Research Article

***rre37* Overexpression Alters Gene Expression Related to the Tricarboxylic Acid Cycle and Pyruvate Metabolism in *Synechocystis* sp. PCC 6803**

Hiroko Iijima, Atsuko Watanabe, Junko Takanobu, Masami Yokota Hirai, and Takashi Osanai

RIKEN Center for Sustainable Resource Science, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan

Correspondence should be addressed to Takashi Osanai; takashi.osanai.aa@riken.jp

Received 4 September 2014; Revised 22 November 2014; Accepted 23 November 2014; Published 28 December 2014

Academic Editor: Anli Geng

Copyright © 2014 Hiroko Iijima et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The tricarboxylic acid (TCA) cycle and pyruvate metabolism of cyanobacteria are unique and important from the perspectives of biology and biotechnology research. Rre37, a response regulator induced by nitrogen depletion, activates gene expression related to sugar catabolism. Our previous microarray analysis has suggested that Rre37 controls the transcription of genes involved in sugar catabolism, pyruvate metabolism, and the TCA cycle. In this study, quantitative real-time PCR was used to measure the transcript levels of 12 TCA cycle genes and 13 pyruvate metabolism genes. The transcripts of 6 genes (*acnB*, *icd*, *ppc*, *pyk1*, *me*, and *pta*) increased after 4 h of nitrogen depletion in the wild-type GT strain but the induction was abolished by *rre37* overexpression. The repression of gene expression of *fumC*, *ddh*, and *ackA* caused by nitrogen depletion was abolished by *rre37* overexpression. The expression of *me* was differently affected by *rre37* overexpression, compared to the other 24 genes. These results indicate that Rre37 differently controls the genes of the TCA cycle and pyruvate metabolism, implying the key reaction of the primary in this unicellular cyanobacterium.

1. Introduction

The tricarboxylic acid (TCA) cycle and pyruvate metabolism are conserved in almost all organisms and are indispensable for cell survival and proliferation. Cyanobacteria were thought to have an incomplete TCA cycle because they lack the 2-oxoglutarate (2-OG) dehydrogenase enzyme [1], but Zhang and Bryant detected a closed and complete TCA cycle in the unicellular cyanobacterium *Synechococcus* sp. PCC 7002 [2]. In this cyanobacterium, 2-OG is converted to succinic semialdehyde, a step catalyzed by a 2-OG decarboxylase, and succinic semialdehyde is converted to succinate by a succinic semialdehyde dehydrogenase [2]. Genes encoding these two enzymes are conserved among cyanobacteria, except in marine species, indicating that the closed TCA cycle is widely maintained in cyanobacteria.

2-OG is a known signaling metabolite in cyanobacteria; its level increases by nitrogen depletion [3]. 2-OG directly interacts with a transcription factor NtcA, promoting its

interaction with promoter DNAs and transcription activation of nitrogen-related genes by NtcA [4, 5]. 2-OG also binds to PII protein, which is a carbon/nitrogen balance sensor, and transduces nitrogen starvation signals [6, 7]. Metabolomic analyses have revealed that, in addition to 2-OG, other organic acids in the TCA cycle, including succinate, malate, and fumarate, are increased by nitrogen depletion in the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter referred to as *Synechocystis* 6803) and halophilic cyanobacterium *Arthrospira platensis* [8–10]. Amino acids derived from pyruvate metabolism and the TCA cycle metabolites, such as alanine, phenylalanine, tyrosine, serine, glycine, valine, and leucine, increase by nitrogen depletion, whereas aspartate, arginine, glutamate, and glutamine decrease [10]. Like glycogen and polyhydroxybutyrate, the pool of organic acids in the TCA cycle may function as carbon storage during nitrogen starvation [10]. The redistribution of carbon sources to various metabolites other than glycogen has been shown in the freshwater

blooming cyanobacterium *Microcystis aeruginosa* PCC 7806 [11].

