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# Genetic manipulation to overexpress *rpaA* altered photosynthetic electron transport in *Synechocystis* sp. PCC 6803

Satomi Arisaka, Haruna Sukigara, and Takashi Osanai*

*Meiji University, School of Agriculture, 1-1-1 Higashimita, Tama-ku, Kawasaki, Kanagawa 214-8571, Japan*

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**Cyanobacteria are a group of prokaryotic organisms that perform oxygenic photosynthesis using a similar photosynthetic apparatus as is used in higher plants and eukaryotic algae. Cyanobacteria are also known to have a circadian rhythm. Here, we evaluated the effects on photosynthesis caused by the genetic manipulation of RpaA, which is a response regulator of a two-component regulatory system responsible for the signal output from circadian clocks. Using the unicellular cyanobacterium *Synechocystis* sp. PCC 6803, photosynthetic activities and transcript levels of photosystem I and photosystems II in the *rpaA*-overexpressing strain were measured, and it was found that the parameters, such as $F_v/F_m$, $F_v'/F_m'$, qP, and ϕII, obtained from chlorophyll fluorescence analysis were decreased by *rpaA* overexpression. These results suggest that *rpaA* overexpression modified photosynthetic electron transport under normal light conditions. Thus, we demonstrated that RpaA regulates photosynthesis in cyanobacteria and can be a potential target of photosynthetic engineering in this cyanobacterium.**



[**Key words:** Chlorophyll fluorescence; Cyanobacteria; Response regulation; Photosynthesis; *Synechocystis*]

Photosynthetic electron transport is one of the most important chemical reactions on Earth, converting light energy to chemical energy. Cyanobacteria are oxygen-evolving photosynthetic bacteria that possess photosystem II and photosystem I (PSII and PSI, respectively), as do higher plants and eukaryotic algae. PSII, located in the thylakoid membrane, catalyzes light-driven oxygen evolution from water and reduction of plastoquinone (1). The PSII complex isolated from thermophilic cyanobacterium *Thermosynechococcus elongatus* was shown to be composed of 17 intrinsic and 3 extrinsic proteins (2,3). The subunits included in the reaction center of PSII are D1, CP47, CP43, D2, α-Cyt b559, β-Cyt b559, and PsbI, and they encode *psbA2/A3*, *B*, *C*, *D1/D2*, *E*, *F*, and *I*, respectively (4,5). The extrinsic proteins PsbO, U, and V are required for stabilizing the $CaMn_4$ cluster and compose the oxygen-evolving complex (3,4). The other peripheral proteins of PSII are encoded by *psbJ*, *L*, *M*, *N*, *P2*, *T*, *W*, *Y*, and *Z*, and a set of assembly factors is necessary for the *de novo* assembly of a functional PSII dimer in cyanobacteria (1). PSI is also located in the thylakoid membrane. It accepts electrons from plastocyanin or cytochrome $c_6$ and transfers them to ferredoxin and ferredoxin $NADP^+$ reductase (FNR), consequently reducing $NADP^+$ to NADPH (6–8). Cyanobacterial PSI exists in a trimeric form, and each monomer consists of nine transmembrane protein subunits (PsaA, PsaB, PsaF, PsaI, PsaJ, PsaK, PsaL, and PsaM) and three stromal protein subunits (PsaC, PsaD, and PsaE) (9). The large heterodimer of PsaA and PsaB is located in the center of the PSI monomer and is associated with the majority of the antenna chlorophylls, the reaction center cofactors, and the other PSI subunits (9,10).

Phycobilisomes are the primary light-harvesting antenna for cyanobacterial photosynthetic electron transport (11). Excess energy absorbed by phycobilisomes is discarded through thermal dissipation, which is called non-photochemical quenching (NPQ) (12). NPQ can be measured from chlorophyll fluorescence using 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) to determine the maximum fluorescence level ($F_m$) in cyanobacteria (13,14). Both NPQ and qN represent non-photochemical quenching, while qP represents photochemical quenching (13,14). The values of $F_v/F_m$, $F_v'/F_m'$, and ϕII represent the maximal photochemical efficiency of PSII, the photochemical efficiency of open PSII centers, and the effective quantum yield of electron transport through PSII, respectively (13,14). Cyanobacterial thermal dissipation is induced by orange carotenoid protein (OCP), unlike the xanthophyll cycle found in higher plants and algae (15). OCP is activated by blue-green light (16). Cyanobacterial NPQ reflects not only thermal dissipation but also a state transition: the distribution of energy from phycobilisomes to PSI (11).

