LIPID BIOSENSOR

# Lipids light up in plant membranes

Phosphatidic acid (PA) is a simple phospholipid of crucial importance in cell biology. Now, a new ratiometric, PA-specific, optogenetic biosensor has been developed to track PA concentration and dynamics at the plant plasma membrane. Using this tool, scientists have revealed a remarkable stress-specific temporal complexity of PA accumulation.

Adiilah Mamode Cassim and Sébastien Mongrand

Phosphatidic acid (PA) represents around 1% of all lipids. It predominantly accumulates at the plasma membrane (PM), but a significant pool is also located in the endoplasmic reticulum (ER) and in plastids. It is the precursor of most phospholipid and triglyceride synthesis, and also a key signalling lipid. PA signalling is at the crossroad of plant responses to a large variety of stimuli, ranging from hormones and biotic and abiotic stresses, to many other developmental and environmental cues. This involves abscisic acid (ABA)-mediated guard cell regulation and response against pathogens[1]. PA also plays a role in vesicular trafficking, secretion and endocytosis. It modulates the activity of effector proteins, such as kinases, phosphatases, phospholipases, transcription factors, NADPH oxidases and microtubule-associated proteins[2]. PA can be generated via different routes: through its main metabolic pathway by the sequential acylation of glycerol-3-phosphate and lyso-PA; through the hydrolytic cleavage mediated by a plethora of plant phospholipase D (PLD) isoforms[3]; through the sequential phosphorylation of diacylglycerol; and finally, through the dephosphorylation of diacylglycerolpyrophosphate.

Intriguingly, PA production can be triggered by opposite stimuli, such as cold and heat or ABA and salicylic acid (hormones that are considered as having antagonistic effects). This property highlights very paradoxical features of PA molecular species, questioning the specificity of the responses controlled by PA. It was proposed that PA regulatory messages are perceived as complex signatures that take into account the site of production, the availability of target proteins and cellular environments[2]. Until now, the study of the role of PA has been strongly impaired by the lack of biosensors efficiently localizing and quantifying the production of PA after stimuli. In a recent paper published in *Nature Plants*, Li et al.[4] developed a ratiometric, PA-specific, optogenetic biosensor (named PAleon) that reports PA concentration and dynamics at the PM.

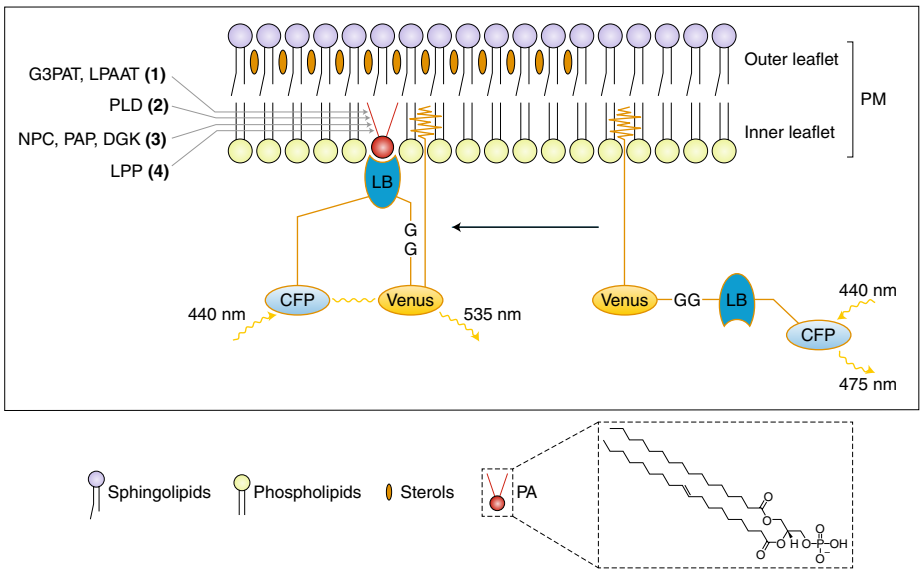


**Fig. 1 | Building a PA-binding biosensor to measure pools of PA in the plant PM.** Simplified PM (constituted of lipids) is shown, with sphingolipid and sterols mostly in the outer leaflet and phospholipids in the inner leaflet. PA is indicated in red. The four pathways leading to PA synthesis are represented on the left sides: (1) glycerol-3-phosphate acyltransferase (G3PAT) and lyso-PA acyltransferase (LPAAT); (2) PLD; (3) non-specific phospholipase (NPC), PA phosphatase (PAP) and diacylglycerol kinase (DGK); and (4) lipid phosphate phosphatase (LPP) of diacylglycerolpyrophosphate.

