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Beyond Membranes: The Evolution of Plant Lipid Signaling

Lipids have many essential functions in plants: They are membrane components and as such play an important role in compartmentation of the cell, membrane curvature, and formation of lipid rafts/microdomains; they participate in vesicle formation and trafficking, and act as a barrier to unrestricted trafficking of molecules. Lipids are also important intermediates in the biosynthesis of secondary metabolites and storage compounds. Even more interesting is their role in signaling. Traditionally, it was assumed that plant signaling lipids function by recruiting or tethering proteins that are part of signaling complexes to the membrane, thus either bringing them into the proximity of, for example, receptors, or by removing them from the signaling cascade. Others act in regulating vesicle budding and fusion, impacting signaling indirectly. The question arises: *Are all these signaling lipids membrane bound?* In recent months, researchers have made great progress in understanding the role of lipids beyond membrane boundaries.

LIPID SIGNALING IN RESPONSE TO DROUGHT/OSMOTIC STRESS

Responding to changes in water availability is essential for plant survival. During drought or osmotic stress, the plant hormone abscisic acid (ABA) is generated and perceived by its receptor PYR/PYL/RCAR. In addition, the phospholipid phosphatidic acid (PA) is produced through an upregulation of phospholipase PLDα1. It interacts with ABI1 (ABSCISIC ACID INSENSITIVE 1), a protein phosphatase 2C, which is a negative regulator of the ABA response (Zhang et al., 2004). This results in PA tethering ABI1 to the membrane, sequestering it away from SNF1-RELATED KINASE 2 (SnRK2), which can now be phosphorylated and, in turn, phosphorylates downstream AREB/ABF transcription factors. The latter bind to ABA-response elements and modify gene expression (Mishra et al., 2006; Fujita et al., 2011). Thus, the lipid PA is an essential component in the mechanism controlling stomatal aperture and, as a result, water-use efficiency.

While these were important findings, the question remains: *Are phospholipids simply membrane tethers that control the availability of signaling proteins or are they (part of) the signaling molecule themselves?* It has been proposed that PA is part of a long-distance protein-lipid signaling complex that mediates a distal response as well (Benning et al., 2012). But what else is out there? In animal systems, lipids are known mobile signaling compounds; extracellular fatty acids and phospholipids can bind to and activate G-protein-coupled receptors or Toll-like receptors, leading to subsequent interactions with PPARs and modification of gene regulation in neighboring as well as in distal cells (McIntyre et al., 2003). These pathways are of critical importance in animal systems but have not been studied in plants. In 2013, Yao et al. (2013) showed that PA binds to the transcription factor WEREWOLF (WER), leading to its translocation into the nucleus. At this point, however, the protein was still considered the sole transcriptional regulator. More progress came from a different research field.

LIPID SIGNALING DURING THE INDUCTION OF FLOWERING

The timing of flower and fruit development is hugely important: Flower too early and no pollinators are present; flower too late and there is insufficient time for seed or fruit maturation. Hence, the transition to flowering has to be finely tuned with the seasons: in response to the appropriate photoperiod, the protein CONSTANS is produced in the leaf. This leads to the production of FLOWERING LOCUS T (FT), a small protein, which enters the sieve elements of the phloem and translocates to the shoot apical meristem. Once there, it interacts with 14-3-3 proteins and transcription factors such as FD to initiate the expression of flower production genes (Andrés and Coupland, 2012). Again, a phospholipid plays an important role in flowering, because FT binds specific phosphatidyl choline (PC) species that are generated in a circadian rhythm (Nakamura et al., 2014). *But can plant lipids also function directly in the regulation of gene expression?* One example, predicted START domain-containing transcription factors are found in plants but their function/ligand remain under investigation (Schrick et al., 2014). In a groundbreaking paper, Nakamura et al. (2019) published a combination of crystallography, computational simulation, mutagenesis, lipid-binding assays, and functional studies to provide evidence that PC is essential for the formation of the soluble florigen activation complex, consisting of FT, 14-3-3, FD, and PC. They proposed that FT-PC binding is crucial for the interaction of the complex with DNA. *This implies that, in this system, the lipid PC is required for transcriptional regulation!*

