Trends in Biochemical Sciences

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Forum

Fine-tuning phosphatidic acid production for optimal plant stress responses

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Phosphatidic acid (PA) is involved in biotic and abiotic stress responses in plants. Here, we summarize quantitative lipidomics and real-time imaging used in PA studies and highlight recent studies of diacylglycerol (DAG) kinase (DGK) 5, an enzyme involved in PA biosynthesis, facilitating fine-tuning PA production for optimal stress responses in plants.

PA metabolism

PA, the simplest phospholipid, is a component of biological membranes and acts as a second messenger in multiple signaling pathways [1]. PA production is mediated by multiple biochemical routes: it can be biosynthesized *de novo* through lysophosphatidic acid acyltransferases (LPAATs) in the glycerol 3-phosphate pathway and can be produced through the phosphorylation of DAG catalyzed by DGKs and the hydrolysis of phospholipids, such as phosphatidylcholine (PC), catalyzed by phospholipase Ds (PLDs). By contrast, PA can be converted back into DAG by specific PA phosphohydrolases (PAHs) and nonspecific lipid phosphate phosphatases (LPPs), and cytidine diphosphate DAG (CDP-DAG) by CDP-DAG synthases (CDSs), respectively. CDP-DAG is the precursor of multiple phospholipids, including phosphatidylinositol and its derivatives, phosphoinositides. In arabidopsis (*Arabidopsis thaliana*) leaf, PA accounts for approximately 2% of all phospholipids, phosphatidylserine (PS) about 1%, and all phosphoinositides less than 1% [2]. Despite the low abundance, PA plays an important role in plant stress signaling and responses, and its content fluctuates under various stress conditions [1].

Enabling technologies for PA studies

The cellular PA level is quite low, about 50–100 μM in arabidopsis plants [3], but it can be quickly produced or removed by its metabolic enzymes in various stress responses. Therefore, cellular PA functions dynamically. In other words, quantifying PA at cellular and subcellular levels is critical to elucidate PA's action in the plant cell. Single-cell lipidomics can be used to analyze diverse phospholipid species and their relative abundance in individual cells [4]. Single-cell lipidomics coupled with single-cell transcriptomics will be powerful in precisely dissecting genes involved in PA metabolism (Figure 1). Additionally, PA content varies in the different cellular organelles. Using subcellular lipidomics, PA content in subcellular compartments, like nuclei [1,5], can be analyzed.

Real-time detection and quantification of PA changes *in situ* are key to our mechanistic understanding and subsequent engineering of PA metabolism-related genes. Lipid biosensors containing specific lipid-binding domains are often used to measure the spatiotemporal dynamics of phospholipids *in vivo* [1,2]. The PA biosensors based on a PA-binding motif from the yeast SNARE protein Spo20p are the first and most widely used [6], and the newly ratiometric PA biosensor PAleon, containing a PA-binding domain sandwiched between the donor and acceptor of the fluorescence resonance energy transfer (FRET) system, can monitor PA concentration changes in living plant cells [3,7,8] (Figure 1).

PA and DGK5 in cellular stress responses

With advanced technologies, recent work demonstrates that PA dynamics mediated by DGKs and PLDs is important for responses to biotic and abiotic stresses. Reactive oxygen species (ROS) play a vital role in plant immunity against pathogen invasion, and PA production mediated by DGKs is associated with ROS signaling [9,10], but how PA levels are fined-tuned in plant immune responses remains elusive. Kong *et al.* recently uncovered one mechanism for regulation: PA binds to and stabilizes respiratory burst oxidase homolog D (RBOHD), a NADPH oxidase, decreasing its ubiquitination to promote ROS production in arabidopsis [8]. The study further showed that PA production from DAG on immune elicitation is regulated by the differential, dual phosphorylation of DGK5 by *Botrytis*-induced kinase 1 (BIK1) and mitogen-activated protein kinase 4 (MPK4) (Figure 2). BIK1 phosphorylates DGK5 at Ser^{506} and increases DGK5's activity, whereas MPK4 phosphorylates DGK5 at Thr^{446} and suppresses its activity. Additionally, they found that DGK5-mediated PA production is involved in both basal and effector-triggered immunity. In another study, Qi *et al.* observed that one of the transcripts of *DGK5*, *DGK5β*, instead of *DGK5α* that encodes proteins lacking the calmodulin-binding domain, mediates PA production under treatment with chitin, a fungus-associated molecular pattern [7]. DGK5β-generated PA also enhances RBOHD stability by suppressing its vacuolar degradation (Figure 2). Taking these findings together, DGK5-mediated PA production contributes to resistance to biotic stresses such as bacterial and fungal pathogens.

