In Arabidopsis thaliana, the vacuolar proton-pumping pyrophosphatase (H⁺-PPase) is highly active in proliferating young tissues. We have previously reported that pyrophosphate (PPi) overaccumulation inhibits cell division in cotyledonary palisade tissue cells, and triggers compensated cell expansion (Ferjani et al., 2011). Quantification of sucrose (Suc) and PPi amounts in the wild type versus fugu5, the H⁺-PPase loss-of-function mutant, demonstrated that it is gluconeogenesis that is inhibited by elevated level of cytosolic PPi. Thus, we provided robust evidence that the major function of H⁺-PPase, during seedling development of Arabidopsis, is the removal of the inhibitory PPi, rather than vacuolar acidification.

Here, careful examination of pavement cells revealed that the fugu5 mutant exhibited a much simpler cell shapes than the wild type, as deduced from Undulation Index values. Also, the number of stomata was significantly increased (~1.7-fold per unit area), whereby stomata cells violated the one-cell-spacing rule. All fugu5 morphological and cellular phenotypic aberrations were rescued by complementation with the yeast cytosolic PPase IPP1, under the control of the Arabidopsis AVP1 promoter. Similar phenotypic analyses in angustifolia (an)-1 and RIC1 ox (for ROP-interactive CRIB motif-containing protein 1 overexpressor) suggested that AN, RIC1 and AVP1/FUGU5 act on genetically independent pathways. Importantly, pavement cells in mutants with defects in the glyoxylate cycle (icl-2 and mls-2) or gluconeogenesis (pck1-2), showed no phenotypes, suggesting that lowered Suc production from triacylglycerol seed reserves is not the cause of fugu5 phenotypes.

In all the double mutants between the above three key genes, pavement cells, stomata and trichome developmental defects were synergistically enhanced. Particularly, in an-1 fugu5-1 and an-1 RIC1 ox, the pavement cells displayed striking 3D-growth phenotype, in addition to aberrant branching and helical arms in RIC1 ox fugu5-1 trichomes. Together, these findings demonstrated that excessive cytosolic PPi levels impair all epidermal developmental programs, probably by restraining the proper distribution and/or dynamism of microtubules, a hypothesis that is now under examination.

Finally, the biological role of the PPi hydrolyzing enzymes, for now, is enigmatic in almost all living organisms, including plants. Our recent breakthrough in this field has shed light, for the first time, on the axial role of the vacuolar H⁺-PPase in PPi homeostasis in plants (Ferjani et al., 2011; 2012; 2014). We believe that this ongoing work will further uncover other mysterious features of PPi, providing novel insights into the link between plant metabolism and development.