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Plants and algae have a large capacity to acclimate themselves to changing environments. We are interested in these acclimation processes, in particular, how efficiently yet safely they harness sunlight for photosynthesis under fluctuating light conditions. Using a model green alga, we are studying the molecular mechanisms underlying photoacclimation of the photosynthetic machinery. We are also applying the knowledge obtained in the studies of a model green alga to various phytoplankton including *Symbiodinium* in corals and sea anemones in tropical oceans, to explore how the environmentally important photosynthetic organisms thrive in their ecological niche.

I. Macroorganization of photosynthetic machinery

Photosystem (PS) II is a multiprotein complex that splits water and initiates electron transfer in photosynthesis. The central part of PSII, the PSII core, is surrounded by light-harvesting complex II proteins (LHCIIs). In higher plants, two or three LHCII trimers are seen on each side of the PSII core whereas only one had been seen in the corresponding positions in a unicellular green alga *Chlamydomonas reinhardtii*. Recently, we re-examined the supramolecular organization of this PSII-LHCII supercomplex in *C. reinhardtii* by solubilizing the thylakoid membranes with n-dodecyl-α-D-maltoside and subjecting them to gel-filtration. This newly-prepared PSII-LHCII supercomplex bound twice as much LHCII than previously reported and retained higher oxygen-evolving activity. Single-particle image analysis of the electron micrographs revealed that the PSII-LHCII supercomplex had a novel supramolecular organization, with three LHCII trimers attached to each side of the core.

II. Acclimation of photosynthesis

Using *C. reinhardtii*, we investigate the molecular mechanisms underlying the acclimation processes of the photosynthetic complexes such as state transitions and non-photochemical quenching by means of biochemistry, molecular genetics, optical spectroscopy, small-angle neutron scattering, and bio-imaging.

2-1 State transitions

Plants respond to changes in light quality by regulating the absorption capacity of their PSs. These short-term acclimations use redox-controlled, reversible phosphorylation of LHCIIs to regulate the relative absorption cross-section of the two photosystems, commonly referred to as state transitions. It is acknowledged that state transitions induce substantial reorganizations of the PSs. However, their consequences on the chloroplast structure are more controversial. Here, we investigate how state transitions affect the chloroplast structure and function using complementary approaches for the living cells of *Chlamydomonas reinhardtii*. Using small-angle neutron scattering, we found a strong periodicity of the thylakoids in State 1, with characteristic repeat distances of approximately 200 Å, which was almost completely lost in State 2. As revealed by circular dichroism, changes in the thylakoid periodicity were paralleled by modifications in the long-range order arrangement of the photosynthetic complexes, which was reduced by approximately 20% in State 2 compared with State 1, but was not abolished. Furthermore, absorption spectroscopy reveals that the enhancement of PSI antenna size during State 1 to State 2 transition (approximately 20%) is not commensurate to the decrease in PSII antenna size (approximately 70%). This implies that a large part of the phosphorylated LHCIIs do not bind to PSI, but instead form energetically quenched complexes, which we have shown to be either associated with PSII supercomplexes or in a free form (Figure 1). Altogether these noninvasive in vivo approaches allowed us to present a more likely scenario for state transitions that explains their molecular mechanism and physiological consequences.

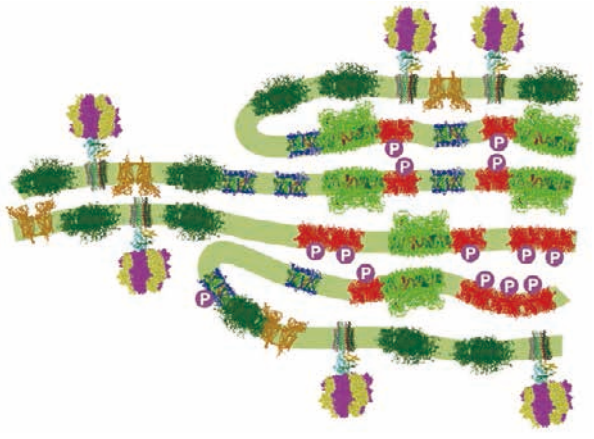

Figure 1. Hypothetical model for chloroplast remodeling during state transitions in *C. reinhardtii*. Side views of the membrane planes showing thylakoid ultrastructure and PS supercomplex composition in State 2. A number of LHCII proteins are phosphorylated, and the thylakoids are partially unstacked and undulated. The periodicity of the thylakoid membranes is weak. Most of the phosphorylated LHCIIs are in an energy-quenching state (red). They either remain associated with PSII so that a large part of the PSII–LHCII supercomplex array is preserved, or are unbound and aggregated.