Under high light conditions, NPQ is activated, but the PSI/PSII ratio also decreases to release excess energy (17). This acclimation is regulated by photosynthetic genes at the transcriptional level. The gene expression of photosynthetic genes in the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 is regulated by an OmpR-type response regulator, RpaB (regulator of phycobilisome association B), which activates the expression of the PSI genes under low light conditions (18,19). RpaA, a homolog of RpaB, which is an OmpR-type response regulator, is involved in energy transfer from the phycobilisome to PSI (18). Furthermore, an *rpaA*-

* Corresponding author. Tel./fax: +81 44 934 7103.
*E-mail address:* tosanai@meiji.ac.jp (T. Osanai).

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overexpressing strain was shown to exhibit altered sugar and amino acid metabolism in response to light/dark transition (20).

RpaA activates the gene expression of the enzymes in the oxidative pentose phosphate (OPP) pathway and glycolysis in subjective dusk (21,22). The cyanobacterial circadian oscillator consists of KaiA, KaiB, and KaiC proteins, and KaiC phosphorylation cycle and transcription/translation feedback loops of *kaiABC* genes are indispensable for generating circadian rhythms (23,24). The two-component regulatory system, histidine kinase SasA/Hik8 and response regulator RpaA, outputs the circadian signal from KaiABC to control gene expression. (25–27). The expression patterns of photosynthetic genes exhibit circadian rhythms during day/night cycles (28). Thus, RpaA is considered to regulate the expression of photosynthetic genes in response to both light and circadian signals. Majeed et al. (29) showed that the *rpaA* knockout strain exhibits reduction of chlorophyll fluorescence from PSI and decline of monomeric PSI and PsaD under only high light conditions.

Here, we clarified the further relationship between RpaA and photosynthetic regulation by analyzing *rpaA* overexpression in the *Synechocystis* 6803 strain, demonstrating that the genetic manipulation of *rpaA* alters photosynthetic activity under normal light conditions in this cyanobacterium.

## MATERIALS AND METHODS

**Bacterial growth conditions** The glucose-tolerant (GT) strain *Synechocystis* 6803, isolated by Williams (30), and the *rpaA*-overexpressing strain, designated as ROX310 (20), were grown in modified BG-11 medium (31) containing 5 mM $NH_4Cl$ (buffered with 20 mM HEPES-KOH, pH 7.8). Among the GT substrains, the GT-I strain was used in this study (32). Liquid cultures were bubbled with 1% (v/v) $CO_2$ in air and incubated at 30°C under continuous white light (approximately 50–70 μmol photons $m^{-2}$ $s^{-1}$). Growth and cell densities were measured at $OD_{730}$ with a UV-2700 UV–VIS spectrophotometer (Shimadzu, Kyoto, Japan). Kanamycin (10 μg/mL) was added to ROX310 during pre-culture.

**RNA isolation and quantitative real-time PCR** RNA isolation was performed as described previously (33). The cDNAs were synthesized with the SuperScript III First-Strand Synthesis System (Life Technologies Japan, Tokyo, Japan) with 2 μg total RNA. Quantitative real-time PCR was performed with the StepOnePlus Real-Time PCR System (Life Technologies Japan) in accordance with the manufacturer's instructions using the primers listed in Table S1. The expression level of *rnpB*, which encodes RNaseP subunit B, was used as an internal standard.

**Immunoblotting** Cells were collected by centrifugation (5800 ×*g* for 2 min), and the supernatant was removed by decantation. Cells were resuspended in residual medium and re-centrifuged at 20,400 ×*g* for 1 min. The supernatant was removed by pipetting, and the cells were suspended in 0.5 mL PBS-T (137 mM NaCl, 2.7 mM KCl, 8.1 mM $Na_2HPO_4$, 1.5 mM $KH_2PO_4$, 0.1% Tween-20) and disrupted by sonication (Vibra cell, VC-750; Sonics & Materials, Inc., Newtown, CT, USA); immunoblotting was performed as described previously (33). Antisera against PsbA, PsbO, and PsaA were purchased from AntiProt (34). Antiserum against OCP was manufactured by Eurofin Co. Ltd. (Tokyo, Japan) by introducing the synthetic peptide $NH_2$-CKLIPERGVTEPAED-COOH into a rabbit.

