第115回　Plant Science Seminar

Pyrophosphate hydrolysis by the vacuolar H^+-PPase promotes gluconeogenesis in germinating oilseeds

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日時　：　10月10日（金）　16:30〜18:00
場所　：　理学部 5号館 8階 813号室

Upon germination, oil-seed plants rely on triacylglycerol (TAG) reserves to sustain their growth during a heterotrophic period that requires β-oxidation, the glyoxylate cycle, and gluconeogenesis activities to convert TAG to sucrose (Suc), which provides energy to developing tissues in the form of ATP. Besides, macromolecular syntheses in proliferating young tissues of the embryo require huge amounts of ATP, which is often hydrolyzed to AMP plus PPi. The loss of the pyrophosphatase (PPase) activity causes growth arrest, and/or developmental defects in many organisms, such as *Escherichia coli*, yeast, and *Caenorhabditis elegans*. In Arabidopsis, the proton-pumping vacuolar pyrophosphatase (H^+-PPase) uses the energy from PPi hydrolysis to acidify the vacuole. The Arabidopsis *fugu5* mutants, previously characterized as having defects in cotyledon development (Ferjani *et al*., 2007), are lacking the AVP1 H^+-PPase (Ferjani *et al*., 2011). The *fugu5* mutants failed to sustain postgerminative heterotrophic growth, which recovered upon Suc supply, or specific PPi removal by the cytosolic IPP1 from yeast under the control of the Arabidopsis *AVP1* promoter in the $AVP1_{pro}$*:IPP1* transgenic lines. This demonstrated that the major function of H^+-PPase in seedling development is the removal of inhibitory PPi rather than vacuolar acidification. Following quantification of sucrose and PPi amounts in etiolated seedlings of the wild type versus *fugu5*, we concluded that elevated level of PPi is most likely to inhibit gluconeogenesis, but the mechanism or metabolic targets of PPi inhibition remained unclear.

Here, our profiling of major metabolites that occur during TAG mobilization showed that the amounts of Glu-1-P and UDP-Glc were 2.0- and 0.5-fold in *fugu5* versus the wild type, while other intermediate metabolites were basically unaffected. This result suggested that UDP-Glucose Pyrophosphorylase (UGPase) is specifically inhibited by cytosolic PPi accumulation in the *fugu5* mutant background. To confirm these findings, we used two independent $AVP1_{pro}$*:IPP1* transgenic lines, in which IPP1 actively hydrolyzes cytosolic PPi, but has no effect on vacuolar acidification. Importantly, the above metabolic defects were reversed in $AVP1_{pro}$*:IPP1*, where Glc-1-P and UDP-Glc were 1.0- and > 2.0-fold compared to the wild type. It is also noticeable that the amounts of UDP-Glc in $AVP1_{pro}$*:IPP1* were > 4.0-fold higher than in *fugu5* muants. It is well established from biochemical *in vitro* experiments that UGPase equilibrates UDP-Glc and its precursor Glc-1-P. Thus, given the readily reversible nature of the Glc-1-P/UDP-Glc reaction, the metabolic target of PPi overaccumulation in *fugu5* is UGPase. PPi metabolism is enigmatic in all living organism, including plants. Our recent reports have supported a pivotal role of the vacuolar H^+-PPase in maintaining cytosolic PPi homeostasis (Ferjani *et al*., 2011; 2014). Also, we proposed gluconeogenesis, which exclusively occur in the cytosol, as a potential target of inhibitory high PPi levels (Ferjani *et al*., 2011). Here, the pinpoint target of PPi has been identified as UGPase, a crucial enzyme that during germination acts prior to Suc production from TAG. Taken together, our results represent an unprecedented breakthrough in this field, which has identified a major missing piece of the modern biochemistry.

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