Positive regulation of sugar catabolic pathways in the cyanobacterium *Synechocystis* sp. PCC 6803 by the group 2 sigma factor SigE

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Running Title: Cyanobacterial Sigma Factor That Activates Sugar Catabolism

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The sigE gene of *Synechocystis* sp. PCC 6803 encodes a group 2 sigma factor for RNA polymerase and has been proposed to function in transcriptional regulation of nitrogen metabolism. Using microarray and Northern analyses, we have now demonstrated that the abundance of transcripts derived from genes important for glycolysis, the oxidative pentose phosphate (OPP) pathway, and glycogen catabolism is reduced in a *sigE* mutant of *Synechocystis* maintained under the normal growth condition. Furthermore, the activities of two key enzymes of the OPP pathway—glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase—encoded by *zwf* and *gnd* genes were also reduced in the *sigE* mutant. The dark-enhancement in both enzyme activity and transcript abundance apparent in the wild type were eliminated by the mutation. In addition, the *sigE* mutant showed a reduced rate of glucose uptake and an increased intracellular level of glycogen. Moreover, it was unable to proliferate under the light-activated heterotrophic growth condition. These results indicate that SigE functions in the transcriptional activation of sugar catabolic pathways in *Synechocystis* sp. PCC 6803.

Cyanobacteria, which constitute one of the largest taxonomic groups of eubacteria, perform oxygenic photosynthesis similar to higher plants and algae. In spite of the diversity in their morphology, physiology and cellular development, all cyanobacteria are able to assimilate inorganic carbon via the reductive pentose phosphate cycle.
by using light energy. Moreover, in the absence of light, cyanobacteria utilize assimilated organic carbon, and some species of cyanobacterium are even capable of utilizing exogenous organic carbon. The cyanobacterium *Synechocystis* sp. PCC 6803 grows photoautotrophically under the light condition while survives in the dark by catabolizing storage carbohydrates such as glycogen. Although the original isolate of this strain was not able to grow in the presence of glucose, a glucose-tolerant (GT) mutant was subsequently isolated (1). In the presence of glucose, this GT strain can grow mixotrophically under the light condition as well as heterotrophically in the dark if provided with a daily pulse of white light. The latter type of growth is referred to as light-activated heterotrophic growth (LAHG) (2).

Similar to other eubacteria, the genome of cyanobacteria encodes multiple sigma factors for RNA polymerase. These factors recognize gene promoters and determine the specificity of transcription by RNA polymerase. In general, eubacterial sigma factors are classified into three categories: Group 1 sigma factors are unique to each strain and correspond to the essential principal sigma factor. Group 2 sigma factors show high sequence similarity to group 1 sigma factors. They also exhibit overlapping promoter recognition specificity with group 1 sigma factors, however are nonessential for growth. Group 3 includes all other types of sigma factor. The existence of multiple sigma factors is thought to allow switching of gene expression patterns in response to changes in the environment or developmental stage (3).

In comparison to other eubacteria, cyanobacteria have unusually large number of group 2 sigma factors (4). Group 2 sigma factors of cyanobacteria were firstly discovered in *Anabaena* sp. PCC 7120, *Synechococcus* sp. PCC 7942, *Synechococcus* sp. PCC 7002 and *Microcystis* sp. (5-8). Genome analyses of *Synechocystis* sp. PCC 6803 and subsequent cyanobacterial genome projects revealed at least four group 2 sigma factors in each strain (9). A similar number of group 2 sigma factors had only previously been found in *Actinomycetes* (10). In *Anabaena* sp. PCC 7120, the genes for the group 2 sigma factors SigB and SigC are induced by nitrogen or sulfur deprivation, suggesting that these factors play a role in adaptation to nutrient limitation (5). In *Synechococcus* sp. PCC 7002, the abundance of the *sigB* transcript is increased by either nitrogen or carbon depletion, whereas that of the *sigC* transcript initially increased, then declined under the same conditions (7). Moreover, SigE of *Synechococcus* sp. PCC 7002 was shown to be responsible for transcription of the *dpsA* gene, which encodes a nucleoid protein, during the stationary phase of growth (11). In the case of nitrogen-fixing cyanobacteria, SigH of *Nostoc*
punctiforme is implicated in symbiosis with plant hosts (12). SigD, SigE, and SigF of Anabaena sp. PCC 7120 contribute to cellular differentiation under diazotrophic growth conditions (13). Four group 2 sigma factors—RpoD2, RpoD3, RpoD4, and SigC—of Synechococcus sp. PCC 7942 are important for circadian rhythmicity of transcription (14, 15).