The expression of the gene encoding a response regulator Rre37 (sll1330) is induced by nitrogen depletion [12]. In the nitrogen-fixing cyanobacterium, *Anabaena* sp. PCC 7120, the expression of an ortholog of Rre37, named NrrA, also increases during nitrogen starvation and NtcA binds to the promoter region of *nrrA* [13, 14]. NrrA regulates glycogen catabolism by controlling the transcription of genes encoding the glycogen catabolism-related enzymes, glycolytic enzymes, and a group 2 sigma factor SigE [15]. *Synechocystis* 6803 Rre37 affects the expression of genes encoding enzymes involved in glycolysis and glycogen catabolism [16, 17]. *Synechocystis* 6803 NtcA binds to the promoter region of *rre37* in a 2-OG dependent manner *in vitro* [17]. Rre37 binds to the promoter regions of genes encoding enzymes involved in glycogen catabolism, glycolysis, and amino acid metabolism [18, 19]. Metabolomic analysis has shown that *rre37* knockout alters the levels of glycogen, sugar phosphates, and organic acids in the TCA cycle [20]. Recent genetic engineering studies using the *rre37*-overexpressing strain ROX370 showed that *rre37* overexpression decreased the levels of glycogen, sugar phosphates, and organic acids in the TCA cycle but increased glycogen catabolism-related enzymes, glycolytic enzymes, and polyhydroxybutyrates [19]. Combining transcriptomic and metabolomic analyses using ROX370 suggests that a possible new cycle, a hybrid of the TCA and ornithine cycle, may be induced during nitrogen starvation [19]. This hybrid cycle enables *Synechocystis* cells to assimilate two molecules of ammonium ions, leading to efficient nitrogen uptake during nitrogen starvation [19]. Thus, the integrity of the TCA cycle and pyruvate metabolism is important for survival during nitrogen starvation, where Rre37 may play a pivotal role in their regulation.

In this study, we examined the expression levels of genes related to the TCA cycle and pyruvate metabolism by quantitative real-time PCR. We found altered levels of transcript levels in response to the nitrogen status, mediated by *rre37* overexpression.

2. Materials and Methods

2.1. Bacterial Strains and Culture Conditions. The glucose-tolerant (GT) strain of *Synechocystis* sp. PCC 6803 [21] and ROX370 [19] were grown in modified BG-11 medium, which consisted of BG-11₀ liquid medium [22] containing 5 mM NH₄Cl (buffered with 20 mM Hepes-KOH, pH 7.8). The GT-I strain was used in this study [23]. Liquid cultures were bubbled with 1% (v/v) CO₂ in air and incubated at 30°C under continuous white light (approx. 50–70 μmol photons m⁻² sec⁻¹). Growth and cell densities were measured at A₇₃₀ with a Hitachi U-3310 spectrophotometer (Hitachi High-Tech., Tokyo, Japan).

2.2. RNA Isolation and Quantitative Real-Time PCR. Cells were diluted to A₇₃₀ = 0.2 in 70 mL of modified BG-11 medium and cultivated overnight. Nitrogen sources were depleted from the medium by filtering the cells and suspending them in BG-11₀ medium. RNA isolation, cDNA synthesis,

and quantitative real-time PCR were performed as described previously [10]. The primers used for quantitative real-time PCR are listed in Table 1.

3. Results

3.1. Transcript Levels of Genes Related to the TCA Cycle in ROX370. The transcripts of 12 genes related to the TCA cycle, *gltA* (sll0401, encoding a citrate synthase), *acnB* (slr0665, encoding an aconitate hydratase), *icd* (slr1289, encoding an isocitrate dehydrogenase), *gabD* (slr0370, encoding a succinic semialdehyde dehydrogenase), *kgd* (sll1981, encoding a 2-OG decarboxylase), *sucC* (sll1023, encoding a succinyl-CoA synthetase beta chain), *sucD* (sll1557, encoding a succinyl-CoA synthetase alpha chain), *sdhA* (slr1233, encoding a succinate dehydrogenase flavoprotein subunit), *sdhB* (sll0823) (encoding a succinate dehydrogenase iron-sulfur subunit), *sdhB* (sll1625) (encoding a succinate dehydrogenase iron-sulfur subunit), *fumC* (slr0018, encoding a fumarase), and *citH* (sll0891, encoding a malate dehydrogenase), were measured using cells grown under nitrogen-replete conditions or nitrogen-depleted conditions for 4 h. The expression of *acnB* and *icd* increased in the GT strain after 4 h of nitrogen depletion (Figure 1). *rre37* overexpression abolished the induction of *acnB* and *icd* expression under nitrogen-depleted conditions (Figure 1). The expression of *kgd* in ROX370 was lower than that of the GT strain under nitrogen-replete conditions (Figure 1). The transcripts of *gabD*, *sucC*, *sucD*, *fumC*, and *citH* in the GT strain decreased by nitrogen-depleted conditions (Figures 1 and 2). A decrease in the levels of transcripts, except *fumC*, was also observed in ROX370 strain after nitrogen starvation (Figure 2). The expression of genes encoding the succinate dehydrogenase subunits, *sdhA*, *sdhB* (sll0823), and *sdhB* (sll1625), was lower in ROX370 than in the GT strain under both nitrogen-replete and nitrogen-depleted conditions (Figure 2).

