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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 fluctuating light conditions. 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 the studies of a model green alga to various phytoplankton including diatoms in the subarctic North Pacific, prasinophytes in the subtropical Mediterranean Sea, and *Symbiodinium* in corals and sea anemones in tropical oceans, to explore how these 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

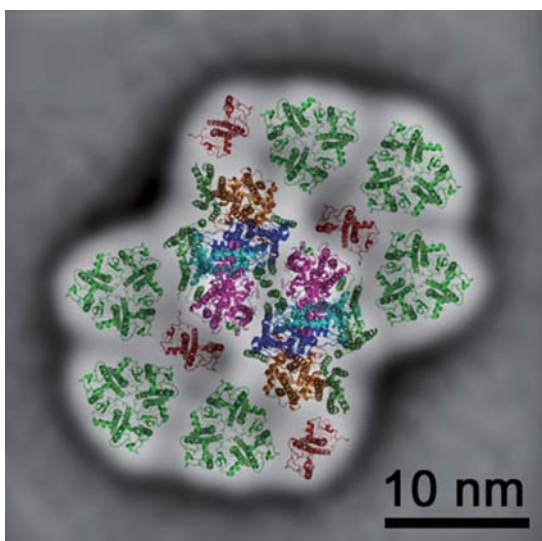


Figure 1. A top view of the PSII-LHCII supercomplex in *C. reinhardtii* as revealed by single particle analysis of electron micrographs.

central part of PSII, the PSII core, is surrounded by light-harvesting complex II proteins (LHCII). In higher plants, two or three LHCII trimers are seen on each side of the PSII core whereas only one is seen in the corresponding positions in a unicellular green alga *Chlamydomonas reinhardtii*. 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 the previously reported supercomplex 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 (Figure 1).

II. Acclimation of photosynthesis

Using *C. 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.

2-1 Non-photochemical 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 photosystem II and I, and energy-dependent quenching (qE), the increased thermal dissipation triggered by lumen acidification. Recently, *npq4*, a mutant strain of *C. reinhardtii* that is qE-deficient and lacks the ancient light-harvesting protein LHCSR3 was reported. Applying the newly-established procedure described above, we isolated the PSII-LHCII supercomplex from both WT *C.*

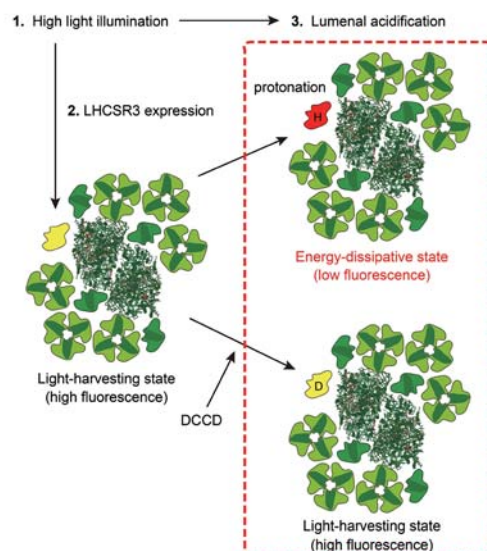


Figure 2. A model for the induction of qE in *C. reinhardtii*.

reinhardtii and the *npq4* mutant grown in either low light or high light. 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 sensitive to 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).

Furthermore, to investigate the respective roles of qE and qT in photoprotection, a mutant (*npq4 stt7-9*) was generated in *C. reinhardtii* by crossing the state transition-deficient mutant (*stt7-9*) with the *npq4* mutant. The comparative phenotypic analysis of the WT, the single mutants and the double mutant reveals that both state transitions and qE are induced in high light. Moreover, the double mutant presents an increased photosensitivity with respect to the single mutants and WT. We suggest therefore that besides qE, state transitions also play a photoprotective role during high light acclimation of the cells.

2-2 Cyclic electron flow

Photosynthetic light reactions establish electron flow in the chloroplast's thylakoid membranes, leading to the production of the ATP and NADPH that participate in carbon fixation. Two modes of electron flow exist—linear electron flow (LEF) from water to NADP⁺ via photosystem (PS) II and PSI in series and cyclic electron flow (CEF) around PSI. Although CEF is essential for satisfying the varying demand for ATP, the exact molecule(s) and operational site are as yet unclear. In the green alga *C. reinhardtii*, the electron flow shifts from LEF to CEF upon preferential excitation of PSII, which is brought about by an energy balancing mechanism between PSII and PSI (state transitions). We isolated a protein supercomplex composed of PSI with its own light-harvesting complex (LHCI), the PSII light-harvesting complex (LHCII), the cytochrome *bf* complex (Cyt *bf*), ferredoxin (Fd)-NADPH oxidoreductase (FNR), and the integral membrane protein PGRL1 from *C. reinhardtii* cells under PSII-favoring conditions. Spectroscopic analyses

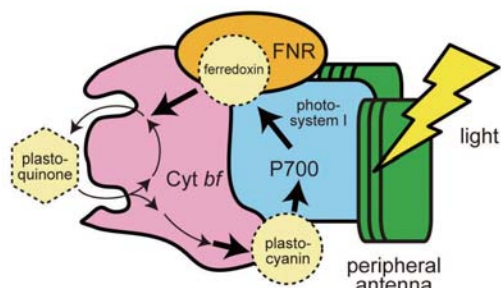


Figure 3. Cyclic electron flow by way of the CEF supercomplex.

indicated that upon illumination, reducing equivalents from downstream of PSI were transferred to Cyt *bf*, while oxidized PSI was re-reduced by reducing equivalents from Cyt *bf*, indicating that this supercomplex is engaged in CEF (Figure 3). Thus, formation and dissociation of the PSI-LHCI-LHCII-FNR-Cyt *bf*-PGRL1 supercomplex not only controlled the energy balance of the two photosystems, but also switched the mode of photosynthetic electron flow.

III. 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.

We isolated and characterized *O. tauri* pigment-protein complexes to understand the diversity and the evolutionary 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.

Our newest project is the study of photoacclimation of *Symbiodinium*, which live in a symbiotic relationship with corals, and other Cnidarians. We are particularly interested in those living with corals and sea anemones and are trying to elucidate how their photosynthetic machinery is acclimated to the variable light and temperature environments in the tropical ocean.

Publication List

[Original papers]

- Alloreant, G., Tokutsu, R., Roach, T., Peers, G., Cardol, P., Girard-Bascou, J., Seigneurin-Berny, D., Petroutos, D., Kuntz, M., Breyton, C., Franck, F., Wollman, F.-A., Niyogi, K.K., Kreiger-Liszky, A., Minagawa, J., and Finazzi, G. (2013). A dual strategy to cope with high light in *Chlamydomonas reinhardtii*. *Plant Cell* 25, 545-557.
- Tokutsu, R., and Minagawa, J. (2013). Energy-dissipative supercomplex of photosystem II associated with LHCSR3 in *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. USA* 110, 10016-10021.

[Review article]

- Minagawa, J. (2013). Dynamic reorganization of photosynthetic supercomplexes during environmental acclimation of photosynthesis. *Front. Plant Sci.* 4, 513.