Synthetic biological study on cyclic electron transport around photosystem I in Arabidopsis

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Abstract（要約）

Light reactions of photosynthesis consist of linear electron transport from water to $NADP^+$ and cyclic electron transport around photosystem I (PSI). In *Arabidopsis thaliana*, PSI cyclic electron transport is mediated by two pathways. The main pathway depends on the PROTON GRADIENT REGULATIOIN 5 (PGR5) protein, whereas the minor pathway is mediated by the chloroplast NADH dehydrogenase-like (NDH) complex. It has not been fully understood how the two pathways independently or cooperatively contribute to ATP synthesis and the regulation of photosynthesis.

Chlororespiration is respiration-like electron transport from the stromal reducing power to oxygen. Plastid terminal oxidase (PTOX) accepts electrons from plastoquinone and reduces molecular oxygen to water. In Arabidopsis, PTOX is required for the carotenoid biosynthesis but its contribution to chlororespiration is almost negligible in mature chloroplasts. In contrast, the high activity of chlororespiration is mediated by CrPTOX2 in *Chlamydomonas reinhardtii*. To study the impact of strong activity of CrPTOX2 in the regulatory network of photosynthetic electron transport in angiosperms, I introduced the gene encoding CrPTOX2 into Arabidopsis wild-type (WT) plants. To clarify the physiological function of PSI cyclic electron transport, I also transformed the Arabidopsis *pgr5* and *crr2* (*chlororespiratory reduction 2*) mutants defective in the two pathways of PSI cyclic electron transport respectively.

In Chapter 1, I characterized the transgenic plants in the WT (CrPTOX2-WT) and *pgr5-1* (CrPTOX2-*pgr5*) background. During the induction of photosynthesis by relatively low light of 130 μmol photons m^{-2} s^{-1}, the high level of photosystem II

(PSII) yield was induced immediately after the onset of actinic light in the CrPTOX2-WT lines because of the efficient operation of CrPTOX2 as a safety valve for electrons. CrPTOX2 does not translocate any protons across the thylakoid membrane but its activity is coupled with the PSII reaction which contributes to the ΔpH formation. Consequently, NPQ was more rapidly induced in the CrPTOX2-WT lines than in the WT. In the *pgr5-1* mutant, the defect in PSI cyclic electron transport impaired the NPQ induction but the introduction of CrPTOX2 contributed to the rapid induction of NPQ, as in the WT background. In addition to the NPQ induction, the lumenal acidification also downregulates the rate of electron transport through the cytochrome b_6f complex (photosynthetic control). Because of the rapid acidification of the thylakoid lumen in the CrPTOX2-WT lines, the PSI reaction center (P700) was oxidized immediately after the onset of actinic light. Although CrPTOX2 also acidified the thylakoid lumen in the *pgr5-1* mutant background, P700 was not oxidized. In addition to the induction of photosynthetic control via the lumenal acidification (donor-side regulation), PGR5 is required for oxidizing P700 in the light. I propose that PGR5 is essential to move the pool of electrons from the acceptor side of PSI to the plastoquinone pool.

In Chapter 2, I investigated the impact of CrPTOX2 accumulation in the *crr2-2* background (CrPTOX2-*crr2*) defective in the chloroplast NDH complex. In the *crr2-2* mutant, oxidation of P700 was delayed during the induction of photosynthesis. In contrast to the *pgr5-1* background, CrPTOX2 rapidly oxidized P700, as in the WT background. Unlike PGR5, the NDH complex does not contribute to the acceptor side regulation significantly. Introduction of CrPTOX2 induced the high level of NPQ than in the CrPTOX2-WT lines. The NDH complex probably leaks protons from the thylakoid membrane via its reverse reaction when the size of ΔpH is extremely high.

CrPTOX2 may efficiently function as a safety valve for electrons under fluctuating light intensity. In Chapter 3, I characterized the transgenic lines under the fluctuating light, as well as the WT and mutant plants. In the CrPTOX2 lines in the WT background, the photosynthetic control was more rapidly induced after the shift from low light (LL) to high light (HL). The Y(NA) parameter of P700 absorbance analysis

reflects the limitation of acceptors from PSI. Although the level of Y(NA) was reduced in the HL periods, it was elevated in the LL periods in the CrPTOX2-WT lines. As during the induction of photosynthesis, the NPQ induction was restored to the WT level in the HL periods in the CrPTOX2-*pgr5* lines but P700 was still reduced. As a result, the introduction of CrPTOX2 did not alleviate the PSI photodamage in the *pgr5-1* mutant background. In the *crr2-2* mutant, the induction of photosynthetic control was partially suppressed in the HL periods. In contrast to the *pgr5-1* background, induction of photosynthetic control was complemented to the higher level than in the WT. Although the NDH complex contributes to the induction of photosynthetic control as well as PGR5, it is not essential for the induction of the acceptor side regulation. The function of the NDH complex is fully complemented by the introduction of CrPTOX2.