3.2. Transcript Levels of Genes Related to the Pyruvate Metabolism in ROX370. The transcripts of 13 genes related to the pyruvate metabolism, *ppc* (sll0920, encoding a phosphoenolpyruvate carboxylase), *pps* (slr0301, encoding a phosphoenolpyruvate synthase), *pyk1* (sll0587, encoding a pyruvate kinase), *pyk2* (sll1275, encoding a pyruvate kinase), *me* (slr0721, encoding a malic enzyme), *ddh* (slr1556, encoding a D-lactate dehydrogenase), *pdhA* (slr1934, encoding a subunit of pyruvate dehydrogenase), *pdhB* (sll1721, encoding a subunit of pyruvate dehydrogenase), *pdhC* (sll1841, encoding a subunit of pyruvate dehydrogenase), *pdhD* (slr1096, encoding a subunit of pyruvate dehydrogenase), *pta* (slr2131, encoding a phosphoacetyltransferase), *ackA* (sll1299, encoding an acetate kinase), and *acs* (sll0542, encoding an acetyl-CoA synthetase), were measured using cells grown under nitrogen-replete conditions or nitrogen-depleted conditions for 4 h. The expressions of *ppc*, *pyk1*, *me*, and *pta* increased after 4 h of nitrogen depletion in the GT strain, but the increases were abolished by *rre37* overexpression (Figures 3 and 4). In particular, *rre37* overexpression affected the expression of *pyk1* and *me*, whose transcripts increased in the GT strain but decreased in the ROX370 strain after nitrogen

TABLE 1: Primer list for quantitative real-time PCR.

