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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 symbiotic dinoflagellate, *Symbiodinium,* that associates with corals and sea anemones, to explore how environmentally important photosynthetic organisms thrive in their ecological niche.

I. 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, 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 (Fig. 1) but not in the supercomplex from

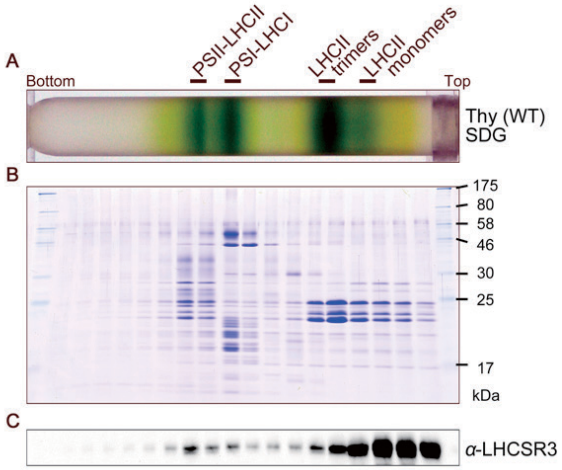

Figure 1. Purification of the PSII-LHCII-LHCSR3 supercomplex from WT C. *reinhardtii*. (A) Thylakoids from WT cells grown under high light conditions were subjected to sucrose density gradient centrifugation (SDG). (B) Polypeptides in the SDG fractions shown in (A) were analyzed by SDS/PAGE. (C) Polypeptides in the SDG fractions were subjected to immunoblotting with an antibody against LHCSR3.

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. However, its molecular mechanism remains unclear.

To investigate how LHCSR3 dissipates excitation energy, thereby protecting the PSII supercomplex, we applied fluorescence decay–associated spectra (FDAS) analysis to a purified PSII–LHCII supercomplex with and without LHCSR3 from *Chlamydomonas reinhardtii*. We found that, when the PSII supercomplex is associated with LHCSR3 under high-light conditions, excitation energy transfer from light-harvesting complexes to chlorophyll binding protein CP43 is selectively inhibited compared with that to CP47, preventing excess excitation energy from overloading the reaction center. By analyzing femtosecond up-conversion fluorescence kinetics, we further found that pH- and LHCSR3-dependent quenching of the PSII—LHCII–LHCSR3 supercomplex is accompanied by a fluorescence emission centered at 684 nm, with a decay time constant of 18.6 ps, which is equivalent to the rise time constant of the lutein radical cation generated within a chlorophyll–lutein heterodimer. These results suggest a mechanism in which LHCSR3 transforms the PSII supercomplex into an energy-dissipative state and provide critical insight into the molecular events and characteristics in LHCSR3-dependent energy quenching (Fig. 2).

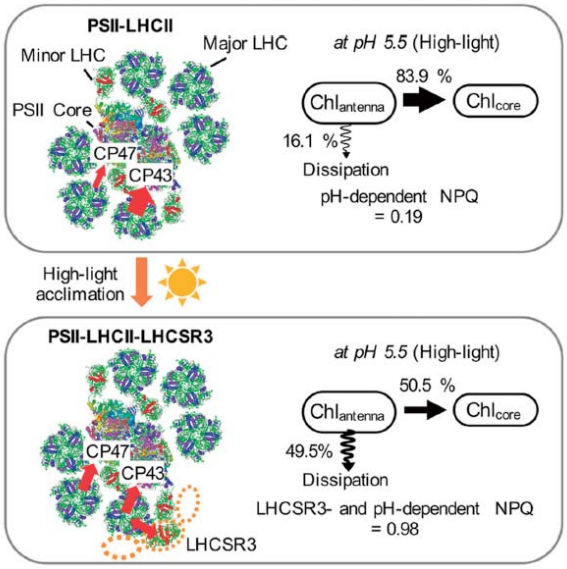

Figure 2. A proposed model of LHCSR3-dependent NPQ in PSII—LHCII–LHCSR3 supercomplexes of C. *reinhardtii*. LHCSR3 is expressed and associated with the PSII supercomplex under high-light conditions. The *circles of orange dots* represent the presumable binding site of LHCSR3. The binding of LHCSR3 to the PSII supercomplex inhibits the excitation energy transfer to CP43. At acidic pH, LHCSR3-dependent NPQ is activated, and it quenches excitation energy of LHCs. NPQ values of PSII–LHCII and PSII—LHCII–LHCSR3 were calculated from the difference between pH 7.5 and 5.5 conditions. Kim et al. (2017) *J. Biol. Chem. 17*, 18951-18960.

