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A phosphatidate phosphatase double mutant provides a new insight into plant membrane lipid homeostasis

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Phospholipids make up the bulk of most eukaryotic cell membranes, but how their synthesis is regulated remains relatively poorly understood in plants. In our article[1] we provide evidence that two Mg^{2+}-dependent phosphatidic acid phosphatase enzymes, called PAH1 and PAH2, are capable of repressing phospholipid biosynthesis at the endoplasmic reticulum in *Arabidopsis thaliana*. The precise mechanism of repression remains unclear and it does appear to vary in several respects from that already described in *Saccharomyces cerevisiae*.[2,3]

The enzyme phosphatidic acid phosphatase (PAP) catalyses the conversion of phosphatidic acid (PA) to diacylglycerol and plays a central role in lipid metabolism by governing the supply of substrates for membrane and storage lipids.[2,3] In higher plants, lipid metabolism is complicated by the fact that fatty acid synthesis occurs within the chloroplast and supplies parallel pathways for glycerolipid synthesis situated in both the chloroplast and endoplasmic reticulum (ER) with trafficking of certain intermediates occurring between the two compartments.[4] The two pathways are termed the prokaryotic and eukaryotic pathways, respectively, and PAPs are thought to be required for both.[4]

Both Nakamura and co-workers[4] and ourselves[1] recently characterized two Mg^{2+}-dependent PAP enzymes from *Arabidopsis thaliana* that are homologous to *Saccharomyces cerevisiae* Pah1p[2] and mammalian lipins.[2] Analysis of the *A. thaliana* double mutant, called *pah1 pah2-1*, revealed that partitioning of substrate between the prokaryotic and eukaryotic pathways is perturbed.[4] In *A. thaliana* leaves both the prokaryotic and eukaryotic pathways contribute approximately equally to the synthesis of galactolipids, which make up the abundant chloroplast thylakoid membranes necessary for photosynthesis, while extra-plastidial membranes are predominantly made up of phospholipids derived from the eukaryotic pathway. In addition, two classes of galactolipid form thylakoid membranes, namely mono- and di-galactosyldiacylglycerol (MGD and DGD), and the prokaryotic pathway contributes predominantly to MGD synthesis, while the eukaryotic pathway mainly supplies DGD synthesis. A relative decrease on a mol% basis in the proportion of galactolipids (and in particular DGD) verses phospholipids in the *pah1 pah2-1* mutant suggested to Nakamura and co-workers that PAH1/2 play a role in the provision of eukaryotic substrate for galactolipid synthesis in leaves.[4]

Our own investigation led us to focus primarily on the effect of the *pah1 pah2-1* mutation on phospholipid, rather than galactolipid synthesis.[1] In addition to its direct metabolic role in lipid biosynthesis, *S. cerevisiae* Pah1p also plays a key regulatory role in phospholipid metabolism.[3] Rather than performing relative measurements of lipid classes in leaves and roots of *pah1 pah2-1* on a mol % basis, we performed absolute measurements on a per unit fresh (or dry) weight basis.[1] This analysis suggested to us that the shift in the ratio of galactolipids to phospholipids was due, at least in part, to an increase in the production of phospholipids. Both the total lipid content and the phospholipid

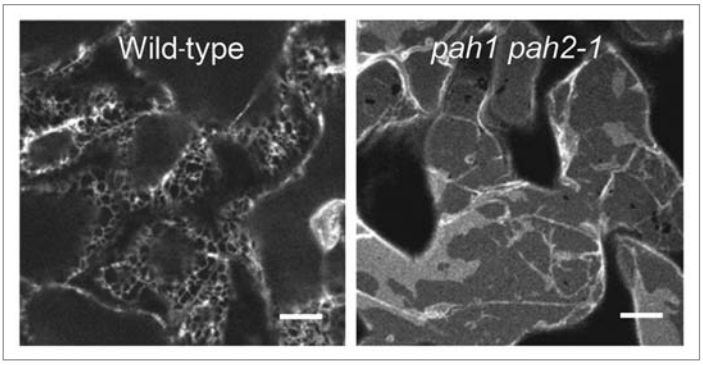

Figure 1. Single plane confocal images of mesophyll cells from wild-type and *pah1 pah2-1* leaves stably transformed with ER lumen-targeted red fluorescent protein expressed under the control of the constitutive 35S promoter. Note that images represent among the most extreme morphological differences observed and that significant variation was seen between cells and lines. Scale bar is 10 μm.

content of *pah1 pah2-1* leaves and roots is greater than wild-type on a per unit fresh weight basis.[1] We also observed an increase in the rate of incorporation of ^{14}C from the radiolabel precursor (*methyl*-^{14}C) choline chloride into the major phospholipid class phosphatidylcholine (PC), significant induction of the expression of several genes encoding enzymes of phospholipid synthesis and gross changes in ER morphology that are consistent with membrane over-expansion.[1] In particular ER sheets (cisternae) appear to be much more extensive in mesophyll cells of *pah1 pah2-1* leaves[1] (**Fig. 1**). Recently it has been shown that this enhanced sheet formation can be partially rescued by overexpression of reticulons,[5] which are integral ER proteins that play a role in ER tubule formation by promoting membrane curvature.[5]

In *S. cerevisiae* the activity of Pah1p controls the expression of multiple genes associated with phospholipid biosynthesis.[3] Pah1p does this by governing the concentration of PA, which in turn controls the localisation of the transcriptional repressor protein Opi1p.[6] When PA levels are high they tether Opi1p to Scs2p at the nuclear-ER membrane, preventing Opi1p translocation into the nucleus where it can interact with the transcription factor Ino2p to inhibit the expression of genes encoding phospholipid biosynthetic enzymes.[6] The mechanism by which PAH1/2 represses phospholipid biosynthesis in *A. thaliana* remains to be determined. Superficially there are parallels between the phenotypes of *A. thaliana pah1 pah2-1*,[1] and *S. cerevisiae pah1*Δ.[2,3] However, it is noteworthy that there are differences in the pathways responsible for phospholipid synthesis in these two species[1] and *A. thaliana* appears to lack homologs of either Opi1p or Ino2p.[1] Further work will therefore be required to understand how PAH1/2 can repress phospholipid synthesis in plants and also to understand the physiological role and significance of this mechanism.

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