Division of Environmental Photobiology

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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 the changing light environment. Using a model green alga, we are studying the molecular mechanisms underlying the photoacclimation of the photosynthetic machinery. We are also applying the knowledge obtained in studies of model green algae to various phytoplankton including diatoms in the subarctic North Pacific, prasinophytes in the subtropical Mediterranean Sea, and *Symbiodinium* in corals in tropical oceans, to explore how these environmentally important photosynthetic organisms thrive in their ecological niche.

I. Acclimation of photosynthesis

Using a unicellular green alga *Chlamydomonas reinhardtii*, we investigate the molecular mechanisms underlying the acclimation processes of the photosynthetic complexes by means of biochemistry, molecular genetics, absorption and fluorescence spectroscopy, and bio-imaging.

1-1 State-transitions

The two photosystems—photosystem I (PSI) and II (PSII)—in the thylakoid membranes function as charge-separation devices. Each has a distinct pigment system with distinct absorption characteristics (PSI has a broad absorption peak in the far-red region as well as peaks in the blue and red regions, whereas PSII has absorption peaks in the blue and red, but not in the far-red region) and a distinct action spectrum. Thus, an imbalance of energy distribution between the two photosystems tends to occur in natural environments, where light quality and quantity fluctuate with time. Since the two photosystems are connected in series under normal conditions, green plants and algae need to constantly balance their excitation levels to ensure optimal efficiency of electron flow. State transitions occur under such conditions to redistribute the harnessed energy to minimize its unequal distribution.

Although state transitions have been widely accepted as a short-term response in plants to acclimate to the fluctuating light conditions, most of the previous investigations were conducted *in vitro*, implying that the real impact on photosynthesis remains to be characterized. This year, we visualized phospho-LHCII dissociation during state transitions using fluorescence lifetime imaging microscopy (FLIM) for the first time *in vivo*, where the fluorescence lifetime in live *C. reinhardtii* cells was monitored under a fluorescence microscope during a transition from State 1 to 2. Initially, the average lifetime of fluorescence emitted between 680-700 nm was 170 psec, which was largely due to the PSII-bound LHCII, but it shifted to 250 psec when the cells were in transition to State 2 after 5 min. Single-cell FLIM further indicated that the dissociated LHCII spreads through the cell during State 2 transitions and forms several large spotted areas. Further biochemical analyses indicated that dissociated phospho-LHCII formed a large aggregated structure, whereas unphosphorylated LHCII did not. Thus, the free phospho-LHCII aggregates appearing during State 2 transitions are in energy-dissipative form.

The molecular mechanism for q_E quenching has been a heated issue during the last two decades, and it still remains controversial. Because the unexpectedly short fluorescence lifetime of the phospho-LHCII aggregates during the state transition described above was not caused by high light illumination, they are not exhibiting q_E quenching, but rather exhibiting q_T (state transition) quenching. However, it is now tempting to speculate that LHCII aggregates are a common site of energy dissipation, i.e., that both q_E and q_T quenching are causally related by the energy-dissipative LHCII aggregates.

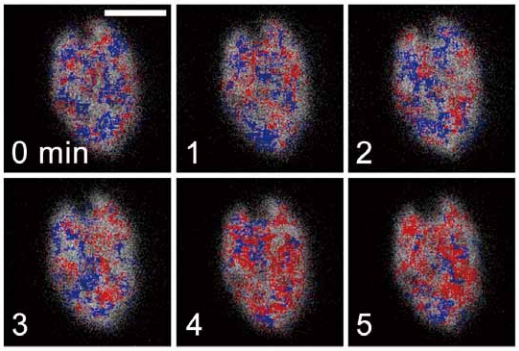

Figure 1. Visualization of the progress of a state 1-to-2 transition by means of FLIM. Blue and red dots correspond to 170 and 250 psec lifetime components, respectively.

1-2 Cyclic electron flow

In eukaryotes, photosynthesis is a process of photochemical energy transduction, which occurs via the conductance of electron flow in the thylakoid membranes of chloroplasts, resulting in the reduction of $NADP^+$ in the stroma and the concomitant generation of a proton motive force across the membranes. The NADPH generated by the electron flow and the ATP synthesized by ATP synthase utilizing the proton motive force are used to fix carbon dioxide in the Calvin-Benson cycle. Linear electron flow (LEF) and cyclic electron flow (CEF) are known as modes of electron flow in photosynthesis. In the linear pathway, electrons are transferred from PSII to $NADP^+$ by way of the cytochrome b_6f complex (Cyt b_6f) and PSI. In the cyclic pathway, however, the exact pathway of electrons that originate in PSI and then return to PSI has not been clear. State transitions have long been considered as a mechanism by which the distribution of light excitation between the two photosystems is regulated. However, the performance of PSI tends to overwhelm PSII under State 2 conditions in *C. reinhardtii* because of its extensive ability to relocate LHCII

proteins; this implies that state transitions might represent a mechanism by which the electron transfer chain in the thylakoid membranes is switched to the mechanism exclusively employed by PSI.

