Regulation of Phospholipid Biosynthesis in Arabidopsis

Phospholipids are major constituents of the membranes that separate cells from their environment and compartmentalize eukaryotic cells into functional units. In yeast (Saccharomyces cerevisiae), the expression of genes involved in phospholipid biosynthesis is regulated by a mechanism that responds to changes in the level of phosphatidic acid (PA), a key precursor of phospholipids (Carman and Henry, 2007). Cellular levels of PA, in turn, are regulated by a Mg2+-dependent PA phosphohydrolase named Pah1p (Han et al., 2006). pah1 deletion mutants have increased levels of PA, elevated expression of phospholipid biosynthesis genes, and expanded nuclear endoplasmic reticulum (ER) membranes (Han et al., 2006; Santos-Rosa et al., 2005).

In an effort to understand how phospholipid biosynthesis is regulated in plants, Eastmond et al. (pages 2796–2811) characterized two phosphohydrolase genes from Arabidopsis, PAH1 and 2, that are similar to yeast PAH1. Real-time PCR revealed that PAH1 and 2 were expressed throughout the plant, and green fluorescent protein (GFP)–tagged PAH1 and 2 transiently expressed in Nicotiana benthamiana leaves localized mainly to the cytosol. His6-tagged PAH1 and 2 heterologously expressed in Escherichia coli were able to dephosphorylate 32P-labeled PA in a Mg2+-dependent manner, confirming that these proteins function as PA phosphohydrolases. Whereas disruption of either PAH1 or 2 had no effect on plant growth, disruption of both genes retarded growth.

Next, the authors investigated the effect of PAH1 and 2 disruption on phospholipid production. Two-dimensional thin layer chromatography followed by gas chromatography analysis revealed a 70 to 100% increase in phospholipid content in the leaves and roots of the pah1 pah2 double mutant. Radiolabel feeding experiments showed that the net rate of [methyl-14C] choline incorporation into phosphatidylcholine, the main phospholipid class in Arabidopsis, was 1.8-fold greater in pah1 pah2 than in the wild type. Furthermore, the expression of several genes involved in phospholipid synthesis was upregulated in pah1 pah2 leaves. This includes PHOSPHORYLETHANOLAMINE N-METHYLTRANSFERASE1, which encodes an enzyme that catalyzes the first committed step of choline synthesis and defines a phosphatidylcholine biosynthesis pathway not found in yeast.

The authors then examined the morphology of the ER and nuclei in pah1 pah2 leaves using targeted GFP markers. Similar to findings in the yeast pah1 deletion mutant (Santos-Rosa et al., 2005), the ER was greatly expanded in pah1 pah2 plants (see figure); however, the nuclei appeared normal.

Thus, PAH1 and 2 function redundantly to repress phospholipid biosynthesis and membrane biogenesis at the ER. Although their role is analogous to that of Pah1p in yeast, they appear to act via a different mechanism and through different target enzymes.

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REFERENCES