Four group 2 sigma factors—SigB, SigC, SigD, and SigE—have been identified in Synechocystis sp. PCC 6803. The expression patterns of sigB and sigD differ during the dark to light transition. Both sigB and sigD transcripts are also induced under various stress conditions (16, 17). SigC activates transcription of glnB, which encodes the carbon-nitrogen sensor PII, in the stationary phase (18, 19). Transcription of sigE increases by nitrogen depletion in a manner dependent on the global nitrogen regulator NtcA (20). Disruption of sigE results in a loss of viability under conditions of nitrogen deprivation as well as in a reduction in the expression of glnN (20), which encodes a type III glutamine synthetase. However, expression of most NtcA-dependent genes is not affected by the sigE mutation, and the relation between SigE and nitrogen regulation is still unclear. Moreover, the abundance of SigE is affected by the light to dark transition (16), suggesting that this sigma factor is important in these aspects of cell physiology which are unrelated to nitrogen availability.

In the present study, we investigated the targets of the regulatory function of SigE in Synechocystis sp. PCC 6803 and found that this sigma factor plays an important role in regulation of sugar catabolic pathways.

EXPERIMENTAL PROCEDURES

Bacterial strains and culture conditions—The GT strain of Synechocystis sp. PCC 6803 (1) and its derivatives were used in this study. Cells were grown in BG-11 medium containing 10 or 5 mM NH4Cl as a nitrogen source for plates and liquid culture, respectively. Medium was also supplemented with 20 mM HEPES-KOH (pH 8.0). The cells were cultured under continuous white light (~70 μmol photons m−2 s−1) at 30°C in an atmosphere of 2% (v/v) CO2. Liquid cultures were aerated with the same gas mixture (21). The dark condition was achieved by wrapping culture vessels with aluminum foil. For LAHG, BG-11 plates further containing 5 mM glucose were incubated in the dark with a daily pulse of white light for 15 min. Cell growth and density were determined by measurement of OD750 with a spectrophotometer (Beckman model DU640). For the construction of a sigE mutant, the sigE (sll1689) coding region of Synechocystis sp. PCC 6803 was isolated by digestion of M13 phage clone ps00320736 (CyanoBase positions 1,301,322 to 1,303,755) (22) with PvuII and was
cloned into the HincII site of pUC118 (TaKaRa). The gene was interrupted at the unique BglII site by insertion of a BamHI kanamycin resistance cassette derived from pUC4K (Pharmacia). The resulting construct was used to transform the GT strain. Colonies resistant to kanamycin (5 μg/ml) were selected, and isolation of a single colony was repeated three times. The disruption of sigE was confirmed by PCR with specific primers (Table SI) and also by immunoblot analysis with specific antiserum. The hik8 mutant was as described (23).

Isolation of RNA and microarray analysis—Cells of mid-exponential phase cultures of *Synechocystis* sp. PCC 6803 (OD750, 0.5 to 0.7) grown in BG-11-based medium were collected by centrifugation at 6,600 × g for 5 min. RNA was isolated from the cells by the previously described acid phenol–chloroform method (24). DNA microarray analysis was performed with Cyanocip version 1.6, which contained PCR fragments of full length or each 1 kbp of COOH-terminal part of the ORFs (TaKaRa), as described (23). Labeling of cDNA with Cy3-dUTP or Cy5-dUTP (Amersham Bioscience) was performed with an RNA Fluorescence Labeling Core Kit (M-MLV version) version 2.0 (TaKaRa). After hybridization, the microarray was rinsed with 0.2 × SSC and then scanned with an array scanner (GMS418, Affymetrix) (18). Signals were quantified and analyzed with ImaGene version 4.0 software (BioDiscovery) as described in (25). Three biologically independent microarray experiments were performed and similar results were obtained.