PA is also involved in plant responses to abiotic stress, including drought, salinity and freezing. The plant hormone abscisic acid (ABA) is crucial to plant abiotic stress responses, and ABA DEFICIENT2 (ABA2) is a key enzyme in the major ABA

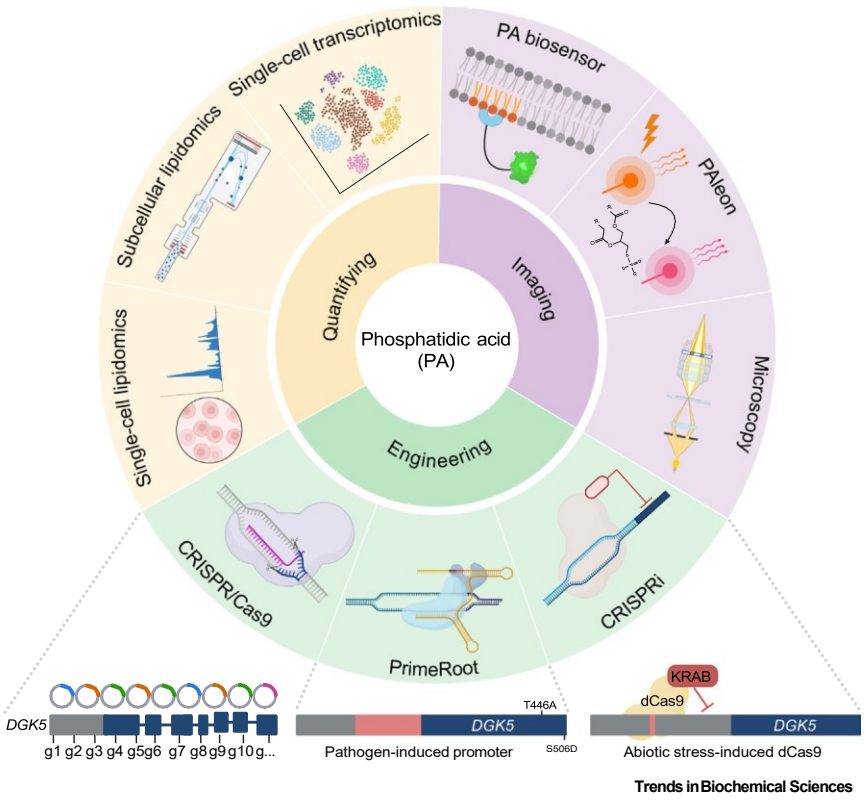

Figure 1. Technologies and tools that facilitate studies and fine-tuning of cellular phosphatidic acid (PA) production for optimal stress responses. Detection and quantification of PA *in situ* enables mechanistic studies of PA dynamics. Single-cell and subcellular lipidomics quantify PA content at the single-cell and organellar levels. Advances in lipid imaging enable us to observe cellular PA changes *in situ*. PA biosensors, based on the yeast SNARE protein Spo20p, are the first and most widely used PA sensors. The ratiometric biosensor PAleon, coupled with advanced microscopy techniques, facilitates the study of PA dynamics. Various genome-editing technologies are capable of genetic engineering of *DGK5* for fine-tuning PA production, facilitated by stress-inducible *cis*-elements identified by single-cell transcriptomics. CRISPR/Cas9 can be used to optimize the coding region and the promoter of *DGK5*. PrimeRoot enables precise insertions of large DNA fragments into plant genomes, and with PrimeRoot an optimized allele of *DGK5* can be engineered into plants. The CRISPR interference (CRISPRi) system, containing a catalytically dead Cas9 (dCas9) fused with the Krüppel-associated box (KRAB) repression domain, can be used to target key *cis*-elements in the promoter of *DGK5* to downregulate its expression. To achieve optimal plant stress responses, coordinated genetic engineering of *DGK5* is needed.

