Full Paper

RpaB, an essential response regulator for high-light stress, is extensively involved in transcriptional regulation under light-intensity upshift conditions in *Synechococcus elongatus* PCC 7942

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In cyanobacteria, transcription of a set of genes is specifically induced by high-light-stress conditions. In previous studies, RpaB, a response regulator of the two-component system, was shown to be involved in this regulation *in vitro* and *in vivo*. In this study, we examined whether RpaB-dependent transcriptional regulation was extensively observed, not only under high-light-stress conditions but also under various light intensities. Transcription of high-light-dependent genes *hliA*, *nblA* and *rpoD3* was transiently and drastically induced during a dark-to-light shift in a manner similar to high-light-stress responses. Moreover, expression of these genes was activated under various light-intensity upshift conditions. Phos-tag SDS-PAGE experiments showed that the phosphorylation level of RpaB was decreased along with transcriptional induction of target genes in all of the light environments examined herein. These results suggest that RpaB may be widely involved in transcriptional regulation under dark-to-light and light-intensity upshift conditions and that high-light-responsive genes may be required in various light conditions other than high-light condition. Furthermore, it is hypothesised that RpaB is regulated by redox-dependent signals rather than by high-light-stress-dependent signals.

Key Words: cyanobacteria; light response; RpaB; *Synechococcus elongatus* PCC 7942; transcriptional regulation; two-component system

Abbreviations: ChAP, chromatin affinity purification; ChIP, chromatin immunoprecipitation; GL, growth light; HL, high light; HLR1, high light regulatory 1; LL, low light; ML, mid-high light

Introduction

Cyanobacteria are classified as gram-negative bacteria and are especially characterised by oxygenic photosynthesis. Because they thrive in various natural environments, they are considered to have a wide variety of environmental responses and mechanisms for the regulation of gene expression. For instance, the two-component regulatory system, one of the major signal transduction mechanisms in bacteria, fungi, and plants, is actually found in cyanobacteria, and this system appears to be required for sensing, adaptation and tolerance for diverse environmental changes, nutrient deficiency or abiotic stresses (Ashby and Houmard, 2006).

Because cyanobacteria are classified as photosynthetic organisms, light is one of the most important environmental signals for their survival and proliferation. However, excess light energy is rather toxic because it damages photosynthetic and cellular components, which is a process known as photoinhibition. Thus, transcriptional regulation during high-light stress in cyanobacteria has been the focus of many studies in recent years. Transcriptomic analysis identified at least 160 high-light-stress-responsive genes in the cyanobacterium *Synechocystis* sp. PCC 6803 (Hihara et al., 2001). In the upstream regions of such genes, the high light regulatory 1 (HLR1) sequence motif is con-

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Table 1. Primers used in northern blot analysis.

Gene	5' primer sequence	3' primer sequence
<i>hliA</i>	atgctagtagcgacggaca	ttagctcaagccgctcgc
<i>nblA</i>	atgctccccctctccc	ctaacttccttgccgaatca
<i>rpoD3</i>	gaggggatccatggccaaaactgaaacccc	ctgctctagaaaactagctagccaagta
<i>rnpB</i>	gaaagtccgggctcccaa	taagccgggtctcttctc

Fig. 1. Transcription induction of RpaB-regulon genes and phosphorylation level of RpaB under high-light stress.

A. Northern blot analysis of RpaB-regulon genes. *Synechococcus* cells were grown under continuous growth light ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and then shifted to high-light conditions ($800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 60 min. Aliquots were harvested at 0, 1, 5, 15, 30 and 60 min after shifting to high light. $10 \mu\text{g}$ (for *hliA*, *nblA*, *rpoD3*) or $1 \mu\text{g}$ (for *rnpB*) of total RNAs were loaded per lane, and a methylene blue staining image of rRNAs served as a loading control. B. Confirmation of the reactivity of anti-RpaB antibody. $10 \mu\text{g}$ of total protein was separated by 12% SDS-PAGE, and RpaB protein level was detected by pre-immune serum (Pre) or anti-RpaB antibody (Ab). C. Phos-tag SDS-PAGE of RpaB protein. *Synechococcus* cells were grown as described above. $10 \mu\text{g}$ of total proteins were separated by 10–12% SDS-PAGE in the presence/absence of Phos-tag acrylamide, and phosphorylation level (+Phos-tag) and accumulation (–Phos-tag) of RpaB were detected using an anti-RpaB antibody. CBB staining image of total proteins served as a loading control.