Northern blot analysis—Total RNA was extracted using the same method as for microarray analysis, and Northern blot analysis was performed as described (26). Gene-specific probes were constructed with specific primers (Table SI) and *Synechocystis* genomic DNA as template (27).

Assay of glucose uptake—Cells were collected at mid-exponential phase by centrifugation at 17,400 × g for 1 min and were resuspended in BG-11 supplemented with 5 mM NH4Cl and 2 mM glucose to obtain an OD750 of 1.0. Glucose uptake was assayed by measurement of the concentration of glucose in the medium with a Glucose CII kit (Wako Pure Chemicals). Portions (100 μl) of the culture were harvested every 2 h and centrifuged at 17,400 × g for 1 min, followed by the determination of glucose concentration of the resulting supernatant.

Analysis of G6PD and 6PGD activities—Glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) activities were measured by monitoring the glucose-6-phosphate- or 6-phosphogluconate-dependent increase in NADPH concentration at A340 with a Beckman DU640 spectrophotometer. For assay of G6PD activity, cells were suspended in 1 ml of buffer A [55 mM Tris-HCl (pH 8.0), 3.4 mM MgCl2] and disrupted by sonication. The
lysate was centrifuged at 17,400 × g for 5 min, and the resulting supernatant was used for the enzyme assay. The reaction was initiated at 25°C in a mixture containing 45 mM Tris-HCl (pH 8.0), 2.8 mM MgCl₂, 1 mM NADP⁺, and 10 mM glucose-6-phosphate, and the change in A₃₄₀ was monitored for 1 min. As a control, the change in A₃₄₀ in the absence of glucose-6-phosphate was measured and subtracted from the experimental values. The activity of 6PGD was measured according to the protocol provided by Oriental Yeast Co. Ltd. with some modifications.

**Determination of glycogen abundance**—Assay of intracellular glycogen was performed as described (28) with some modifications. Cells (~6 × 10⁸) were suspended in 100 μl of 3.5% (v/v) sulfuric acid and boiled for 40 min. Glucose produced by acid hydrolysis was quantified with the use of o-toluidine according to the protocol recommended by Sigma Diagnostics.

**Production of rabbit antiserum to SigE and immunoblot analysis**—The ORF of sigE was amplified by PCR with specific primers (Table S1) and cloned into pET21b (Novagen) with the use of the 5’ NdeI and 3’ XhoI sites included in the primers. After confirming the structure by sequencing, the resulting plasmid was used to transform Escherichia coli BL21 (DE3), and expression of the hexahistidine-tagged recombinant SigE protein was induced in cells cultivated at 37°C in LB medium by exposure to 1 mM isopropyl-β-D-thiogalactopyranoside (Wako Pure Chemicals) for 2 h. Cells were harvested from 500 ml cultures by centrifugation (2,400 × g for 10 min), resuspended in 25 ml of sonication buffer [50 mM sodium phosphate (pH 8.0), 300 mM NaCl], and disrupted by sonication. The SigE protein was present in inclusion bodies and was collected by centrifugation of the crude extract at 10,000 × g for 15 min. The resulting pellet was washed once with 25 ml of sonication buffer containing 4% (v/v) Triton X-100, dissolved in 5 ml of denaturation buffer [6 M guanidine hydrochloride, 0.1 M sodium phosphate, 0.01 M Tris-HCl (pH 8.0)], incubated at 37°C for 1 h, and centrifuged again at 10,000 × g for 15 min to remove debris. The recombinant protein was purified from the resulting supernatant by affinity chromatography with Ni-NTA agarose (Qiagen). After application of the sample and washing of the column with denaturation buffer, proteins were eluted with a solution containing 8 M urea, 0.1 M sodium phosphate, and 0.01 M Tris-HCl and a pH gradient of 8.0 to 4.5. SigE was eluted at pH 4.5 and its abundance was quantified with a Bio-Rad protein assay. The purified protein (1 mg) was mixed with the same amount of Freund’s complete adjuvant (Sigma) and injected into a rabbit for the generation of polyclonal antibodies. Immunoblot analysis with the resulting antiserum was performed as described (29).
RESULTS