biosynthesis pathway. A new study shows that DGK5 binds to ABA2 and generates PA to suppress ABA2's activity as well as promote ABA2's nuclear sequestration [5] (Figure 2). The results indicate that DGK5-mediated lipid phosphorylation suppresses ABA biosynthesis and negatively regulates arabidopsis's response to abiotic stress, including drought and high salinity. It is also reported that the arabidopsis *dgk5* knockout mutant showed improved tolerance and decreased PA production in response to freezing stress [11].

Spatiotemporally fine-tuning PA production by engineering *DGK5* may yield optimal plant stress responses

Cellular PA dynamics is regulated by multiple enzymes encoded by many different genes [1]. Here, we focus on *DGK5* engineering for PA production to achieve optimal stress responses in plants. With detailed biochemical studies of the role of DGK5 in PA production and cellular stress responses [5,7,8], we learn that DGK5 is involved in a tradeoff in the response to abiotic and biotic stress conditions. A tradeoff between two traits is not uncommon in plants. Another example is the immunity–growth tradeoff, in which the energy balance tilting towards one causes loss in the other.

One of the strategies to break the tradeoff is to use genome editing to fine-tune gene function. For example, from a rice mutant collection, Sha *et al.* identified a mutant named *rbl1*, which shows robust immunity but a severe yield penalty, a typical immunity–growth tradeoff [12]. The causal gene *RBL1* encodes a PA-metabolizing enzyme converting PA into CDP-DAG. Through multiplexed genome editing of *RBL1*, an optimal allele named $RBL1^{\Delta 12}$, harboring a 12-bp deletion in the coding region, was obtained. $rbl1^{\Delta 12}$ shows broad-spectrum disease resistance without affecting the yield, breaking the immunity–growth tradeoff [12]. Similarly, we believe that targeted multiplexed mutagenesis mediated by genome editing can be applied to *DGK5* to fine-tune its function to achieve both abiotic and biotic stress resilience, or at least to achieve resilience to one stress without negatively affecting the other (Figure 1). More efficiently, we can use promoter editing in fine-tuning the expression of *DGK5*, the consequence of which can be more predictable, as *cis*-elements in the promoter and their effects on gene expression are readily predictable with advanced bioinformatic tools [13].

Plant stress responses need to be spatiotemporally regulated to achieve optimal outcomes. Stress-induced or -suppressed expression of *DGK5* is one such strategy. For example, we can use a pathogen-induced promoter to drive an engineered *DGK5* allele that mimics the BIK1-