Gene name	
<i>rnpB</i>	Forward primer 5'-AAAGGGTAAGGGTGCAAAGG-3' Reverse primer 5'-AATTCCTCAAGCGGTTCAC-3'
<i>gltA</i>	Forward primer 5'-ATCGAGGGTGAGCCATGTG-3' Reverse primer 5'-GCGAATGCCCCGGTACT-3'
<i>acnB</i>	Forward primer 5'-TCACCCTCGCCCCAAAAA-3' Reverse primer 5'-GTGCCGGGACGAATACCTT-3'
<i>icd</i>	Forward primer 5'-CCCCGGCTCTGTGATCCT-3' Reverse primer 5'-TGCCAGCCCATAAATTCCA-3'
<i>gabD</i>	Forward primer 5'-TGCGCAAGTAGAACAACCATTT-3' Reverse primer 5'-TGGCCGCCACAACGA-3'
<i>kgd</i>	Forward primer 5'-CCATTTCCAAGGCCAAAAAC-3' Reverse primer 5'-GCTTCGGCTCGGATGGT-3'
<i>sdhA</i>	Forward primer 5'-GTCTGGCCCCTGATACCAA-3' Reverse primer 5'-GAACGGATGGGATGGGTTT-3'
<i>sdhB</i> (sll0823)	Forward primer 5'-TCAGATCAAATGGCAACAGGAT-3' Reverse primer 5'-TGGCATTACGGCAATTCTTG-3'
<i>sdhB</i> (sll1625)	Forward primer 5'-TGCAGTATGCGGGTTAATGG-3' Reverse primer 5'-TTCACTGCCCACATTTTCCTT-3'
<i>sucC</i>	Forward primer 5'-CCCTCAAACGGTTGCAAATT-3' Reverse primer 5'-GCCCGCACCTGGGATT-3'
<i>sucD</i>	Forward primer 5'-GGGCGCAAAAATCAAACG-3' Reverse primer 5'-AGTTGGTTGGCCACAATGGT-3'
<i>fumC</i>	Forward primer 5'-GAATGTTTTGCAGGCATCACTAAA-3' Reverse primer 5'-GGGCACTGCGTCCATCA-3'
<i>citH</i>	Forward primer 5'-CTGAAATTGCCGCCTTACTACA-3' Reverse primer 5'-AAGAGGCCGGCGCATAA-3'
<i>ppc</i>	Forward primer 5'-CCACCACCACAGCCCTACTAA-3' Reverse primer 5'-GTCGGAATAGCCCACCATAATTT-3'
<i>pps</i>	Forward primer 5'-TCACTGACCGGGCTATTTCTT-3' Reverse primer 5'-CCACCGCAAAAATGGTCAAA-3'
<i>pyk1</i>	Forward primer 5'-CGTGGCCAACGCTATTTTG-3' Reverse primer 5'-CGATTCCCCCGATAACATCA-3'
<i>pyk2</i>	Forward primer 5'-ATGCCGGCTCTGTGCAA-3' Reverse primer 5'-GGGCGACTGGTGAGGGTAT-3'
<i>me</i>	Forward primer 5'-CGGAGCCACCGATATTTGG-3' Reverse primer 5'-TGCGATGTTTGCCACAA-3'
<i>ddh</i>	Forward primer 5'-AGCAAACCACCCCATCA-3' Reverse primer 5'-CAAGGTTGAGTTGGGCATCA-3'
<i>pdhA</i>	Forward primer 5'-CACGAGCGGGCAACGT-3' Reverse primer 5'-TGTTGAACACACTGGCTTTTTTG-3'
<i>pdhB</i>	Forward primer 5'-CCGCATGCGTCACCATT-3' Reverse primer 5'-GGTCGTAGCCTTCTTTTTCCAA-3'
<i>pdhC</i>	Forward primer 5'-GGGCAACCCTTGGCCTAGT-3' Reverse primer 5'-GCTTGGGCTTCGGCAAT-3'
<i>pdhD</i>	Forward primer 5'-AAAATCCAATCTGACCTGACCAA-3' Reverse primer 5'-CCCCGGATGGTATCGACTT-3'
<i>pta</i>	Forward primer 5'-GACGCCCCTCCCCTGTT-3' Reverse primer 5'-AAACGGGCGGAAGTTTCAT-3'
<i>ackA</i>	Forward primer 5'-CCTGGTGGGCCATCGA-3' Reverse primer 5'-AAAGTGGCTTCGGCATGATC-3'
<i>acs</i>	Forward primer 5'-TGGCGGCGGTAAATGC-3' Reverse primer 5'-CGCCGGTTTCCGTTTG-3'

FIGURE 1: Quantitative real-time PCR analysis of transcription in GT and ROX370. Relative transcript levels of 6 genes involved in the TCA cycle pathway (*gltA*, *acnB*, *icd*, *gabD*, *kdg*, and *sucC*) are described. Data represent the mean $\pm$ SD from four independent experiments. Transcript levels were calibrated relative to that of corresponding levels in GT under nitrogen-replete conditions (set at 100%). Asterisks indicate statistically significant differences between GT and ROX370 (Student's *t*-test; **P* < 0.05, ***P* < 0.005).

FIGURE 2: Quantitative real-time PCR analysis of transcription in GT and ROX370. Relative transcript levels of 6 genes involved in the TCA cycle pathway (*sucD*, *sdhA*, *sdhB* (sll0823), *sdhB* (sll1625), *fumC*, and *citH*) are described. Data represent the mean $\pm$ SD from four independent experiments. Transcript levels were calibrated relative to that of corresponding levels in GT under nitrogen-replete conditions (set at 100%). Asterisks indicate statistically significant differences between GT and ROX370 (Student's *t*-test; * *P* < 0.05, ** *P* < 0.005).