served (Eriksson et al., 2000; Kappell et al., 2006). Among the candidates for a trans-acting factor that binds the HLR1 element, RpaB, one of the response regulators of the two-component systems in *Synechocystis* PCC 6803, has been identified (Kappell and van Waasbergen, 2007). RpaB has been shown to bind to the HLR1 element on various high-light-responsive promoters by gel-shift analysis in *Synechocystis* PCC 6803 (Kadowaki et al., 2016; Kappell and van Waasbergen, 2007; Riediger et al., 2019; Seino et al., 2009; Takahashi et al., 2010) as well as in *Synechococcus elongatus* PCC 7942 (Kato et al., 2011; López-Redondo et al., 2010; Seki et al., 2007). Furthermore, a dynamic change in the RpaB binding pattern with target promoters under high-light-stress conditions has been observed *in vivo* by ChIP (Hanaoka and Tanaka, 2008) and ChAP (Kadowaki et al., 2016) analyses. These evidences suggest that RpaB is the typical transcription factor that is required for high-light-stress response.

RpaB and its paralogous protein RpaA, both of which are OmpR-type DNA-binding response regulator proteins, have been initially identified as regulators for energy transfer from photosynthetic light-harvesting antennae to the photosystem (PS)I and PSII reaction centres (Ashby and Mullineaux, 1999). This regulation to balance energy trans-

fer is called ‘state transition’ and is typically found in fluctuating light conditions (van Thor et al., 1998). Therefore, we hypothesised that RpaB is widely involved in transcriptional regulation not only under high-light stress but also during various states of fluctuating light conditions, such as a dark-to-light shift. To our knowledge, there are no previous reports describing the extended function of RpaB in diverse light environments.

In this report, we show that the expression of RpaB-dependent, high-light-responsive genes (hereafter described as RpaB-regulon genes) is actually induced under various light conditions in *S. elongatus* PCC 7942. RpaB may therefore be involved in transcriptional regulation under fluctuating light conditions.

Materials and Methods

Bacterial strain, medium and growth conditions. The wild-type *Synechococcus elongatus* PCC 7942 strain was cultivated in BG-11 liquid medium (Rippka, 1988) with 2% CO_2 aeration at 30°C under continuous illumination with fluorescent light ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). To change the light conditions, liquid cell cultures were exposed to various intensities of light (10, 50, 100 or $800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 1 h after acclimatisation to darkness or

Fig. 2. Transcription induction of RpaB-regulon genes and phosphorylation level of RpaB under a dark-to-light shift.

Synechococcus cells grown under continuous growth light ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) were kept in the dark for 12 h and then exposed again to growth light ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 60 min. Aliquots were harvested at 0, 1, 5, 15, 30 and 60 min after shifting to growth light. A. Northern blot analysis of RpaB-regulon genes. 10 μg (for *hliA*, *nblA*, *rpoD3*) or 1 μg (for *rnpB*) of total RNAs were loaded per lane, and a methylene blue staining image of rRNAs served as a loading control. B. Phos-tag SDS-PAGE of RpaB. In this, 10 μg of total proteins were separated by 10–12% SDS-PAGE in the presence/absence of Phos-tag acrylamide, and phosphorylation level (+Phos-tag) and accumulation (–Phos-tag) of RpaB were detected using an anti-RpaB antibody. CBB staining image of total proteins served as a loading control.

weaker intensities of light (0, 10 or $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 12 h.

RNA extraction and northern blot analysis. *S. elongatus* cells at mid-log phase (normally, OD_{750} was set approximately 0.5) were harvested at 0, 1, 5, 15, 30 or 60 min after the light shift and stored at -80°C until use. Extraction of total cellular RNA and northern blot analysis were performed as described previously (Los et al., 1993). DNA probes for *hliA*, *rpoD3*, *nblA* and *rnpB* were DIG-labelled from corresponding DNA fragments that were amplified by PCR using the specific primer sets listed in Table 1.

Phos-tag SDS-PAGE and immunoblot analysis. Protein extraction from *S. elongatus* cells was performed as reported previously (Kim et al., 2015). Protein concentration was estimated using the BCA protein assay kit (Thermo Fisher Scientific). SDS-PAGE and immunoblotting using acrylamide gels with or without Phos-tag acrylamide (FUJIFILM Wako Pure Chemical) were conducted as described previously (Kim et al., 2015). The rabbit polyclonal anti-RpaB antibody was prepared from the recombinant RpaB protein that was purified as described previously (Seki et al., 2007) to detect accumulation and phosphorylation levels of RpaB.

