学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Mg-dechelatase Encoded by Chlamydomonas *Stay-Green* is Involved in the Formation of Photosystem II but not in Chlorophyll Degradation
(Stay-Green 遺伝子の産物 Mg-脱離酵素はクラミドモナスではクロロフィルの分解ではなく光化学系 II の形成に関与する)

The STAY-GREEN (*SGR*) gene encodes a Mg-dechelatase that catalyzes the conversion of chlorophyll (Chl) *a* to pheophytin (Pheo) *a* which is the first step in Chl degradation pathway. Unquestionably, Pheo *a* is an indispensable molecule in photosystem (PS) II reaction center, which suggests the relationship between SGR function and PSII formation. To investigate the physiological functions of Chlamydomonas SGR, two Chlamydomonas *sgr* null mutants were prepared by screening an insertion-mutant library. The two Chlamydomonas *sgr* mutants had reduced maximum quantum efficiency of PSII and Pheo *a* levels. The two Chlamydomonas *sgr* mutants also showed reduced photoheterotrophic growth rate but still could photoautotrophiclly grow. These phenotypes were complemented by the introduction of the Chlamydomonas *SGR* gene.

Blue-native polyacrylamide gel electrophoresis and immunoblotting analysis were carried out with these two Chlamydomonas *sgr* mutants and all the results showed the reduced PSII levels and the unchanged levels of PSI and light-harvesting Chl *a*/*b* complex. These results were also verified by low temperature fluoresce spectroscopy of whole Chlamydomonas cells and by the Chl/P700 ratios measurement. High performance liquid chromatography analysis showed that Chlamydomonas *sgr* mutants have reduced Chl levels during the photoheterotrophic growth. The light sensitivity of Chlamydomonas *sgr* mutants was observed under high light conditions.

In order to examine whether SGR is involved in chlorophyll degradation in Chlamydomonas, Chl degradation was induced by nitrogen starvation under both photoheterotrophic and photoautotrophic conditions. Chl was similarly degraded in wild type and Chlamydomonas *sgr* mutants under these conditions, indicating that *SGR* is not required for Chl degradation but primarily contributes to the PSII formation by supplying Pheo *a* in Chlamydomonas. Chlamydomonas SGR partly rescued the stay green phenotype of Arabidopsis *sgr 1 2 l* triple mutant, which completely lack SGR activity, indicating that Chlamydomonas SGR has same enzymatic properties as Arabidopsis SGR.

In contrast, in the Arabidopsis *sgr* triple mutants, PSII was normally synthesized and stay green phenotype was observed. These results suggest that the Arabidopsis *SGR* participates in Chl degradation, while the Chlamydomonas *SGR* participates in PSII formation in spite of the same catalytic property. This is the first report which clarified that Pheo *a* is not spontaneously synthesized but enzymatically synthesized for PSII formation.

In this thesis, several hypotheses about formation and supply of Pheo *a* were discussed. This thesis shows that Mg-dechelatase plays a fatal role in PSII formation, but not in Chl degradation in Chlamydomonas. This research advances our understanding of PSII formation and the functional diversification of *SGR* in green lineage. In this thesis, the function of *SGR* is discussed from an evolutionary viewpoint.