FIGURE 3: Quantitative real-time PCR analysis of transcription in GT and ROX370. Relative transcript levels of 6 genes involved in pyruvate metabolism (*ppc*, *pps*, *pyk1*, *pyk2*, *me*, and *ddh*) are shown. Data represent the mean $\pm$ SD from four independent experiments. Transcript levels were calibrated relative to that of corresponding levels in GT under nitrogen-replete conditions (set at 100%). Asterisks indicate statistically significant differences between GT and ROX370 (Student's *t*-test; * $P < 0.05$, ** $P < 0.005$).

FIGURE 4: Quantitative real-time PCR analysis of transcription in GT and ROX370. Relative transcript levels of 7 genes involved in pyruvate metabolism (*pdhA*, *pdhB*, *pdhC*, *pdhD*, *pta*, *ackA*, and *acs*) are shown. Data represent the mean $\pm$ SD from four independent experiments. Transcript levels were calibrated relative to that of corresponding levels in GT under nitrogen-replete conditions (set at 100%). Asterisks indicate statistically significant differences between GT and ROX370 (Student's *t*-test; * $P < 0.05$, ** $P < 0.005$).

FIGURE 5: The metabolic map around the TCA and pyruvate metabolism in *Synechocystis* 6803.

depletion (Figure 3). The transcript levels of *pps*, *pyk2*, *ddh*, *pdhABCD*, *ackA*, and *acs* decreased in the GT strain by nitrogen depletion; the levels of these transcripts, except for *ddh* and *ackA*, also decreased in the ROX370 strain (Figures 3 and 4).

4. Discussion

Quantitative real-time PCR analysis demonstrated that the levels of transcripts of 6 genes related to the TCA cycle and pyruvate metabolism increased under conditions of nitrogen depletion in the wild-type strain, but these increases were abolished by *rre37* overexpression (Figures 1–4). The results showed that the transcript levels of the genes whose expression is induced by nitrogen depletion particularly were altered by *rre37* overexpression (Figures 1, 3, and 4). These results indicate that the proper amount of Rre37 proteins is important in the transcriptional activation of these genes during nitrogen starvation, consistent with a previous study [17]. Among the 25 genes tested in this study, the transcription of *icd* was activated by NtcA [24], and the other 24 genes did not seem to be regulated by NtcA [25]. A previous study suggested that transcription of *me* is positively regulated by Rre37, although further biochemical analysis is required to validate this [19]. In addition to *in vitro* analysis, chromatin immunoprecipitation (ChIP) showed that, in *Anabaena* sp. PCC 7120, 55 genes involved in primary metabolism, including *pps*, are potentially regulated by NtcA [26]. Using genetic analyses, we demonstrated that Rre37 is involved in the transcriptional regulation of the genes related to the TCA cycle and pyruvate metabolism. Future studies with ChIP analyses may uncover whether Rre37 regulates genes in the TCA cycle and pyruvate metabolism directly or indirectly.

The expression of *rre37* is affected by environmental conditions and genetic modifications. SigE is a global regulator of sugar catabolism activation [27, 28], and *sigE* overexpression increases polyhydroxybutyrate and hydrogen production [29, 30]. Rre37 protein levels are upregulated by *sigE* knockout [17]. Another study has shown that knockout of a gene encoding the transcription factor SyAbrB2 decreases the transcript levels of *sigE*, *gnd* (encoding 6-phosphogluconate dehydrogenase), *pyk1*, *pyk2*, and *icd* [31], implying that Rre37 levels may be affected by *SyabrB2* knockout. Thus, several transcriptional regulators, including NtcA, SigE, and SyAbrB2, are involved in the regulation of *rre37* transcription either directly or indirectly. Imamura et al. demonstrated that sigma factors are differently associated with the promoters of nitrogen-related genes in response to growth phase and external nutrient conditions [32]. The role of PipX, a small protein binding to NtcA in a 2-OG dependent manner [33], should be considered in *rre37* transcription during nitrogen starvation. Thus, multiple factors control Rre37 levels, leading to the regulation of primary metabolism in *Synechocystis* 6803. Elucidation of the regulatory mechanism of *rre37* transcription is complicated but is necessary for understanding of the primary metabolism of *Synechocystis* 6803. This is because transcript analyses have revealed that multiple transcriptional regulators, including Rre37, are involved in regulating the expression of metabolic enzymes, as also evidenced in this study.