Results

RpaB-dependent gene expression under high light condition

First, we checked the expression pattern of typical RpaB-regulon genes under an experimental shift from growth light (GL: $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) to high light (HL: $800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). In this study, we selected *hliA*, *nblA* and *rpoD3* as high-light-dependent genes and *rnpB*

as a high-light-independent negative control gene. Expression of all three RpaB-regulon genes was transiently induced after the shift to high light (Fig. 1A), as described previously for similar conditions (Kato et al., 2011; López-Redondo et al., 2010; Seki et al., 2007).

To assess the protein accumulation and phosphorylation levels of RpaB, we raised a rabbit polyclonal antibody from the RpaB recombinant protein expressed in *E. coli*. A specific signal at approximately 25 kDa was detected at which no signals appeared for the pre-immune serum control (Fig. 1B). The phosphorylation level of RpaB during high-light stress then was examined using this antibody. While accumulation of total RpaB was constant from 0 to 60 min of high light, phosphorylated RpaB appeared more abundantly at 0 min and rapidly decreased after the shift to high light (Fig. 1C), as reported previously (Moronta-Barrios et al., 2012).

RpaB-dependent gene expression upon dark-to-light shift

We next examined the expression patterns of RpaB-regulon genes under a shift from darkness to growth light. *S. elongatus* cells maintained under growth light (GL: $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) were exposed to darkness (0 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 12 h and again transferred to light (GL: $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) conditions. Remarkably, the expression of RpaB-regulon genes *hliA*, *nblA* and *rpoD3* was clearly induced after growth light treatment (Fig. 2A). To examine whether RpaB is involved in transcriptional regulation during dark-to-light shift, Phos-tag SDS-PAGE analysis was again performed. Phosphorylated protein levels of RpaB were higher in darkness and rapidly decreased in a light-dependent manner, although the total RpaB protein level remained constant (Fig. 2B). These results suggest that RpaB-dependent expression of

Fig. 3. Transcription induction of RpaB-regulon genes and phosphorylation level of RpaB under various light intensities following dark adaptation.

Synechococcus cells grown under continuous growth light ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) were kept in the dark for 12 h and then shifted to (A) high light (HL: $800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), (B) mid-high light (ML: $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), or (C, D) low light (LL: $10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 60 min. Aliquots were harvested at 0, 1, 5, 15, 30 and 60 min after shifting to each light condition. A–C. Northern blot analysis of RpaB-regulon genes. $10 \mu\text{g}$ (for *hliA*, *nblA*, *rpoD3*) or $1 \mu\text{g}$ (for *rnpB*) of total RNAs were loaded per lane, and a methylene blue staining image of rRNAs served as a loading control. D. Phos-tag SDS-PAGE of RpaB. In this, $10 \mu\text{g}$ of total proteins were separated by 10–12% SDS-PAGE in the presence/absence of Phos-tag acrylamide, and phosphorylation level (+Phos-tag) and accumulation (–Phos-tag) of RpaB were detected using an anti-RpaB antibody. CBB staining image of total proteins served as a loading control.

hliA, *nblA* and *rpoD3* occurs not only during high-light stress but also during the dark-to-light shift that occurs at dawn.

We further performed gene expression analyses under high light (HL: $800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), mid-high light (ML: $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and low light (LL: $10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) after dark adaptation ($0 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 12 h. In each case, expression of *hliA*, *nblA* and *rpoD3* was induced after illumination, and the peak time of transcription induction were rather different (Figs. 3A–C). Phosphorylation levels of RpaB again decreased after the transition from darkness to low light (shown as representative of each light intensity), albeit only slightly in this condition (Fig. 3D). This observation suggests that the extent of transcriptional induction of RpaB-regulon genes and dephosphorylation level of RpaB could be dependent on light intensities exposed after dark adaptation.

RpaB-dependent gene expression under various light-intensity upshift condition

Because RpaB-dependent transcriptional derepression of high-light-responsive genes was widely observed under various intensities of light following dark adaptation, we hypothesised that this regulation would also be seen during light-intensity upshift conditions that naturally occur during the daytime. To explore this possibility, *S. elongatus* cells were acclimatised in growth light (GL: $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) or low light (LL: $10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 12 h, followed by cultivation under higher light intensities per group: mid-high light (ML: $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) or growth light (GL: $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), respectively. In brief, gene expression analyses for LL-to-GL (Fig. 4A), LL-to-ML (Fig. 4B) and GL-to-ML transitions (Fig. 4C) were performed. We found that expression of *hliA*, *nblA* and *rpoD3* was similarly induced

Fig. 4. Transcription induction of RpaB-regulon genes and phosphorylation level of RpaB under various light-intensity upshift conditions.