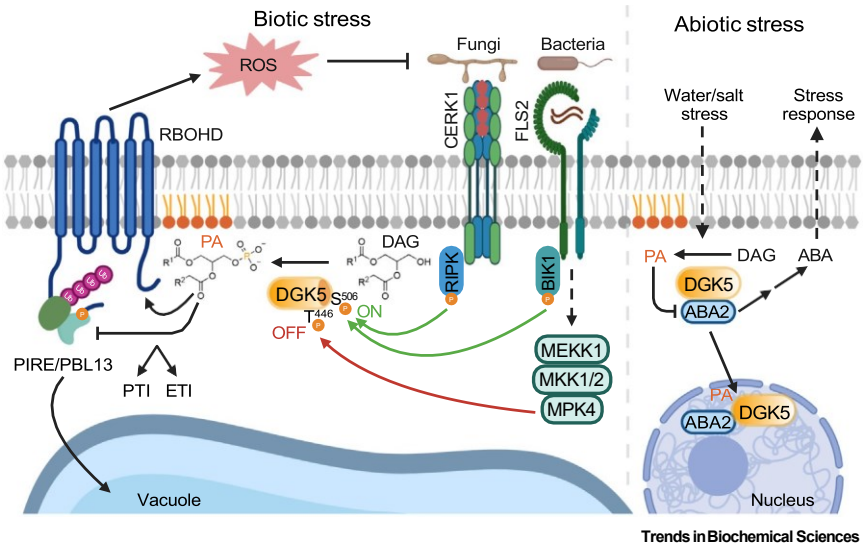

Figure 2. Diacylglycerol (DAG) kinase 5 (DGK5) and phosphatidic acid (PA) for abiotic and biotic stress responses. Under biotic stress, bacterial flg22 and fungal chitin are recognized by receptors FLAGELLIN SENSING 2 (FLS2) and chitin elicitor receptor kinase 1 (CERK1), respectively, which activate BOTRYTIS-INDUCED KINASE 1 (BIK1) and RPM1-induced protein kinase (RIPK). Phosphorylated BIK1 and RIPK activate DGK5 by phosphorylating Ser^{506} to produce PA, but phosphorylated mitogen-activated protein kinase 4 (MPK4) induced by bacterial infection suppresses DGK5 activity by phosphorylating Thr^{446}. The dual phosphorylation of DGK5 regulates PA homeostasis. PA binds to respiratory burst oxidase homolog D (RBOHD) (an NADPH oxidase), stabilizes RBOHD by suppressing AvrPphB susceptible 1-like 13 (PBL13)/PBL13 interacting RING domain E3 ubiquitin ligase (PIRE)-mediated vacuolar degradation of RBOHD, and increases its plasma membrane localization, promoting reactive oxygen species (ROS) production. The calmodulin-binding domain is key to DGK5's functions. Under abiotic stress, DGK5-mediated PA production inhibits the activity of abscisic acid (ABA) insensitive 2 (ABA2), a dehydrogenase in ABA biosynthesis, and decreases ABA production. Additionally, PA promotes the nuclear localization of ABA2, reducing ABA production. Abbreviations: ETI, effector-triggered immunity; MKK, MPK kinase; MEKK, MKK kinase; PTI, pattern-triggered immunity.

phosphorylated variant ($DGK5^{S506D}$) and alters the MPK4-phosphorylated residue ($DGK5^{T446A}$) to enhance plant immunity [8] (Figure 1). The above-optimized allele can be integrated into a genomic safe harbor site, enabled by the genome-editing technology named PrimeRoot that can precisely insert large DNA fragments (~11 kb) into genomes [14]. For abiotic stress responses, CRISPR interference (CRISPRi), containing a catalytically inactive dCas9 fused to a transcriptional repression domain, and CRISPR/Cas13a-mediated RNAi [15], are feasible approaches to downregulate *DGK5* expression via guide RNAs specifically binding to key *cis*-elements in the promoter or mRNAs of *DGK5*, and abiotic stress-induced expression of the systems is preferred (Figure 1). In summary, to optimally modulate the opposite roles of DGK5 in biotic and abiotic stress responses, orchestrated precise genetic engineering is needed.

Concluding remarks

The function of phospholipids is multifaceted and mutation of phospholipid metabolism-related genes often causes significant consequences, but the biochemical mechanisms of these resultant changes are difficult to dissect, as phospholipids are highly interconnected and multiple phospholipid metabolism pathways are entangled, as well as there being constant phospholipid replenishment from membrane trafficking [2]. However, with technical advances, three recent studies, together with others [1], showcase the exact role of the PA-producing gene *DGK5* in cellular abiotic and biotic stress responses. These advances facilitate the design of precise metabolic engineering to achieve optimal stress responses and stress-resilient crops.

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Declaration of interests

There are no interests to declare.

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