2-2 Energy-dependent quenching of excess energy (qE quenching)

Absorption of light in excess of the capacity for photosynthetic electron transport is damaging to photosynthetic organisms. Several mechanisms exist to avoid photodamage, which are collectively referred to as non-photochemical quenching (NPQ). This term comprises at least two major processes: state transitions (qT), the change in the relative antenna sizes of PSII and PSI as described in the previous section, and energy-dependent quenching of excess energy (qE), the increased thermal dissipation triggered by lumen acidification. Recently, we isolated the PSII-LHCII supercomplex from both WT *C. reinhardtii* and the *npq4* mutant, which is qE-deficient and lacks the ancient light-harvesting protein LHCSR. LHCSR3 was present in the PSII-LHCII supercomplex from the high light-grown WT but not in the supercomplex from the low light-grown WT or the *npq4* mutant. The purified PSII-LHCII supercomplex containing LHCSR3 showed a normal fluorescence lifetime at a neutral pH (7.5) by single-photon counting analysis but exhibited a significantly shorter lifetime (energy-quenching) at pH 5.5, which mimics the acidified lumen of the thylakoid membranes in high light-exposed chloroplasts. The switching from light-harvesting mode to energy-dissipating mode observed in the LHCSR3-containing PSII-LHCII supercomplex was inhibited by DCCD (dicyclohexylcarbodiimide), a protein-modifying agent specific to protonatable amino acid residues. We conclude that the PSII-LHCII-LHCSR3 supercomplex formed in high light-grown *C. reinhardtii* cells is capable of energy dissipation upon protonation of LHCSR3 (Figure 2).

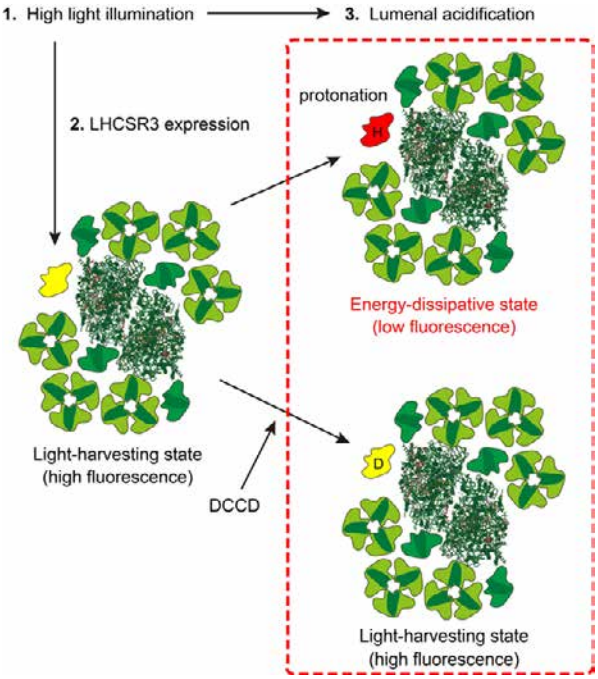

Figure 2. A model for the induction of qE in *C. reinhardtii*.

Although it has been known that two very closely related genes (*LHCSR3.1* and *LHCSR3.2*) encoding LHCSR3 protein and another paralogous gene *LHCSR1* are present in the *C. reinhardtii* genome, it was unclear how these isoforms are differentiated in terms of transcriptional regulation and functionalization. We showed that transcripts of both of the isoforms, *LHCSR3.1* and *LHCSR3.2*, are accumulated under high light stress. Reexamination of the genomic sequence and gene models along with a survey of sequence motifs suggested that these two isoforms shared an almost identical but still distinct promoter sequence and a completely identical polypeptide sequence, with more divergent 3'-untranscribed regions. Transcriptional induction under high light condition of both isoforms was suppressed by treatment with a PSII inhibitor, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), and a calmodulin inhibitor W7.

