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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. 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 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. However, the environmental cue that triggers the expression of LHCSR3 protein has been elusive.

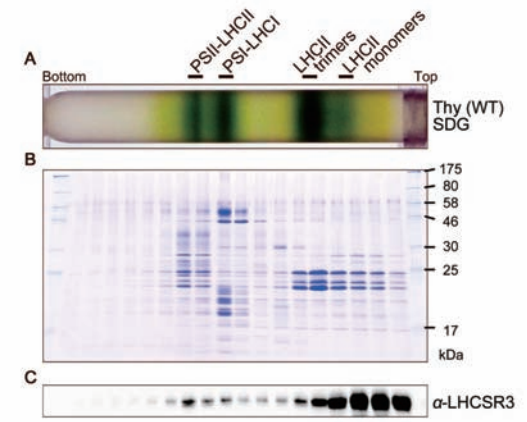

Figure 1. Purification of the PSII-LHCII-LHCSR3 supercomplex from WT *C. reinhardtii*. (A) Thylakoids from WT cells grown under high light conditions were subjected to sucrose density gradient centrifugation (SDG). (B) Polypeptides in the SDG fractions shown in (A) were analyzed by SDS/PAGE. (C) Polypeptides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

In plants and algae, light serves both as the energy source for photosynthesis and as a biological signal that triggers cellular responses via specific sensory photoreceptors. Red light is perceived by bilin-containing phytochromes and blue light by the flavin-containing cryptochromes and/or phototropins (PHOTs), the latter containing two photosensory light, oxygen, or voltage (LOV) domains. Photoperception spans several orders of light intensity, ranging from far below the threshold for photosynthesis to values beyond the capacity of photosynthetic CO_2 assimilation. We revealed that PHOT controls qE by inducing the expression of LHCSR3 in high light intensities. This control requires blue-light perception by LOV domains on PHOT, LHCSR3 induction through PHOT kinase, and light dissipation in photosystem II via LHCSR3. Mutants deficient in the PHOT gene display severely reduced fitness under excessive light conditions, indicating that the sensing, utilization, and dissipation of light is a concerted process that plays a vital role in microalgal acclimation to environments of variable light intensities. Here we demonstrated the existence of a molecular link between photoreception, photosynthesis, and photoprotection in the green alga *Chlamydomonas reinhardtii*.

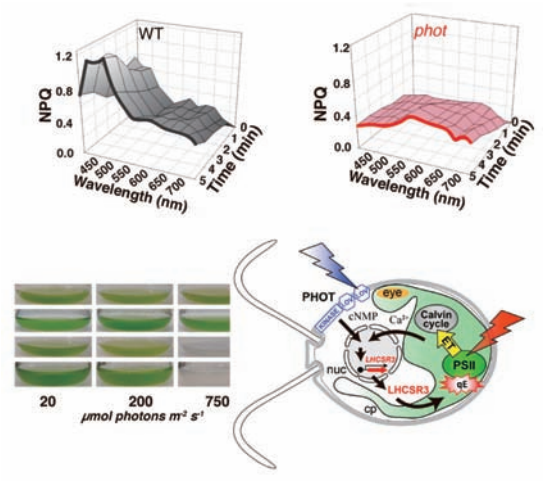

Figure 2. PHOT controls induction of LHCSR3 and qE and is crucial for survival of *C. reinhardtii* in high light. NPQ in WT (A) and *phot* (B) cells after exposure for 4 h to different wavelengths of high light). (C) Erlenmeyer flasks containing WT, *acry*, *phot* and *npq4* cells after 16 h of exposure to light of 20, 200 and 750 µmol photons m^{-2} s^{-1}. (D) Schematic representation of the relationship between photoreception, photosynthesis and photoprotection in *C. reinhardtii*. cp, chloroplast; cNMP, cyclic nucleotide mono phosphate (cAMP or cGMP); ET, electron transport; eye, eyespot; nuc, nucleus; PSII, photosystem II.

