

Involvement of the modulation of proton motive force in the regulation of photosynthesis

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In photosynthesis, light-driven electron transport stores energy in an electrochemical gradient of protons and other ions across the thylakoid membrane, termed proton motive force (*pmf*). The *pmf* consists of proton gradient (ΔpH) and membrane potential ($\Delta\psi$) and is ultimately used to drive ATP synthesis in $\text{CF}_0\text{-CF}_1\text{-ATP synthase}$ (ATP synthase). In addition to this central role of *pmf* in the ATP synthesis, the ΔpH component of *pmf* is also involved in the regulation of photosynthetic electron transport via the downregulation of both light energy utilization in photosystem (PS) II and activity of the cytochrome *b₆f* complex. However, it is still under debate whether thylakoids can store a substantial fraction of *pmf* as $\Delta\psi$. And, differential parsing of *pmf* into ΔpH and $\Delta\psi$ will have large effects on the regulatory behavior of the chloroplast (Cruz et al., 2001). Therefore, it is essential to know how the size and the partitioning of *pmf* between ΔpH and $\Delta\psi$ are controlled, for a better understanding of the regulation of photosynthesis in response to fluctuating light conditions. In this work, I address to clarify the regulatory mechanisms of *pmf* on the basis of two viewpoints: (1) The role of cyclic electron transport around photosystem (PS) I in the regulation of *pmf* (Chapter 1). (2) The requirement of a thylakoid membrane localized K^+/H^+ antiporter KEA3 in modulating the two components (ΔpH and $\Delta\psi$) of *pmf* in photosynthesis (Chapter 2).

In Chapter 1, electrochromic shift (ECS), which was used to monitor *pmf* *in vivo*, was analyzed in the Arabidopsis *pgr5* mutant, two NDH-defective mutants (*ndhs* and *crr4-2*), and their double mutants (*ndhs pgr5* and *crr4-2 pgr5*), to assess the contribution of two cyclic electron transport pathways around PSI (one depending on PGR5/PGRL1 and another one depending on NDH) to *pmf* formation. In *pgr5*, the total size of *pmf*, represented by ECS_t , was greatly reduced compared with that in the wild type (WT),

accompanied by enhanced g_H^+ , a parameter representing the proton conductivity of ATP synthase, especially at high light intensities. Moreover, a disturbed regulation of g_H^+ in the dark was observed in *pgr5*. By contrast, ECS_t was slightly lower in the NDH-deficient mutants than that in the WT, but g_H^+ was unaffected. In the two double mutants, ECS_t was even lower than that in *pgr5*. These results suggest that both PGR5/PGRL1- and NDH-dependent pathways contribute to *pmf* formation but with different extents.

In Chapter 2, I report the identification of a novel mutant allele of *KEA3*, whose amino acid alteration mutation showed semidominant or dominant nature depending on different air conditions. This mutant showed similar but distinct non-photochemical quenching (NPQ) phenotype with the knockout (KO) allele in the artificial air (CO₂-free, low O₂) and ambient air, respectively. The experimentally determined membrane topology of *KEA3* only supported the NPQ phenotype of the KO allele in the ambient air. In addition, the protein level of *KEA3* was unaffected in the missense mutant. Through the analysis of the complexed phenotypes in two mutant alleles, I discuss on a fine regulation of *KEA3* activity required for optimizing photosynthesis.

Taken together with the results of both chapters, I discuss on how the size and two components of *pmf* are regulated in photosynthesis.