Despite a similar response to high light, the inhibitory effects of DCMU and W7 to *LHCSR1* transcript accumulation were limited compared to *LHCSR3* genes. These results suggest that the transcription of *LHCSR* paralogs in *C. reinhardtii* are regulated by light signals and differentially modulated via photosynthetic electron transfer and calmodulin-mediated calcium signaling pathway(s) (Figure 3).

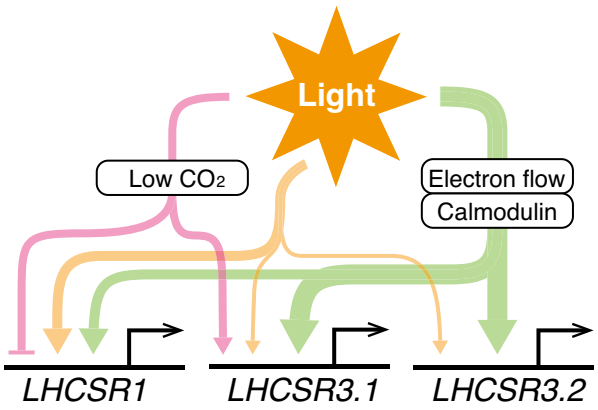

Figure 3. A model of transcriptional regulation of *LHCSR* genes in *C. reinhardtii*.

III. Ecophysiology of micro algae

Our new projects are the study of photoacclimation of dinoflagellates that can live in a symbiotic relationship with cnidarians, and the study of oil-producing *Chlamydomonas*. We are particularly interested in a dinoflagellate *Symbiodinium* living with corals and sea anemones (Figure 4), and the oil-producing *Chlamydomonas* grown under natural pond-like environments. We are trying to elucidate how their photosynthetic machinery acclimates to variable light and temperature conditions.

Figure 4. Fluorescence image of the tiny sea anemone *Aiptasia*, a model system for studies of dinoflagellate (*Symbiodinium*)-cnidarian symbiosis. Each red dot is a cell of *Symbiodinium*.

Publication List

〔Original papers〕

- Maruyama, S., Tokutsu, R., and Minagawa, J. (2014). Transcriptional regulation of the stress-responsive light harvesting complex genes in *Chlamydomonas reinhardtii*. Plant Cell Physiol. *55*, 1304-1310.
- Nagy, G., Ünnep, R., Zsiros, O., Tokutsu, R., Takizawa, K., Porcar, L., Moyet, L., Petroutsos, D., Garab, G., Finazzi, G., and Minagawa, J. (2014). Chloroplast remodeling during state transitions in *Chlamydomonas reinhardtii* as revealed by non-invasive techniques in vivo. Proc. Natl. Acad. Sci. USA *111*, 5042-5047.
- Shibata, Y., Katoh, W., Chiba, T., Namie, K., Ohnishi, N., Minagawa, J., Nakanishi, H., Noguchi, T., and Fukumura, H. (2014). Development of a novel cryogenic microscope with numerical aperture of 0.9 and its application to photosynthesis research. Biochim. Biophys. Acta *1837*, 880-887.
- Takahashi, H., Okamuro, A., Minagawa, J., and Takahashi, Y. (2014). Biochemical characterization of photosystem I associating light-harvesting complexes I and II isolated from State-2 cells of *Chlamydomonas reinhardtii*. Plant Cell Physiol. *55*, 1437-1449.

〔Review articles〕

- Finazzi, G., and Minagawa, J. (2014). High light acclimation in green microalgae. *In* "Non-Photochemical Quenching and Energy Dissipation in Plants, Algae, and Cyanobacteria" (B. Demmig-Adams, G. Garab G, W. Adams III and Govindjee, Eds.), pp.445-469, Advances in Photosynthesis and Respiration Vol.40, Springer, Dordrecht.
- Johnson, G. N., Cardol, P., Minagawa, J., and Finazzi, G. (2014). Regulation of electron transport in photosynthesis. *In* "Plastid Biology" (Theg, S., Wollman, F.-A., Eds.)" pp.437-464, Advances in Plant Biology Vol. 5, Springer, Dordrecht.