We analyzed ROX370, overexpressing *rre37* strain, and found that the expression of genes related to primary metabolism is widely affected (Figures 1–4) [19]. We could not distinguish between the direct and indirect effects of *rre37* overexpression in the altered transcription seen in the ROX370 strain. Therefore, we could not elucidate the

molecular mechanisms underlying transcriptional regulation by Rre37. However, these results demonstrate that genetic engineering of a transcription factor rewires *Synechocystis* 6803 metabolism, which is beneficial for biotechnological applications for producing high-value products. In addition, the expression pattern of *me* was different from the other 24 genes tested (Figures 1–4), and these results may indicate the key enzyme of the primary metabolism in this cyanobacterium. Accumulation of organic acids in the later half of the TCA cycle during nitrogen starvation has been demonstrated [10], and the transcript results suggest that the malic enzyme may play important roles in their accumulation (Figure 5). The metabolomic technique is indispensable for the analysis of these mutants and provides significant information regarding primary metabolism in *Synechocystis* 6803 as previously described [10, 28]. This is significant, considering that both carbon and nitrogen signals mediated by transcription factors are important in the regulation of primary metabolism and carbon uptake in other cyanobacteria [34].

5. Conclusion

The TCA cycle and pyruvate metabolism are important for interaction between carbon and nitrogen metabolism. Detailed analysis of their regulatory mechanisms is valuable in understanding the biology of metabolism of cyanobacteria, in biotechnological applications for producing valuable products [35], and in environmental microbiology for avoiding water pollution [36]. The transcript levels analysis in this study demonstrated the differential control of the transcript levels of the TCA and pyruvate metabolism, possibly indicating key enzymes in primary metabolism of this unicellular cyanobacterium.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This work was supported by the Ministry of Education, Culture, Sports, Science, and Technology, Japan, by a grant to Takashi Osanai from ALCA (project name “Production of Cyanobacterial Succinate by the Genetic Engineering of Transcriptional Regulators and Circadian Clocks”), the Japan Science and Technology Agency.

