Title of Project : Comprehensive study of excessive light stress response in plants

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【Purpose and Background of the Research】

The photosynthetic apparatus is designed to efficiently use the energy of light to fix CO_2. However, photosynthetic apparatuses exposed to excess amount of light are often damaged by reactive oxygen species produced by overexcited chlorophylls. Ironically, plants are not good at direct sunlight. This has led to the development of a mechanism called non-photochemical quenching (NPQ), which is an emergency measure for the light-harvesting antenna (LHCII) to prevent the transfer of excess amount of excitation energy to the reaction center of photosystem II (PSII) (Fig. 1). As the physiological importance of NPQ has been recognized, research has been conducted worldwide, but details of the mechanism are still unclear. In this study, we will elucidate how the NPQ system is induced after absorbing a signal of excess light, and how the NPQ system safely dissipates excess amount of energy at the level of supramolecular complexes using a unicellular green alga *Chlamydomonas reinhardtii*. Furthermore, we will expand the analysis to the macrostructural regulation of the supramolecular complexes, and clarify how NPQ is implemented in plants in general from an evolutional context.

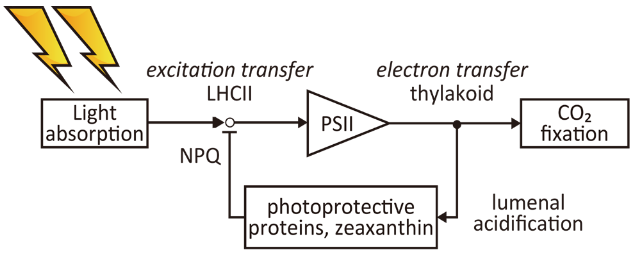

Fig. 1. A schematic representation of NPQ. Excess light energy collected by LHCII causes excessive electron transfer from PSII, which acidifies the lumen of the thylakoid membrane. After a complex process, NPQ is triggered, resulting in negative feedback on

【Research Methods】

The present study is divided into three sections to elucidate the entire photoprotection reaction by NPQ.

I. Excess light signaling system

The induction of the NPQ system in the green alga *Chlamydomonas reinhardtii* has been shown to involve photoreceptors (phototropin, UVR8), ubiquitin E3 ligases (COP1, SPA1, DET1, etc.), and transcription factors (CONSTANS, etc.) (e. g. Petroutsos et al., 2016). We will elucidate the entire signaling pathway linking these factors to the chloroplast-derived signal(s) using various mutants.

II. Mechanism of excess energy dissipation

Although NPQ in the green alga *C. reinhardtii* differs from that in land plants and is not usually dependent on zeaxanthin, the basic NPQ mechanism is likely to be common among green plants, and the analysis of *C. reinhardtii* will elucidate such details. The energy dissipation process in zeaxanthin bound to the photoprotective protein of *C. reinhardtii* will be analyzed in detail using various mutants. The research will be extended to the analysis of land plants.

III. Structural basis of NPQ

The PSII supercomplex structure of *C. reinhardtii* in its resting state has recently been reported (Sheng et al. 2019). In this study, PSII supercomplexes of *C. reinhardtii* in the NPQ state will be prepared from wild-type and various mutant strains, and their structures will be determined by cryo-EM. In addition, the dynamics of PSII-LHCII supercomplex in the thylakoid membranes in spinach, *Arabidopsis thaliana*, and *C. reinhardtii* will be visualized by high-speed AFM to identify the structural determinants of NPQ.

【Expected Research Achievements and Scientific Significance】

This study will clarify the following: 1) the excess light signaling system in green algae, 2) the mechanism of thermal dissipation by zeaxanthin, and 3) the structural basis of NPQ. Although both aquatic algae and terrestrial plants have NPQ functions, there are similarities and differences in their mechanisms, and such details will be clarified. This research is expected to give an answer to the fundamental question of how to conduct photosynthesis safely, and also to reveal part of the reason why plants have been able to advance on land.

【Publications Relevant to the Project】

・ Petroutsos, D., et al. (2016) A blue light photoreceptor mediates the feedback regulation of photosynthesis. ***Nature*** 537: 563-566.
・ Sheng, X., et al. (2019) Structural insight into light harvesting for photosystem II in green algae. ***Nat. Plants*** 5: 1320-1330.

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