II. Photoprotection mechanism in symbiotic algae

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* within corals is a major producer in coral reef ecosystems, which represent one of the most biologically rich environments on earth. When the photosynthesis in *Symbiodinium* is damaged, corals bleach and the sustainability of the reefs is endangered. Photosynthesis in *Symbiodinium* is sensitive to small increases in seawater temperature, resulting in photoinhibition of photosynthesis. The thermal sensitivity of *Symbiodinium* to photoinhibition differs among *Symbiodinium* strains but its mechanism has not been well understood.

Previous studies have demonstrated that increase in seawater temperature enhances cyclic electron flow (CEF), which sustains photoprotective thermal energy dissipation (qE), in *Symbiodinium*. However, the result was still controversial. Furthermore, it was uncertain whether this ability differs among *Symbiodinium* strains. We therefore examined the effect of increased temperature on CEF using different *Symbiodinium* strains. The light-dependent reduction of the primary electron donor PSI, i.e., P700+, was enhanced in all *Symbiodinium* strains by increasing temperatures, indicating CEF was induced by heat, which is accompanied by qE activation. However the critical temperatures for inducing CEF were different among *Symbiodinium* strains. The clade A strains with greater susceptibility to photoinhibition, OTcH-1 and Y106, exhibited higher CEF activities under moderate heat stress than a more phototolerant clade B strain Mf1.05b, suggesting that the observed CEF induction was not a preventive protection mechanism, but was a stress response in *Symbiodinium*.

III. Species specificity in coral-algae symbiosis

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.

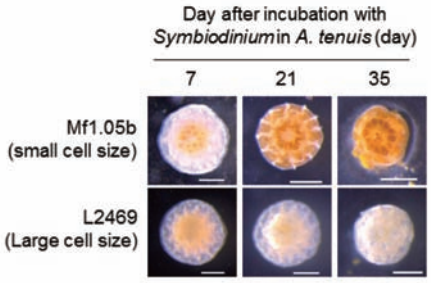

Figure 3. Infection of different Symbiodinium strains into corals. Aposymbiotic primal polyps of *A. tenuis* (scale bars, 500 µm) were separately incubated with small (Mf1.05b) and large (L2469) *Symbiodinium* strains. Uptake of *Symbiodinium* into coral polyps was monitored using a stereomicroscope.

Publication List:

〔Original Papers〕

- Wang, L., Yamano, T., Takane, S., Niikawa, Y., Toyokawa, C., Ozawa, S., Tokutsu, R., Takahashi, Y., Minagawa, J., Kanesaki, Y., Yoshikawa, H., and Fukuzawa, H. (2016). Chloroplast-mediated regulation of CO_2-concentrating mechanism by Ca^{2+}-binding protein CAS in the green

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- Petroutsos, D.*, Tokutsu, R.*, Maruyama, S., Flori, S., Greiner, A., Magneschi, L., Cusant, L., Kottke, T., Mittag, M., Hegemann, P., Finazzi, G., and Minagawa, J. (2016). A blue light photoreceptor mediates the feedback regulation of photosynthesis. Nature *537*, 563-566. (*: Co-first authors)
- Ueki, N., Ide, T., Mochiji, S., Kobayashi, Y., Tokutsu, R., Ohnishi, N., Yamaguchi, K., Shigenobu, S., Tanaka, K., Minagawa, J., Hisabori, T., Hirono, and M., Wakabayashi, K. (2016). Eyespot-dependent determination of the phototactic sign in *Chlamydomonas reinhardtii*. Proc. Natl. Acad. Sci. USA *113*, 5299-5304.
- Aihara, Y., Takahashi, S., and Minagawa, J. (2016). Heat induction of cyclic electron flow around photosystem I in the symbiotic dinoflagellate *Symbiodinium*. Plant Physiol. *171*, 522-529.
- Watanabe, C.K., Yamori, W., Takahashi, S., Terashima, I., and Noguchi, K. (2016). Mitochondrial alternative pathway-associated photoprotection of photosystem II is related to the photorespiratory pathway. Plant Cell Physiol. *57*, 1426-1431.
- Yamamoto, H., Takahashi, S., Murray, R.B., and Shikanai, T. (2016). Artificial remodelling of alternative electron flow by flavodiiron proteins in *Arabidopsis*. Nature Plants *2*, 16012.