References

- [1] T. Kaneko, S. Sato, H. Kotani et al., “Sequence analysis of the genome of the unicellular cyanobacterium *synechocystis* sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions,” *DNA Research*, vol. 3, no. 3, pp. 109–136, 1996.
- [2] S. Zhang and D. A. Bryant, “The tricarboxylic acid cycle in cyanobacteria,” *Science*, vol. 334, no. 6062, pp. 1551–1553, 2011.
- [3] M. I. Muro-Pastor, J. C. Reyes, and F. J. Florencio, “Cyanobacteria perceive nitrogen status by sensing intracellular 2-oxoglutarate levels,” *The Journal of Biological Chemistry*, vol. 276, no. 41, pp. 38320–38328, 2001.
- [4] R. Tanigawa, M. Shirokane, S.-I. Maeda, T. Omata, K. Tanaka, and H. Takahashi, “Transcriptional activation of NtcA-dependent promoters of *Synechococcus* sp. PCC 7942 by 2-oxoglutarate *in vitro*,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 7, pp. 4251–4255, 2002.
- [5] M. F. Vázquez-Bermúdez, A. Herrero, and E. Flores, “2-oxoglutarate increases the binding affinity of the NtcA (nitrogen control) transcription factor for the *Synechococcus glnA* promoter,” *FEBS Letters*, vol. 512, no. 1–3, pp. 71–74, 2002.
- [6] O. Fokina, V.-R. Chellamuthu, K. Forchhammer, and K. Zeth, “Mechanism of 2-oxoglutarate signaling by the *Synechococcus elongatus* P_{II} signal transduction protein,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 46, pp. 19760–19765, 2010.
- [7] K. Zeth, O. Fokinas, and K. Forchhammers, “Structural basis and target-specific modulation of ADP sensing by the *Synechococcus elongatus* PII signaling protein,” *Journal of Biological Chemistry*, vol. 289, no. 13, pp. 8960–8972, 2014.
- [8] T. Hasunuma, F. Kikuyama, M. Matsuda, S. Aikawa, Y. Izumi, and A. Kondo, “Dynamic metabolic profiling of cyanobacterial glycogen biosynthesis under conditions of nitrate depletion,” *Journal of Experimental Botany*, vol. 64, no. 10, pp. 2943–2954, 2013.
- [9] W. Hauf, M. Schlebusch, J. Hüge, J. Kopka, M. Hagemann, and K. Forchhammer, “Metabolic changes in *Synechocystis* PCC6803 upon nitrogen-starvation: excess NADPH sustains polyhydroxybutyrate accumulation,” *Metabolites*, vol. 3, no. 1, pp. 101–118, 2013.
- [10] T. Osanai, A. Oikawa, T. Shirai et al., “Capillary electrophoresis-mass spectrometry reveals the distribution of carbon metabolites during nitrogen starvation in *Synechocystis* sp. PCC 6803,” *Environmental Microbiology*, vol. 16, no. 2, pp. 512–524, 2014.
- [11] S. Meissner, D. Steinhauser, and E. Dittmann, “Metabolomic analysis indicates a pivotal role of the hepatotoxin microcystin in high light adaptation of *Microcystis*,” *Environmental Microbiology*, 2014.
- [12] T. Osanai, S. Imamura, M. Asayama et al., “Nitrogen induction of sugar catabolic gene expression in *Synechocystis* sp. PCC 6803,” *DNA Research*, vol. 13, no. 5, pp. 185–195, 2006.
- [13] S. Ehira and M. Ohmori, “NrrA, a nitrogen-responsive response regulator facilitates heterocyst development in the cyanobacterium *Anabaena* sp. strain PCC 7120,” *Molecular Microbiology*, vol. 59, no. 6, pp. 1692–1703, 2006.
- [14] S. Ehira and M. Ohmori, “NrrA directly regulates expression of hetR during heterocyst differentiation in the cyanobacterium *Anabaena* sp. strain PCC 7120,” *Journal of Bacteriology*, vol. 188, no. 24, pp. 8520–8525, 2006.
- [15] S. Ehira and M. Ohmori, “NrrA, a nitrogen-regulated response regulator protein, controls glycogen catabolism in the nitrogen-fixing cyanobacterium *Anabaena* sp. strain PCC 7120,” *The Journal of Biological Chemistry*, vol. 286, no. 44, pp. 38109–38114, 2011.
- [16] Y. Tabei, K. Okada, and M. Tsuzuki, “Sll1330 controls the expression of glycolytic genes in *Synechocystis* sp. PCC 6803,” *Biochemical and Biophysical Research Communications*, vol. 355, no. 4, pp. 1045–1050, 2007.