II. Species specificity in coral-algae symbiosis

Reef-building corals harbor endosymbiotic dinoflagellates of the genus *Symbiodinium* and rely on the energy that the algae generate from photosynthesis for their growth and survival. *Symbiodinium* are genetically diverse and their physiological characteristics (e.g., stress sensitivity) differ among phylotypes. Therefore, corals need to recruit *Symbiodinium* phylotypes that suit the environment in order to survive and adapt to changes (e.g., global change and warming). Interestingly, each coral species associate only with specific *Symbiodinium* phylotypes, consequently the diversity of symbionts available differs among coral species. However, the mechanism regulating the diversity of compatible symbionts in cnidarian organisms, including coral, was unknown.

We studied how corals select symbionts and what determines symbiont diversity in each coral species. In our study, we focus on the difference of cell size among *Symbiodinium* strains. Using a model *Aiptasia-Symbiodinium* system, we first found that, of *Symbiodinium* strains tested, only large-sized strains failed to infect the *Aiptasia* host. This size-dependency was supported by experiments using fluorescent microspheres of different sizes. We then tested the uptake of different sized *Symbiodinium* strains into aposymbiotic polyps from two different coral species. *Acropora tenuis* showed the same preference as *Aiptasia*, with no infection by the large-sized *Symbiodinium* strains. However, for *Cyphastrea serailia* all *Symbiodinium* strains tested, including the large-sized strains, were able to infect the host. Our results demonstrated that the infectivity of each *Symbiodinium* strains in a host is primarily determined by their cell size and that the diversity of symbionts in each host species is determined by their maximum acceptable symbiont cell size. We proposed that corals with a higher maximum threshold for symbiont cell size may have the opportunity to associate with more diverse *Symbiodinium* phylotypes. Such coral species may be better able to adapt to changing environmental conditions, and more specifically might be more suited to avoiding bleaching under increasing ocean temperatures.

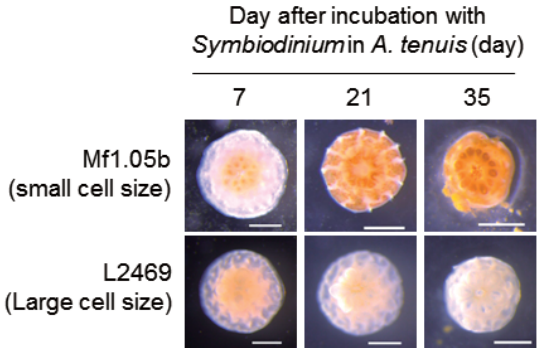

Figure 3. Infection of different Symbiodinium strains into corals. Aposymbiotic primal polyps of A. *tenuis* (scale bars, 500 μm) were separately incubated with small (Mf1.05b) and large (L2469) *Symbiodinium* strains. Uptake of *Symbiodinium* into coral polyps was monitored using a stereomicroscope. Biquand et al. (2017) *ISME J. 11*, 1702-1712.

Publication List:

〔Original Papers〕

- Biquand, E., Okubo, N., Aihara, Y., Rolland, V., Hayward, D., Hatta, M., Minagawa, J., Maruyama, T., and Takahashi, S. (2017). Acceptable symbiont cell size differs among cnidarian species and may limit symbiont diversity. ISME J. *11*, 1702-1712.
- Ho, S.-H., Nakanishi, A., Kato, Y., Yamasaki, H., Chang, J.-S., Misawa, N., Hirose, Y., Minagawa, J., Hasunuma, T., and Kondo, A. (2017). Dynamic metabolic profiling together with transcription analysis reveals salinity-induced starch-to-lipid biosynthesis in alga *Chlamydomonas* sp. JSC4. Sci. Rep. *7*, 45471.
- Kim, E., Akimoto, E., Tokutsu, R., Yokono, M., and Minagawa, J. (2017). Fluorescence lifetime analyses reveal how the high light-responsive protein LHCSR3 transforms PSII light-harvesting complexes into an energy-dissipative state. J. Biol. Chem. *292*, 18951-18960.
- Takizawa, K., Minagawa, J., Tamura, M., Kusakabe, N., and Narita, N. (2017). Red-edge position of habitable exoplanets around M-dwarfs. Sci. Rep. *7*, 7561.