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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 environmentally important photosynthetic organisms thrive in their ecological niche.

I. Acclimation of photosynthesis

Using a green unicellular alga *C. reinhardtii*, we investigate the molecular mechanisms underlying the acclimation processes of the photosynthetic complexes such as state transitions, non-photochemical quenching, photoinhibition, and cyclic electron flow by means of biochemistry, molecular genetics, various physiological analyses, and optical spectroscopy.

1-1 State transitions

Photosynthetic organisms 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. Each of the two charge-separation devices—PSI and PSII—in the thylakoid membranes has a distinct pigment system with unique absorption characteristics. Thus, an imbalance of energy distribution between the two photosystems tends to occur in natural environments, where light quality and quantity fluctuate with time. Because the two photosystems are functionally connected in series under normal conditions, plants and algae must constantly balance their excitation levels to ensure the optimal efficiency of electron flow. State transitions occur under such conditions to balance the light-harvesting capacities of the two photosystems, thereby minimizing the unequal distribution of light energy. State 1 occurs when PSI is preferentially excited and the light-harvesting capacities of PSII and PSI are increased and decreased, respectively, to adjust the excitation imbalance; this state is indicated by a higher chlorophyll fluorescence yield at room temperature. Conversely, State 2 occurs when PSII is preferentially excited and the light-harvesting capacities of PSII and PSI are decreased and increased, respectively, to readjust the excitation imbalance; this state can be monitored as a lower chlorophyll fluorescence yield at room temperature (Figure 1).

In *C. reinhardtii* WT cells, the modulation of CEF normally occurs in parallel with state transitions. When the light-induced reduction of Cyt *bf* was probed in State 1- and State

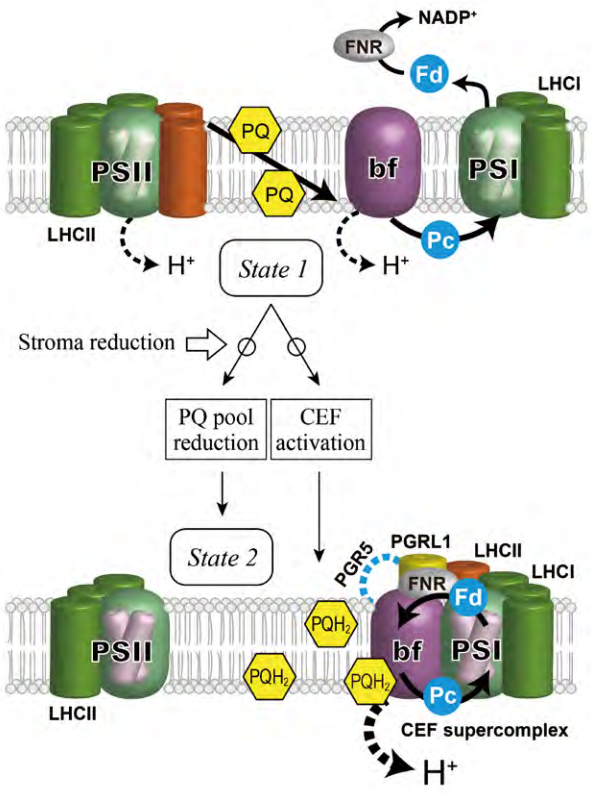

Figure 1. Schematic representation of the regulation of electron flow and state transitions in *C. reinhardtii*. Upper: when PSI is preferentially excited, the stroma of chloroplast and the PQ pool are oxidized. Under these conditions, LHCIIs are bound to PSII (State 1). The photosynthetic electron flow proceeds in linear electron flow (LEF) mode, generating NADPH as well as a proton gradient across the thylakoid membrane that is used for ATP production. Middle: when the stroma is reduced, first, cyclic electron flow (CEF) is activated by the association of Cyt *bf* and FNR with PSI to form a super-supercomplex (CEF supercomplex). PGRL1 and possibly PGR5 are also associated with the CEF supercomplex; second, PQ pool is reduced and migration of the mobile LHCIIs (*orange*) from PSII to PSI occurs; Lower: the cells are in State 2, and the photosynthetic electron flow proceeds in CEF mode.

2-adapted cells, a differential sensitivity to the addition of the PSII inhibitor DCMU was observed. In the presence of DCMU, the reduction of Cyt *bf* was suppressed in State 1 but not in State 2. An identical sensitivity to an inhibitor of Cyt *bf*, DBMIB, was observed in both State 1 and State 2, suggesting that PSII-independent Cyt *bf* reduction occurs only in State 2. However, state transitions and CEF/LEF switching are not mechanically linked in *C. reinhardtii* but the two phenomena are rather coincidental. Under anaerobic conditions, the independent knockdown of three thylakoid membrane proteins, PGRL1, CAS, and ANR1, resulted in decreased CEF activity, but the ability to undergo state transitions was unaffected. This was further supported by the low CEF activity in a State 2-locked mutant of *C. reinhardtii*. Although the lateral migration of mobile LHCIIs occurred in the ptox2 mutant, which was State 2-locked due to a lack of plastid terminal oxidase 2, the effects on $P700^+$ re-reduction were negligible. CEF was thus proposed to be correlated with a reduced state of the stroma.

1-2 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 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, 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.