- [17] M. Azuma, T. Osanai, M. Y. Hirai, and K. Tanaka, "A response regulator Rre37 and an RNA polymerase sigma factor SigE represent two parallel pathways to activate sugar catabolism in a cyanobacterium *Synechocystis* sp. PCC 6803," *Plant and Cell Physiology*, vol. 52, no. 2, pp. 404–412, 2011.
- [18] D. Liu and C. Yang, "The nitrogen-regulated response regulator *nrRa* controls cyanophycin synthesis and glycogen catabolism in the cyanobacterium *Synechocystis* sp. PCC 6803," *The Journal of Biological Chemistry*, vol. 289, no. 4, pp. 2055–2071, 2014.
- [19] T. Osanai, A. Oikawa, K. Numata et al., "Pathway-level acceleration of glycogen catabolism by a response regulator in the cyanobacterium *Synechocystis* species PCC 6803," *Plant Physiology*, vol. 164, no. 4, pp. 1831–1841, 2014.
- [20] A. Joseph, S. Aikawa, K. Sasaki et al., "Rre37 stimulates accumulation of 2-oxoglutarate and glycogen under nitrogen starvation in *Synechocystis* sp. PCC 6803," *FEBS Letters*, vol. 588, no. 3, pp. 466–471, 2014.
- [21] J. G. K. Williams, "Construction of specific mutations in photosystem II photosynthetic reaction center by genetic engineering methods in *Synechocystis* 6803," *Methods in Enzymology*, vol. 167, pp. 766–778, 1988.
- [22] R. Rippka, "Isolation and purification of cyanobacteria," *Methods in Enzymology*, vol. 167, pp. 3–27, 1988.
- [23] Y. Kanesaki, Y. Shiwa, N. Tajima et al., "Identification of substrain-specific mutations by massively parallel whole-genome resequencing of *Synechocystis* sp. PCC 6803," *DNA Research*, vol. 19, no. 1, pp. 67–79, 2012.
- [24] M. Isabel Muro-Pastor, J. C. Reyes, and F. J. Florencio, "The NADP⁺-isocitrate dehydrogenase gene (*icd*) is nitrogen regulated in cyanobacteria," *Journal of Bacteriology*, vol. 178, no. 14, pp. 4070–4076, 1996.
- [25] Z. Su, V. Olman, F. Mao, and Y. Xu, "Comparative genomics analysis of NtcA regulons in cyanobacteria: regulation of nitrogen assimilation and its coupling to photosynthesis," *Nucleic Acids Research*, vol. 33, no. 16, pp. 5156–5171, 2005.
- [26] S. Picossi, E. Flores, and A. Herrero, "ChIP analysis unravels an exceptionally wide distribution of DNA binding sites for the NtcA transcription factor in a heterocyst-forming cyanobacterium," *BMC Genomics*, vol. 15, no. 1, article 22, 2014.
- [27] T. Osanai, M. Imashimizu, A. Seki et al., "ChlH, the H subunit of the Mg-chelatase, is an anti-sigma factor for SigE in *Synechocystis* sp. PCC 6803," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 16, pp. 6860–6865, 2009.
- [28] T. Osanai, A. Oikawa, M. Azuma et al., "Genetic engineering of group 2 σ factor SigE widely activates expressions of sugar catabolic genes in *Synechocystis* species PCC 6803," *The Journal of Biological Chemistry*, vol. 286, no. 35, pp. 30962–30971, 2011.
- [29] T. Osanai, K. Numata, A. Oikawa et al., "Increased bioplastic production with an RNA polymerase sigma factor SigE during nitrogen starvation in *Synechocystis* sp. PCC 6803," *DNA Research*, vol. 20, no. 6, pp. 525–535, 2013.
- [30] T. Osanai, A. Kuwahara, H. Iijima et al., "Pleiotropic effect of sigE over-expression on cell morphology, photosynthesis and hydrogen production in *Synechocystis* sp. PCC 6803," *The Plant Journal*, vol. 76, no. 3, pp. 456–465, 2013.
- [31] Y. Kaniya, A. Kizawa, A. Miyagi et al., "Deletion of the transcriptional regulator *cyAbrB2* deregulates primary carbon metabolism in *Synechocystis* sp. PCC 6803," *Plant Physiology*, vol. 162, no. 2, pp. 1153–1163, 2013.
- [32] S. Imamura, K. Tanaka, M. Shirai, and M. Asayama, "Growth phase-dependent activation of nitrogen-related genes by a control network of group 1 and group 2 σ factors in a cyanobacterium," *Journal of Biological Chemistry*, vol. 281, no. 5, pp. 2668–2675, 2006.
- [33] J. Espinosa, K. Forchhammer, S. Burillo, and A. Contreras, "Interaction network in cyanobacterial nitrogen regulation: PipX, a protein that interacts in a 2-oxoglutarate dependent manner with PII and NtcA," *Molecular Microbiology*, vol. 61, no. 2, pp. 457–469, 2006.
- [34] R. López-Igual, S. Picossi, J. López-Garrido, E. Flores, and A. Herrero, "N and C control of ABC-type bicarbonate transporter Cmp and its LysR-type transcriptional regulator CmpR in a heterocyst-forming cyanobacterium, *Anabaena* sp.," *Environmental Microbiology*, vol. 14, no. 4, pp. 1035–1048, 2012.
- [35] N. C. Bhatt, A. Panwar, T. S. Bisht, and S. Tamta, "Coupling of algal biofuel production with wastewater," *The Scientific World Journal*, vol. 2014, Article ID 210504, 10 pages, 2014.
- [36] B. A. Neilan, L. A. Pearson, J. Muenchhoff, M. C. Moffitt, and E. Dittmann, "Environmental conditions that influence toxin biosynthesis in cyanobacteria," *Environmental Microbiology*, vol. 15, no. 5, pp. 1239–1253, 2013.