Microarray analysis for identification of potential SigE targets—To provide insight into SigE function, we constructed a targeting vector by inserting a kanamycin resistance cassette into the Bg/II site of the gene and used the resulting construct to transform the wild-type (GT) strain of *Synechocystis* sp. PCC 6803 (Fig. 1A). The disruption of endogenous sigE in the transformed strain, designated G50, was confirmed by PCR (Fig. 1B) and immunoblot analysis with antiserum specific for SigE (Fig. 1C). The growth of G50 under photoautotrophic or photomixotrophic conditions did not appear to differ from that of the wild type (data not shown).

To compare the transcriptomes of GT and G50, we extracted total RNA from both strains cultured under the normal growth condition and processed it for microarray analysis with a gene chip including fragments of cyanobacterial ORF (Fig. 1D). Of the 3,076 genes represented on the array, the expression level of 67 genes was reduced over two-fold by the sigE mutation (Table SII). The down-regulated genes included several genes whose products contribute to sugar catabolism, including enzymes that participate in glycolysis, the oxidative pentose phosphate (OPP) pathway, or glycogen breakdown (Table I). These results thus suggest that the expression of these genes is dependent on SigE.

Positive regulation of glycolysis by SigE—The genes whose expression was reduced by the sigE mutation and whose products contribute to glycolysis included those for phosphofructokinase [pfkA (*sll1196*)], glyceraldehyde-3-phosphate dehydrogenase (*gap1*), and pyruvate kinase (*pyk1*) (Table I). Northern hybridization confirmed that the abundance of transcripts of these three genes was reduced in G50 cells under the normal growth condition (Fig. 2A). The genome of *Synechocystis* sp. PCC 6803 contains two structural genes for each of these enzymes. Phosphofructokinase and pyruvate kinase catalyze irreversible reactions in glycolysis. Induction of the expression of either of the two structural genes for each of these enzymes would be expected to increase glycolytic flux. Of the two genes for glyceraldehyde-3-phosphate dehydrogenase, *gap1* encodes an NAD+-dependent enzyme that has been shown to be responsible for the glycolytic reaction (30). Induction of transcription of *pfkA* (*sll1196*), *gap1*, and *pyk1* by SigE thus likely increases the rate of glycolysis in *Synechocystis* sp. PCC 6803.

Given that sugar catabolism is especially important in the absence of light, we also examined the effects of a shift from light to dark on the amounts of the transcripts of these three genes. Northern blot analysis revealed that, for GT cells, the abundance of *pyk1* transcripts was increased slightly, whereas that of *pfkA* (*sll1196*)
and $gap1$ transcripts remained unchanged 1 h after the dark shift. However, the amount of $pyk1$ transcripts was decreased slightly and that of $pfkA$ ($sll1196$) and $gap1$ transcripts was reduced markedly after 4 h (Fig. 2A). Consistent with a role for SigE in regulation of the expression of these genes, in G50, although a slight induction of $pyk1$ expression was observed after the dark shift, the transcript levels of all three genes remained reduced compared with those in the wild-type (Fig. 2A).