PAleon is based on the translational fusion of five domains: cyan fluorescent protein (CFP) bound to a PA lipid-binding domain (LB) of the NADPH oxidase, named respiratory burst oxidase homologue protein D; a flexible linker containing two glycines; a yellow fluorescent protein, named VENUS; and a C-terminal farnesylation signal of the protein K-Ras4B, which targets the whole chimaeric protein to the PM (Fig. 1). The authors generated a mutated PAleon (mPAleon), in which the four PA-binding arginine residues were replaced by alanines, to function as a negative control. After PA binding, PAleon undergoes a conformational change resulting in a Förster resonance energy transfer (FRET) between CFP and VENUS. PAleon was shown to be sensitive in vivo to physiological concentrations of PA, thereby enabling the visualization of PA dynamics at subcellular resolution in *Arabidopsis*.

To test the validity of PAleon, the authors first exogenously supplied PA to the system and recorded a dramatic increase of FRET after 500 s of exposure, while no other phospholipids induced FRET. In a more physiological system, the authors applied ABA to plants and observed PA changes in the guard cells. PA accumulation was

increased within 200 s after ABA exposure and reached saturation at 300 s, moments preceding stomatal closure. Salt stress also induces specific patterns of PA accumulation at the same rate in three different root tissues: the root tip, differentiation zone and maturation zone. Treatment with an inhibitor of PLD efficiently hindered PA accumulation in all three zones to near basal levels. Consistently, PA accumulation was impaired in *pldα1* mutants. By comparing PA changes under salt stress and osmotic stress, the authors revealed that the remarkable temporal complexity of PA accumulation is highly stress-specific. Finally, authors rationalized that changes in intracellular pH would modulate the protonation of PA polar head, the free phosphate group. Consequently, PA may function as a pH sensor during salt tolerance management in plants.

PAleon is a biosensor that was developed to study the role of lipids in planta among other biosensors, such as the ones detecting phosphoinositides[5] and diacylglycerol[6]. Besides the PM, PAleon will also pave the way for the study of PA by changing the C-terminal farnesylation domain to target other organelles, such as mitochondria or plastids. In this latter case, PA is a fine regulator of the plastidial lipids as it allosterically regulates the monogalactosyldiacylglycerol synthase responsible for the synthesis of galactolipids in the plastid envelope. The direct quantification of the pool of plastidial PA in vivo would be essential to better understand the regulation of these metabolic pathways[7]. PAleon could also find its use in the study of the nuclear membrane or vesicular pathways. In addition, this new tool will probably help to gain insight into the paradoxical effect of PA raised in the beginning of this article. How can PA be synthesized both by cold and heat stress, but trigger opposite physiological responses? PAleon could help solve this enigma by precisely localizing and quantifying PA after exposure to specific stimuli. ❐

Adiilah Mamode Cassim and Sébastien Mongrand*

*Laboratoire de Biogènese Membranaire, UMR 5200, CNRS, University of Bordeaux, Bordeaux, France.*
**e-mail: sebastien.mongrand@u-bordeaux.fr*

Published online: 26 August 2019
https://doi.org/10.1038/s41477-019-0494-9

## References

1. Testerink, C. & Munnik, T. *Trends Plant Sci.* **10**, 368–375 (2005).
2. Pokotylo, I., Kravets, V., Martinec, J. & Ruelland, E. *Prog. Lipid Res.* **71**, 43–53 (2018).
3. Wang, G., Ryu, S. & Wang, X. in *Lipases and Phospholipases: Methods in Molecular Biology* Vol. 861 (ed. Sandoval, G.) 123–137 (Humana Press, 2012).
4. Li, W. et al. *Nat. Plants* https://doi.org/10.1038/s41477-019-0497-6 (2019).
5. Simon, M. L. et al. *Plant J.* **77**, 322–337 (2014).
6. Vermeer, J. E. M. et al. *Plant Cell Physiol.* **58**, 1196–1207 (2017).
7. Dubots, E. et al. *Biochimie* **94**, 86–93 (2012).

## Competing interests

The authors declare no competing interests.