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 (Δψ) and is ultimately used to drive ATP synthesis in CF_0-CF_1-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 b_6f complex. However, it is still under debate whether thylakoids can store a substantial fraction of *pmf* as Δψ. And, differential parsing of *pmf* into ΔpH and Δψ 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 Δψ 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 Δψ) 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 (CO_2-free, low O_2) 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.