherent negative charge, phosphate groups induce a significant alteration in the charge of proteins upon phosphorylation. This change in charge subsequently influences the conformation of the protein, thereby impacting its functional activity. Structural studies could offer valuable insights into the contrasting effects observed when DGK5 is phosphorylated at serine 506 and threonine 446, shedding light on how these modifications influence DGK5's enzymatic activity in PA biosynthesis. PLD and DGK5 both possess the enzymatic capacity to synthesize PA. Confronted with a spectrum of environmental stressors, it is imperative to elucidate the regulatory mechanisms governing the plant's strategic selection of either PLD or DGK5 for PA biosynthesis as an adaptive response to fluctuating environmental conditions. It is well known that ROS scavenger enzymes, including superoxide dismutase, ascorbate peroxidase, and catalase, and non-enzymatic mechanisms, such as ascorbate, glutathione, carotenoids, prolines, flavonoids, and phenolics, are involved in the removal of ROS (Zandi and Schnug, 2022). It remains to be determined how these ROS elimination mechanisms coordinate with PLD, DGK5, RIPK, BIK1, and MPK4 to optimize pathogen-induced ROS burst.

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