1-3 Photodamage to photosystem II

Light, the driving force of photosynthesis, damages photosynthetic machinery, primarily photosystem II (PSII), and it results in photoinhibition. A new photodamage model, the two-step photodamage model, suggests that photodamage to PSII initially occurs at the oxygen evolving complex (OEC) because of light energy absorbed by manganese and that the PSII reaction center is subsequently damaged from light energy absorbed by photosynthetic pigments due to the limitation of electrons to the PSII reaction center. However, it was uncertain whether this model is applicable to photodamage to PSII under visible light as manganese absorbs visible light only weakly. In our study, we demonstrated using PSII membrane fragments isolated from spinach leaves that visible light damages OEC prior to photodamage to the PSII reaction center. This finding supports that the two-step photodamage model is applicable to photodamage to PSII by visible light.

1-4 Cyclic electron flow in plants

CEF around PS I is difficult to quantify. In our study, a new method was introduced to measure CEF in wild-type and *pgr5* and *ndh* mutants of *Arabidopsis*. We obtained the linear electron flux ($LEFO_2$) through both photosystems and the total electron flux through PS I (ETR1) in *Arabidopsis* in CO_2-enriched air. $\Delta Flux = ETR1 - LEFO_2$ is an upper estimate of CEF, which consists of two components, an antimycin A-sensitive, PGR5 (proton gradient regulation 5 protein)-dependent component and an insensitive component facilitated by a chloroplastic nicotinamide adenine dinucleotide dehydrogenase-like complex (NDH). Our results demonstrated that (1) 40% of the absorbed light was partitioned to PS I; (2) at high irradiance a substantial antimycin A-sensitive CEF occurred in the wild type and the *ndh* mutant; (3) at low irradiance a sizable antimycin A-sensitive CEF occurred in the wild type but not in the *ndh* mutant, suggesting an enhancing effect of NDH in low light; and (4) in the *pgr5* mutant, the wild type, and *ndh* mutant treated with antimycin A, a residual ΔFlux existed at high irradiance, attributable to charge recombination and/or pseudo-cyclic electron flow. Therefore, in low-light-acclimated plants exposed to high light, ΔFlux has contributions from various paths of electron flow through PS I.

II. 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 3), 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. Furthermore, our interest has been expanded to higher plants.

2-1 Diversification of light harvesting complex gene family via intra- and intergenic duplications in the coral symbiotic alga *Symbiodinium*

The unicellular dinoflagellate alga *Symbiodinium* is known to be an endosymbiont of cnidarian animals including corals and sea anemone, and provide carbohydrates generated through photosynthesis to host animals. Although *Symbiodinium* possesses a unique light-harvesting complex (LHC) gene family called chlorophyll *a*-chlorophyll c_2-peridinin protein complex (acpPC), the genome-level gene diversity and evolutionary trajectories have not been investigated. We show phylogenetic analysis revealing that

many of the acpPC/LHCs were encoded in highly duplicated genes with the multi-subunit polyprotein structures in the nuclear genome of *Symbiodinium minutum*. This provided an extended list of the LHC gene family in a single organism, including 82 loci encoding polyproteins composed of 164 LHC subunits recovered in the phylogenetic tree. In *S. minutum*, 5 phylogenetic groups of the Lhcf-type gene family, which is exclusively conserved in algae harboring secondary plastids of red algal origin, and 5 groups of the Lhcr-type, of which members are known to be associated with PSI in red algae and secondary plastids of red algal origin were identified. Notably, members classified to a phylogenetic group of the Lhcf-type (group F1) are highly duplicated, which can explain the presence of an unusually large number of LHC genes in this species. While some gene units were homologous to other units within single loci of the polyprotein genes, intergenic homologies between separate loci were conspicuous in other cases, implying that gene unit 'shuffling' by gene conversion and/or genome rearrangement might have been a driving force for the diversification. These results suggest that, through vigorous intra- and intergenic gene duplication events, the genomic framework of the photosynthesis has been forged in the coral symbiont dinoflagellate algae.

Figure 2. 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*.

2-2 Novel characteristics of photodamage to PSII in a high-light-sensitive *Symbiodinium* phylotype

Symbiodinium is genetically diverse, and acquiring suitable *Symbiodinium* phylotypes is crucial for the host to survive in specific environments, such as high-light conditions. The sensitivity of *Symbiodinium* to high light differs among *Symbiodinium* phylotypes, but the mechanism that controls light sensitivity has not yet been fully resolved. In the present study using high-light-tolerant and -sensitive *Symbiodinium* phylotypes, we examined what determines sensitivity to high light. In growth experiments under different light intensities, *Symbiodinium* CS-164 (clade B1) and CCMP2459 (clade B2) were identified as high-light-tolerant and -sensitive phylotypes, respectively. Measurements of the maximum quantum yield of photosystem II (PSII) and the maximum photosynthetic oxygen production rate after high-light exposure demonstrated that CCMP2459 is more sensitive to photoinhibition of PSII than CS-164, and tends to lose maximum photosynthetic activity faster. Measurement of photodamage to PSII under light of different wavelength ranges demonstrated that PSII in both *Symbiodinium* phylotypes was significantly more sensitive to photodamage under shorter wavelength regions of light spectra (<470 nm). Importantly, PSII in CCMP2459, but not CS-164, was also sensitive to photodamage under the regions of light spectra around 470-550 and 630-710 nm, where photosynthetic antenna proteins of *Symbiodinium* have light absorption peaks. This finding indicates that the high-light-sensitive CCMP2459 has an extra component of photodamage to PSII, resulting in higher sensitivity to high light. Our results demonstrate that sensitivity of PSII to photodamage differs among *Symbiodinium* phylotypes and this determines their sensitivity to high light.

Publication List:

〔Original Papers〕

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