**Measurement of respiratory and photosynthetic activities** Chlorophyll levels in the cells were determined by a methanol extraction method (35,36). Cells containing 10 μg chlorophyll were resuspended in 1 mL $BG\text{-}11_0$ liquid medium, supplemented with 5 mM $NH_4Cl$, and incubated at 30°C within the chamber of an Oxytherm Clark-type oxygen electrode (Hansatech Instruments, King's Lynn, UK). Cells were incubated in dark conditions while monitoring oxygen consumption for 8 min. The rate of oxygen consumption during the final 3 min of the dark incubation was used to calculate respiration activity. The rate of oxygen evolution was measured after the addition of 10 μL of 1 M $NaHCO_3$ and exposure to white light of 60, 200, 500, 700, and 1050 μmol photons $m^{-2}$ $s^{-1}$. The rate of oxygen evolution was calculated during the final 3 min of the 5-min measurement period.

**Chlorophyll fluorescence** Chlorophyll fluorescence was measured with an AquaPen-C AP-C 100 fluorometer (Photon Systems Instruments, Drasov, Czech Republic). Chlorophyll levels were determined as described above, and cells were diluted to 0.3 μg $mL^{-1}$ chlorophyll in 2 mL $BG\text{-}11_0$ medium supplemented with 5 mM $NH_4Cl$. Chlorophyll fluorescence was measured in accordance with the

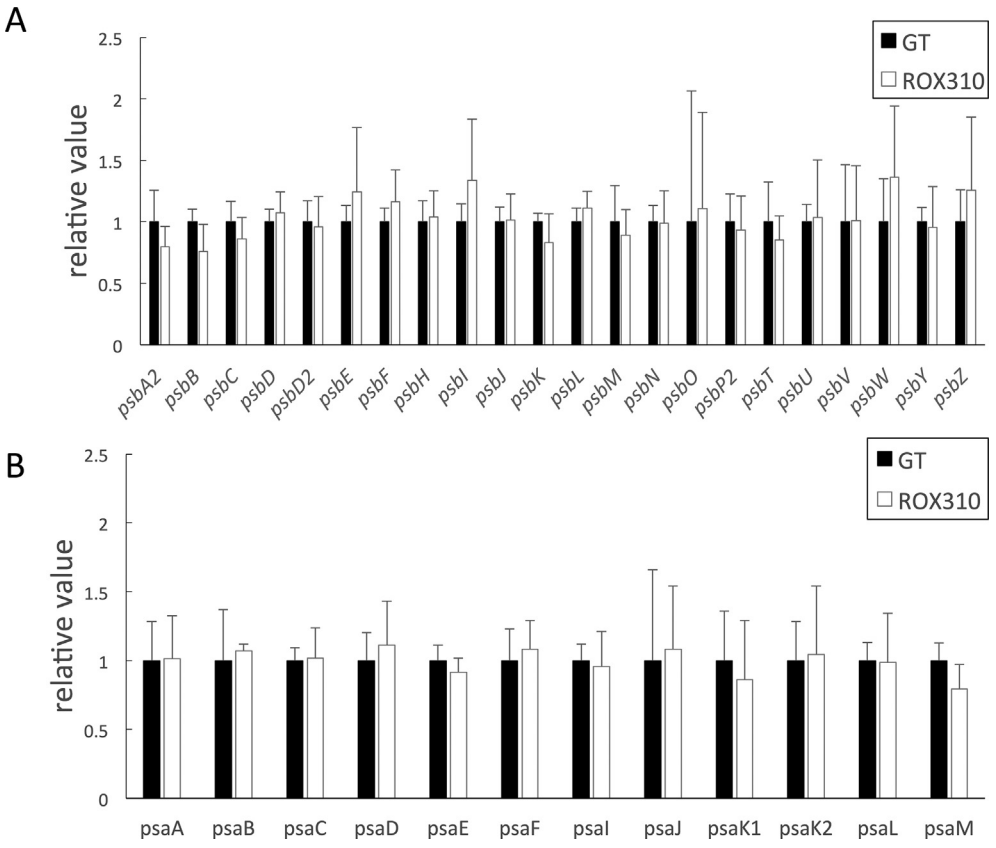


FIG. 1. Transcript levels of (A) 22 genes encoding photosystem II proteins and (B) 12 genes encoding photosystem I proteins in GT and *rpaA*-overexpressing (ROX310) cells. Data are the mean ± SD from four independent experiments. The levels were calibrated relative to the value obtained in the GT strain, which was set at 1. Differences between GT and *rpaA*-overexpressing cells were analyzed with Student's *t*-test.

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