LIPID SIGNALING DURING SEED GERMINATION

In a further advance, Cai et al. (2020) recently published data showing the role of lipids, particularly PA, in the regulation of seed oil metabolism and seedling development. In humans, dietary lipids are taken up into the cell and either stored in lipid droplets or cleaved by lipases. This generates fatty acids,

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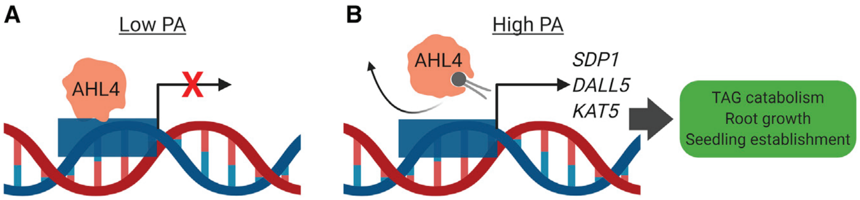

Figure 1. Possible Mechanism of AHL4-PA Interaction in the Regulation of TAG Catabolism during Seed Germination.

(A) At low phosphatidic acid (PA) concentrations, AHL4 binds to the AT-rich promoter region (blue box) and downregulates gene expression.

(B) At high levels of PA, the lipid interacts with AHL4, thus interfering with its binding to the promotor. Removal of AHL4 leads to the expression of genes involved in TAG catabolism, which in turn provides energy and building blocks for seedling establishment.

which can function as ligands of transcription factors such as PPARα1, which then upregulates β-oxidation (Pawlak et al., 2015). Based on the finding in yeast that PA is involved in the transcriptional regulation of phospholipid metabolism, the group led by Xuemin Wang searched for PA-binding transcription factors in *Arabidopsis* (Kim et al., 2019). Among those was a nuclear localized protein with a short DNA-binding AT-hook motif that modulates chromatin. Proteins in this AT-hook motif-containing (AHL) family function in the regulation of plant growth and development and stress response. In a systematic multipronged approach, Cai et al. (2020) showed that AHL4 very specifically binds PA but no other glycerolipids, with a preference for PA species containing at least one unsaturated acyl chain. They confirmed their findings using a "lipid pulldown" approach that identified 34:2-PA, 34:3-PA, and 36:4-PA as *in vivo* AHL4 ligands.

To study the effect of AHL4 on plants, Cai et al. (2020) obtained T-DNA knockout lines and generated overexpression and complementation lines. Loss of *AHL4* function led to increased seed germination and early seedling establishment, while *AHL4*-overexpression lines required sucrose for germination. They showed that the latter is due to altered expression of several genes associated with triacylglycerol (TAG) catabolism (Cai et al., 2020), some of which contain AT-rich regions in their promoters known to interact with the AT domain of AHL proteins. A combination of electrophoretic mobility shift assay, mutagenesis, and chromatin immunoprecipitation followed by—PCR analysis revealed that AHL4 binds to the promoter regions of at least three genes, *SDP1* (*sugar dependent 1,* a TAG lipase), *DALL5* (*defective in anther dehiscence 1 [DAD1]-like lipase 5*), and *KAT5* (*3-ketoacyl-CoA thiolase 5*; β-oxidation). The presence of PA but no other phospholipid interfered with the AHL4-DNA interaction, suggesting that both AHL4 and PA are necessary for regulating gene expression. To mimic these conditions *in vivo*, *Arabidopsis* lines with higher or lower PA content (PA phosphohydrolase-KD/*pah* and phospholipase D-KD/*pld*-, respectively) were analyzed. Nuclei showed a concomitant PA increase in *pah1/2* and decrease *pld* lines. *SDP1*, *DALL5*, and *KAT5* expression was higher in high-PA lines *(pah1/2)* and lower in low-PA lines *(pld)* and seedling development was affected accordingly. This change was not due to changes in the localization of AHL4, however; it was solely due to its interaction with PA and DNA. *Taken together, this suggests that AHL4 is a negative regulator of the expression of oil catabolism and β-oxidation during seedling development and that PA is not simply a membrane tether but directly regulates gene expression by interfering with the binding of AHL4 with DNA* (Figure 1). *As such, it was proposed that PA is simultaneously a sensor and regulator of cellular metabolism.* With these emerging data, lipid signals are graduating from a supporting role to the stars of the show.

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