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Molecular studies on high light acclimation in *Cyanobacteria*

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Acclimation to light environments is one of the most important responses in photosynthetic organisms to optimize the photosynthetic apparatus. A most typical response to high light is down-regulation of antenna complexes and reaction centers to reduce light excitation itself. Secondly, photosystem stoichiometry (ratio of photosystem I (PSI) to photosystem II (PSII)) is readjusted upon shift to high light, reflecting that two photosystems having distinct antenna are tandemly coupled with each other. Thirdly, protecting enzymes such as superoxide dismutase and catalase are induced to protect against reactive oxygen species, which can be generated by recombination of excess excitation or excess reducing equivalents with oxygen at the donor side of PSII. However, very little has been known about molecular mechanisms of those high light responses in *cyanobacteria* or plants.

We have been studying the light acclimation in the unicellular cyanobacterium, Synechocystis sp. PCC 6803. This cyanobacterium is naturally transformable to incorporate exogenous DNA into a genome with homologous recombination. When low-light-grown (20 $\mu E \cdot m^{-2} \cdot s^{-1}$) cells of a kind of wild-type strain (GT strain) were transferred to high light (200 $\mu E \cdot m^{-2} \cdot s^{-1}$), accumulation of chlorophyll and photosystems was suppressed. Furthermore, accumulation of photosystem I (PSI) and photosystem II (PSII) was separately modulated. The PSI/PSII stoichiometry was kept high in the low-light-grown cells, probably due to smaller antenna size of PSI compared with that of PSII, whereas it declined remarkably upon the shift to high light. This has been assumed as an acclimation to balance excitation of PSI with PSII under different light irradiances. We have isolated a mutant, which does not show adjustment of the photosystem stoichiometry but showed suppressed accumulation of antenna and reaction centers upon shift to high light. By complementation of the mutant phenotype, we identified a novel gene, pmgA, which showed no clear homology to any known genes but a motif of ATP-binding (1).

high light
500 - 2000 $\mu Ein^{-2}s^{-1}$

We introduced disruption of pmgA into GT strain and could demonstrate that the pmgA mutants could reduce the cellular content of both photosystems but failed to modulate the photosystem stoichiometry upon the shift to high light. The major difference between wild type GT strain and the mutants was cellular PSI content but not PSII content (2). This suggests that functional pmgA is specifically involved in regulated accumulation of PSI complex in response to high light. This seems to be consistent with previous observations that accumulation of PSI rather than PSII is regulated in response to various environmental changes in *cyanobacteria* (3). Interestingly, the pmgA mutants was originally isolated as a strain which showed better photoautotrophic growth than the wild type GT strain at high light irradiances. Consistently, photosynthetic activity as well as PSI and PSII activities on a per cell basis were higher in the mutants than in wild type. Active PSII centers measured by PAM pulse-amplitude modulated (PAM) fluorometer was rapidly inactivated in wild type cells by brief illumination with very high light (2000 $uE/m^2/s$), while that of the mutant was stable. This clearly demonstrated that the higher PSI content relative to PSII is quite effective in protection against PSII photoinhibition, possibly by keeping the acceptor side of PSII more oxidative. Thus, the higher PSI content in the mutants could lead to higher activity of whole photosynthetic rates and eventually better growth than wild type under high light conditions.

If the pmgA mutant can grow better than wild type under high light conditions, we must explain the reason why *cyanobacteria* have developed the pmgA-mediated high light acclimation to modulate the photosystem stoichiometry. We found that wild type but not the pmgA mutants could survive especially under the long-term exposure to high light. With PAM fluorometer measurement, we could detect significant acceleration in photoinhibition of PSII in the mutant cells but not in wild type. Furthermore, prior to the photoinhibition, we detected marked increase in an apparent PSI activity of mutant cells supported with diaminodurene, ascorbic acid and methylviologen. It was inferred that membrane permeability was modified in the mutant under prolonged high light conditions. These suggest that the pmgA-induced modulation of the photosystem stoichiometry in wild type is to allow curable PSII photoinhibition, which finally down-regulates electron flows. On the other hand, the pmgA mutants, which does not modulate the stoichiometry, could prevent from the PSII photoinhibition under short-term high light conditions but

could not avoid a yet unidentified critical photodamage under long-term high light.

To extend our understanding of the light-induced modulation of photosystem stoichiometry, we analyzed the high light responses in an original wild type (PCC strain). Although PCC strain showed higher PSI/PSII ratio than GT strain under low light conditions, it responded to high light like GT strain to modulate the photosystem stoichiometry. Notably, PCC strain was clearly more sensitive to high light than GT strain and lost most of chlorophyll (4). It was known that a 154bp segment present in PCC strain was absent in GT strain (5). Since the segment harbors a putative translation initiation codon of a downstream hypothetical gene, slr2031, we examined possible correlation of functionality of slr2031 and differences in phenotype between PCC and GT strain. We disrupted slr2031 gene in PCC strain and found that suppression of pigmentation under high light conditions was no longer observed in the mutant like GT strain. In addition, the slr2031-disrupted mutant was more resistant to high light. Gliding motility of PCC strain was greatly reduced in the disruption mutant. These results seem to suggest that thehigh light responses and motility are regulated by a single gene. Since slr2031 supposedly encodes a PP2C-type protein phosphatase, which often acts in various signal transduction pathways in many organisms, it may be a regulatory component of a similar pathway to respond to some environmental signal. We also introduced a strong promoter just upstream of slr2031 into PCC strain (overexpression mutant). Expectedly, the overexpression mutant showed more pronounced responses to high light than PCC strain, although differences in the photosystem stoichiometry or motility varied depending on the time after transfer to the high light conditions. These results may suggest that the putative gene product of slr2031 plays some regulatory role in the high light responses in cooperation with pmgA.

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