Synechococcus cells grown under continuous growth light ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) were kept in LL (A, B) or GL (C, D) conditions for 12 h and then shifted to GL (A) or ML (B–D) conditions for 60 min. Aliquots were harvested at 0, 1, 5, 15, 30 and 60 min after shifting to each light condition. A–C. Northern blot analysis of RpaB-regulon genes. In this, $10 \mu\text{g}$ (for *hliA*, *nblA*, *rpoD3*) or $1 \mu\text{g}$ (for *rnpB*) of total RNAs were loaded per lane, and a methylene blue staining image of rRNAs served as a loading control. D. Phos-tag SDS-PAGE of RpaB. $10 \mu\text{g}$ of total proteins were separated by 10–12% SDS-PAGE in the presence/absence of Phos-tag acrylamide, and phosphorylation level (+Phos-tag) and accumulation (–Phos-tag) of RpaB were detected using an anti-RpaB antibody. CBB staining image of total proteins served as a loading control.

under all three conditions. In addition, under the GL-to-ML condition, phosphorylated RpaB was transiently dephosphorylated after changing the light intensity (Fig. 4D). These data suggest that RpaB-dependent transcriptional regulation is not restricted to high-light condition but extensively observed in a wide variety of light-intensity upshift conditions.

Discussion

In the present study, we showed that transcriptional induction of *hliA*, *nblA* and *rpoD3*, which was previously considered to be a high-light-stress-specific event, also occurred in a dark-to-light shift as well as during various light-intensity upshift conditions. Northern blot analysis indicated that expression of these genes was clearly activated under all light conditions examined. This finding suggests that RpaB-regulon genes are required not only for tolerance of high-light stress but also for various light-

intensity upshift conditions in the daytime. Although some other transcriptional factor(s) are supposed to be also participated in this regulation, our Phos-tag SDS-PAGE analyses suggested that the essential response regulator of the cyanobacterial two-component system, RpaB, also appears to be involved in diverse light-dependent transcriptional regulation in addition to its previously reported function during high-light stress (Hanaoka and Tanaka, 2008; Kato et al., 2011; López-Redondo et al., 2010; Seki et al., 2007). RpaB has been initially identified as a regulator of state transition that controls the association of phycobilisomes with reaction centres of PSI and PSII (Ashby and Mullineaux, 1999). Considering that state transition is generally observed in fluctuating light conditions, RpaB appears to be involved in regulation during various intensities of light. In addition, RpaB is required for the output pathway of the circadian clock system (Espinosa et al., 2015; Hanaoka et al., 2012). Taken together with the results of our present study, RpaB could be required

for widespread environmental responses.

The corresponding histidine kinase for RpaB is speculated to be NblS (van Waasbergen et al., 2002) in *S. elongatus* PCC 7942 by yeast two-hybrid assays (Kato et al., 2012). The orthologous factor of NblS in *Synechocystis* PCC 6803 is DspA/Hik33 (Tu et al., 2004), and it has been reported as a sensor to multiple stress conditions, including high-light stress (Kanesaki et al., 2007; Marin et al., 2003; Mikami et al., 2002; Paithoonrangsarid et al., 2004; Shoumskaya et al., 2005). Therefore, NblS could be a stress-responsive histidine kinase. In this study, we indicated that the expression of RpaB-regulon genes such as *hliA* is induced under various light-intensity upshift conditions, possibly dependent on RpaB. However, it is still unclear whether NblS also senses light-intensity upshift conditions as tested herein or, more specifically, only abiotic stress conditions, including high light. RpaB has been reported to be regulated by redox signal by interacting with thioredoxin (Kadowaki et al., 2015). Because all light-shift conditions that we examined in this study could be accompanied with a certain degree of change in the redox state of the photosynthetic electron transport chain, a change in the phosphorylation level of RpaB as well as transient transcription induction of target genes might be regulated in a manner dependent upon cellular redox level.

RpaB is conserved in phycobilisome-containing red algal plastids as well as in cyanobacteria (Ashby et al., 2002). The role of RpaB in response to various intensities of light other than high-light-stress conditions therefore may be conserved among both photosynthetic prokaryotes and eukaryotes, beyond the endosymbiotic evolution event believed to establish chloroplasts.