**Positive regulation of the OPP pathway by SigE**—Microarray analysis suggested that transcription of OPP pathway genes was reduced by the $sigE$ mutation (Table I). Northern blot analysis confirmed that both under the normal growth condition and after the dark shift the abundance of transcripts derived from $gnd$ (which encodes 6PGD, a key enzyme of the OPP pathway) and those derived from $tal$ (which encodes transaldolase, another enzyme of the OPP pathway) was greatly reduced in G50 cells compared with GT cells (Fig. 2B). The amounts of transcripts derived from $zwf$ (which encodes G6PD, another key enzyme of the OPP pathway) and from $opcA$ (which encodes a protein that is conserved only among cyanobacteria and is required for oligomerization of G6PD) were also reduced by the $sigE$ mutation under both light and dark conditions (Fig. 2B). Consistent with these differences in transcript levels, the activities of both G6PD and 6PGD were decreased by the $sigE$ mutation under the normal growth condition and after the dark shift (Fig. 2, C and D). In GT cells, the enhancement in the activities of these enzymes 4 h after dark shift was also reduced (G6PD) or actually reversed by the $sigE$ mutation (Fig. 2, C and D). These results thus indicate that metabolic flux through the OPP pathway is down-regulated in G50 cells.

**Reduced rate of glucose uptake in the $sigE$ mutant**—Current observations indicate that glycolysis and the OPP pathway are positively regulated by SigE, suggesting that the rate of glucose catabolism might be reduced by the $sigE$ mutation. To examine this possibility, we cultured GT and G50 in medium supplemented with glucose and measured the rate of glucose uptake by the cells. As predicted, in comparison to wild-type, the rate of glucose uptake in G50 cells was reduced (by ~20 to 30%) (Fig. 3), suggesting that the rate of glucose catabolism is indeed reduced by the $sigE$ mutation.

**Positive regulation of glycogen catabolism by SigE**—Microarray analysis suggested that the abundance of transcripts derived from the genes for glycogen isoamylase [$glgX$ ($slr0237$)] and glycogen phosphorylase [$glgP$ ($sll1356$)] were reduced by the $sigE$ mutation (Table I). In eubacteria, most glycogen is degraded through the action of glycogen phosphorylase. However glycogen isoamylase is also required for the
complete digestion of glycogen (31). The genome of *Synechocystis* sp. PCC 6803 contains two *glgX* and two *glgP* genes, and we examined the abundance of the transcripts of all four genes by Northern analysis. Consistent with the microarray data, the amounts of *glgX* (*slr0237*) and *glgP* (*sll1356*) transcripts were reduced in G50 under the normal growth condition (Fig. 4A). The *sigE* deficiency did not markedly affect the levels of *glgX* (*slr1857*) and *glgP* (*slr1367*) transcripts under the normal growth condition. However, the amount of *glgP* (*slr1367*) transcripts in the dark condition was reduced in G50 compared with that in GT (Fig. 4A). We further examined whether these effects of the *sigE* mutation resulted in a difference in the extent of glycogen accumulation between GT and G50 cells. Under the normal growth condition, the amount of glycogen in G50 was greater than that in GT (Fig. 4B). Transfer of both strains to the dark resulted in reduction in the amount of glycogen. However, the rate of glycogen utilization was reduced by ~10 to 20% in the *sigE* mutant (Fig. 4B). These results indicated that glycogen catabolism was reduced by the *sigE* mutation.

**Impaired heterotrophic growth and dark survival of the *sigE* mutant**—Finally, we examined G50 for phenotypes related to sugar catabolism. GT and G50 cells were grown to mid-logarithmic phase, and serial dilutions of the cultures were then spotted onto plates supplemented with 5 mM glucose and were incubated under the LAHG condition. In contrast to GT cells, G50 cells were incapable of proliferation under this condition (Fig. 5A). We also compared the abilities of the two strains to survive in the dark by spotting them onto plates supplemented with glucose as for the LAHG condition and then incubating them in darkness for 4 days. The cells were then exposed to light and their growth was monitored. The appearance of visible colonies was markedly delayed by the *sigE* mutation (Fig. 5B). Although it is unclear whether this effect of the mutation was due to a loss of cell viability or to lengthening of the latent growth period, both phenotypes are consistent with the defect in sugar catabolism in G50 cells.