This year, we solubilized thylakoid membranes from *C. reinhardtii* cells under State 2 conditions and loaded them onto a sucrose density gradient. A "super-supercomplex" (CEF supercomplex) with a molecular weight of approximately 1.5 million composed of the PSI-LHCI supercomplex with LHCIIs, Cyt *bf*, Fd-NADPH oxidoreductase (FNR), and the integral membrane protein PGRL1 was detected in a fraction heavier than the PSI-LHCI supercomplex. Spectroscopic analyses indicated that upon illumination, reducing equivalents downstream of PSI were transferred to Cyt *bf*, while the oxidized PSI was re-reduced by reducing equivalents from Cyt *bf*, indicating that this supercomplex is engaged in CEF. Thus, CEF takes place in a protein supercomplex where steps in LEF are rearranged to undergo an alternative pathway for the flow of electrons.

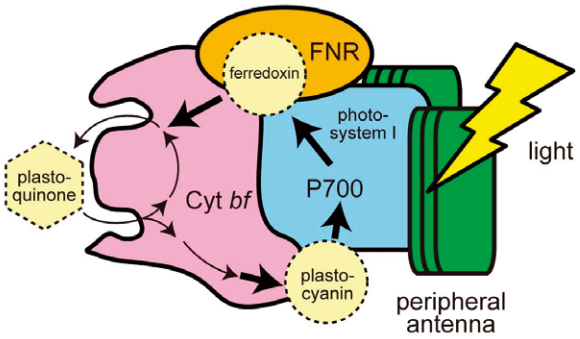

Figure 2. Cyclic electron flow by way of the CEF supercomplex.

II. Ecophysiology of marine phytoplankton

Prasinophyceae are a broad class of early-branching eukaryotic green algae. These picophytoplankton are found ubiquitously throughout the ocean and contribute considerably to global carbon-fixation. *Ostreococcus tauri*, as the first sequenced prasinophyte, is a model species for studying the functional evolution of light-harvesting systems in photosynthetic eukaryotes.

This year, we isolated and characterized *O. tauri* pigment-protein complexes to understand the diversity and the evolutional traits of the light-harvesting systems in a primitive green alga. Two PSI fractions were obtained by sucrose density gradient centrifugation in addition to free LHC fraction and PSII core fractions. The smaller PSI fraction contains the PSI core proteins, LHCI, which are conserved in all green plants, Lhcp1, a prasinophyte-specific LHC protein, and the minor, monomeric LHCII proteins CP26 and CP29. The larger PSI fraction contained the same antenna proteins as the smaller, with the addition of Lhca6 and Lhcp2, and a 30% larger absorption cross-section. When *O. tauri* was grown under high-light conditions, only the smaller PSI fraction was present. The two PSI preparations were also found to be devoid of far-red chlorophyll fluorescence (715-730 nm), a signature of PSI in oxygenic phototrophs. These unique features of *O. tauri* PSI may reflect primitive light-harvesting systems in green plants and their adaptation to marine ecosystems.

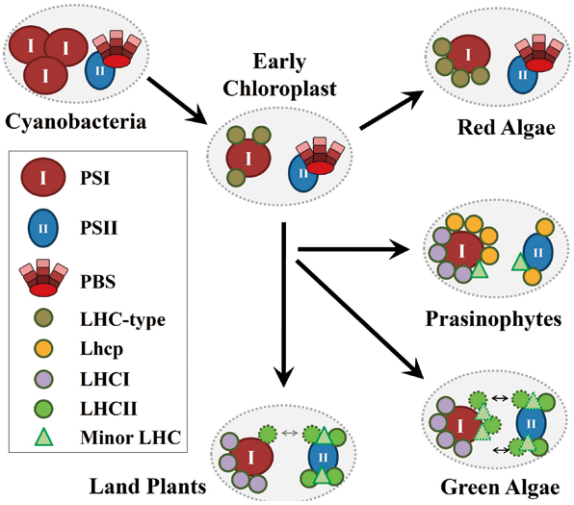

Figure 3. Evolutionary model of LHC affinity in photosynthetic eukaryotes as revealed by biochemical study of the LHC systems in *O. tauri*.

Publication List

〔Original papers〕

- Hohmann-Marriott, M.F., Takizawa, K., Eaton-Rye, J.J., Mets, L., and Minagawa, J. (2010). The redox state of the plastoquinone pool directly modulates minimum chlorophyll fluorescence yield in *Chlamydomonas reinhardtii*. FEBS Lett. *584*, 1021-1026.
- Iwai, M., Takizawa, K., Tokutsu, R., Okamuro, A., Takahashi, Y., and Minagawa, J. (2010). Isolation of the elusive supercomplex that drives cyclic electron flow in photosynthesis. Nature *464*, 1210-1213.
- Iwai, M., Yokono, M., Inada, N., and Minagawa, J. (2010). Live-cell imaging of photosystem II antenna dissociation during state transitions. Proc. Natl. Acad. Sci. USA *107*, 2337-2342.
- Swingley, W.D., Iwai, M., Chen, Y., Ozawa, S.I., Takizawa, K., Takahashi, Y., and Minagawa, J. (2010). Characterization of photosystem I antenna proteins in the prasinophyte *Ostreococcus tauri*. Biochim. Biophys. Acta *1797*, 1458-1464.