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References

- Ashby, M. K. and Houmard, J. (2006) Cyanobacterial two-component proteins: structure, diversity, distribution and evolution. *Microbiol. Mol. Biol. Rev.*, **70**, 472–509.
- Ashby, M. K. and Mullineaux, C. W. (1999) Cyanobacterial *ycf27* gene products regulate energy transfer from phycobilisomes to photosystems I and II. *FEMS Microbiol. Lett.*, **181**, 253–260.
- Ashby, M. K., Houmard, J., and Mullineaux, C. W. (2002) The *ycf27* genes from cyanobacteria and eukaryotic algae: distribution and implications for chloroplast evolution. *FEMS Microbiol. Lett.*, **214**, 25–30.
- Eriksson, J., Salih, G. F., Ghebremedhin, H., and Jansson, C. (2000) Deletion mutagenesis of the 5' *psbA2* region in *Synechocystis* 6803: identification of a putative *cis* element involved in photoregulation. *Mol. Cell. Biol. Res. Commun.*, **3**, 292–298.
- Espinosa, J., Boyd, J. S., Cantos, R., Salinas, P., Golden, S. S. et al. (2015) Cross-talk and regulatory interactions between the essential response regulator RpaB and cyanobacterial circadian clock output. *Proc. Natl. Acad. Sci. USA*, **112**, 2198–2203.
- Hanaoka, M. and Tanaka, K. (2008) Dynamics of RpaB-promoter interaction during high light stress, revealed by chromatin immunoprecipitation (ChIP) analysis in *Synechococcus elongatus* PCC 7942. *Plant J.*, **56**, 327–335.
- Hanaoka, M., Takai, N., Hosokawa, N., Fujiwara, M., Akimoto, Y. et al. (2012) RpaB, another response regulator operating circadian clock-dependent transcriptional regulation in *Synechococcus elongatus* PCC 7942. *J. Biol. Chem.*, **287**, 26321–26327.
- Hihara, Y., Kamei, A., Kanehisa, M., Kaplan, A., and Ikeuchi, M. (2001) DNA microarray analysis of cyanobacterial gene expression during acclimation to high light. *Plant Cell*, **13**, 793–806.
- Kadowaki, T., Nishiyama, Y., Hisabori, T., and Hihara, Y. (2015) Identification of OmpR-family response regulators interacting with thioredoxin in the Cyanobacterium *Synechocystis* sp. PCC 6803. *PLoS One*, **10**, e0119107.
- Kadowaki, T., Nagayama, R., Georg, J., Nishiyama, Y., Wilde, A. et al. (2016) A feed-forward loop consisting of the response regulator RpaB and the small RNA PsrR1 controls light acclimation of Photosystem I gene expression in the Cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Cell Physiol.*, **57**, 813–823.
- Kanesaki, Y., Yamamoto, H., Paithoonrangsarid, K., Shoumskaya, M., Suzuki, I. et al. (2007) Histidine kinases play important roles in the perception and signal transduction of hydrogen peroxide in the cyanobacterium, *Synechocystis* sp. PCC6803. *Plant J.*, **49**, 313–324.
- Kappell, A. D. and van Waasbergen, L. G. (2007) The response regulator RpaB binds the high light regulatory 1 sequence upstream of the high-light-inducible *hliB* gene from the cyanobacterium *Synechocystis* PCC 6803. *Arch. Microbiol.*, **187**, 337–342.
- Kappell, A. D., Bhaya, D., and van Waasbergen, L. G. (2006) Negative control of the high light-inducible *hliA* gene and implication for the activities of the NblS sensor kinase in the cyanobacterium *Synechococcus elongatus* strain PCC 7942. *Arch. Microbiol.*, **186**, 403–413.
- Kato, H., Kubo, T., Hayashi, M., Kobayashi, I., Yagasaki, T. et al. (2011) Interactions between histidine kinase NblS and the response regulators RpaB and SrrA are involved in the bleaching process of the cyanobacterium *Synechococcus elongatus* PCC 7942. *Plant Cell Physiol.*, **52**, 2115–2122.
- Kato, H., Watanabe, S., Nimura-Matsune, K., Chibazakura, T., Tozawa, Y. et al. (2012) Exploration of a possible partnership among orphan two-component system proteins in cyanobacterium *Synechococcus elongatus* PCC 7942. *Biosci. Biotechnol. Biochem.*, **76**, 1484–1491.
- Kim, Y.-I., Boyd, J., Espinosa, J., and Golden, S. S. (2015) Detecting KaiC phosphorylation rhythms of the cyanobacterial circadian oscillator *in vitro* and *in vivo*. *Methods Enzymol.*, **551**, 153–173.
- López-Redondo, M. L., Moronta, F., Salina, P., Espinosa, J., Cantos, R. et al. (2010) Environmental control of phosphorylation pathways in a branched two-component system. *Mol. Microbiol.*, **78**, 475–489.
- Los, D., Horvath, I., Vigh, L., and Murata, N. (1993) The temperature-dependent expression of the desaturase gene *desA* in *Synechocystis* PCC6803. *FEBS Lett.*, **318**, 57–60.
- Marin, K., Suzuki, I., Yamaguchi, K., Ribbeck, K., Yamamoto, H. et al. (2003) Identification of histidine kinases that act as sensors in the perception of salt stress in *Synechocystis* sp. PCC 6803. *Proc. Natl. Acad. Sci. USA*, **100**, 9061–9066.
- Mikami, K., Kanesaki, Y., Suzuki, I., and Murata, N. (2002) The histidine kinase Hik33 perceives osmotic stress and cold stress in *Synechocystis* sp PCC 6803. *Mol. Microbiol.*, **46**, 905–915.
- Moronta-Barrios, F., Espinosa, J., and Contreras, A. (2012) *In vivo* features of signal transduction by the essential response regulator RpaB from *Synechococcus elongatus* PCC 7942. *Microbiology*, **158**, 1229–1237.
- Paithoonrangsarid, K., Shoumskaya, M. A., Kanesaki, Y., Satoh, S., Tabata, S. et al. (2004) Five histidine kinases perceive osmotic stress and regulate distinct sets of genes in *Synechocystis*. *J. Biol. Chem.*, **279**, 53078–53086.
- Riediger, M., Kadowaki, T., Nagayama, R., Georg, J., Hihara, Y. et al. (2019) Biocomputational analyses and experimental validation identify the regulon controlled by the redox-responsive transcription factor RpaB. *iScience*, **15**, 316–331.
- Rippka, R. (1988) Isolation and purification of cyanobacteria. *Methods Enzymol.*, **167**, 3–27.
- Seino, Y., Takahashi, T., and Hihara, Y. (2009) The response regulator RpaB binds to the upstream element of photosystem I genes to work for positive regulation under low-light conditions in *Synechocystis* sp. strain PCC 6803. *J. Bacteriol.*, **191**, 1581–1586.
- Seki, A., Hanaoka, M., Akimoto, Y., Masuda, S., Iwasaki, H. et al. (2007)

- Induction of a group 2 sigma factor, RPOD3, by high light and the underlying mechanism in *Synechococcus elongatus* PCC 7942. *J. Biol. Chem.*, **282**, 36887–36894.
- Shoumskaya, M. A., Paithoonrangsarit, K., Kanesaki, Y., Los, D. A., Zinchenko, V. V. et al. (2005) Identical Hik-Rre systems are involved in perception and transduction of salt signals and hyperosmotic signals but regulate the expression of individual genes to different extents in *synechocystis*. *J. Biol. Chem.*, **280**, 21531–21538.
- Takahashi, T., Nakai, N., Muramatsu, M., and Hihara, Y. (2010) Role of multiple HLR1 sequences in the regulation of the dual promoters of the *psaAB* genes in *Synechocystis* sp. PCC 6803. *J. Bacteriol.*, **192**, 4031–4036.
- Tu, C. J., Shrager, J., Burnap, R. L., Postier, B. L., and Grossman, A. R. (2004) Consequences of a deletion in *dspA* on transcript accumulation in *Synechocystis* sp. strain PCC6803. *J. Bacteriol.*, **186**, 3889–3902.
- van Thor, J. J., Mullineaux, C. W., Matthijs, H. C. P., and Hellingwerf, K. J. (1998) Light-harvesting and state transitions in cyanobacteria. *Bot. Acta*, **111**, 430–443.
- van Waasbergen, L. G., Dolganov, N., and Grossman, A. R. (2002) *nblS*, a gene involved in controlling photosynthesis-related gene expression during high light and nutrient stress in *Synechococcus elongatus* PCC 7942. *J. Bacteriol.*, **184**, 2481–2490.