**Regulatory relationship between SigE and Hik8**—Very recently, it has been reported that a histidine kinase Hik8 (*sll0750*) positively regulates the expression of sugar catabolic and anabolic genes in *Synechocystis* sp. PCC 6803 (32). They also showed that transcript levels of *cph1* [encoding a cyanobacterial phytochrome (*slr0473*)] and *rcp1* [encoding a response regulator cotranscribed with *cph1* (*slr0474*)] were decreased by the *hik8* disruption (32). In the current investigation, microarray analysis also suggested the SigE dependence of these gene expressions (Table SII), which was further confirmed by Northern hybridization analysis (Fig. 6A). Since target genes of Hik8 were partially identical to
those of SigE, we examined the regulatory relationship between SigE and Hik8. The result of microarray analysis indicated that the hik8 transcript levels were almost identical in GT and G50 cells [Expression ratio (G50/GT) was 0.976; Table SII], suggesting that Hik8 expression is not under the control of SigE. We also found that the amount of SigE was not affected by the hik8 mutation (Fig. 6B), indicating that Hik8 is not required for the SigE expression. Thus, Hik8 and SigE presumably activate these gene transcriptions independently or cooperatively.

DISCUSSION

In the current investigation, microarray and Northern analyses have revealed that the abundance of transcripts derived from genes important for glycolysis, the OPP pathway, and glycogen catabolism is decreased in a sigE mutant (G50) of Synechocystis sp. PCC 6803 (Fig. 7). Furthermore, the rate of glucose utilization was reduced and glycogen accumulation was increased in the mutant cells, indicating that SigE positively regulates sugar catabolism in Synechocystis.

The expression of various genes other than those important for sugar catabolism was also reduced in G50 cells compared with the parental GT strain. It is therefore possible that the changes in expression of some of these other genes contribute to the inability of the mutant cells to grow under the LAHG condition and/or to the increased sensitivity of the cells to the dark. However, in Nostoc sp., mutants deficient in the OPP pathway have previously been shown to be unable to grow under the heterotrophic condition (33), and to manifest a decreased viability after incubation in the dark in Synechococcus sp. PCC 7942 (34). It is thus likely that the defect in the OPP pathway in G50 may be partly responsible for these growth phenotypes of the sigE mutant. Indeed, the OPP pathway was shown to be a major route of glucose catabolism under the heterotrophic condition in Synechocystis sp. PCC 6803 (35), and the major role of the OPP pathway is to provide a reductant (NADPH) under this growth condition.

Although SigE is a transcriptional regulator implicated in the activation of sugar catabolic pathways in Synechocystis, transcription of these sugar catabolic genes was still apparent and, in some cases, was still increased by a dark shift even in the absence of SigE, suggesting that other transcriptional regulators also participate in the control of their expression. Hik8 that was recently found to control common regulatory targets with SigE (32) is a probable candidate for such regulation (Fig. 8), and this kind of complexity may increase the metabolic versatility of cyanobacteria.

Some genes for glycolytic and glycogen catabolic enzymes are present in two copies in the
genome of *Synechocystis* sp. PCC 6803. However, with the exception of the two glyceraldehyde-3-phosphate dehydrogenases, the functional differences between the encoded isozymes have not been clarified. Whereas Gap1 catalyzes the glycolytic reaction, Gap2 catalyzes the reverse reaction in gluconeogenesis (30). We discovered that SigE activates the transcription of gap1 but not that of gap2 (Table I), suggesting that SigE contributes only to catabolism, not to anabolism, of glucose. There are also two phosphofructokinase genes (*sll0745, sll1196*) and two pyruvate kinase genes (*sll0587, sll1275*) in *Synechocystis* sp. PCC 6803, and the transcription of only one of each of these pairs of genes (*sll1196, sll0587*) was decreased by the *sigE* mutation (Table I, Fig. 2A, and data not shown). These results thus suggest that members of each of these pairs of isozymes are deployed preferentially under specific physiological conditions. With the use of in silico analysis, Mrazek *et al.* predicted that each of the two phosphofructokinase genes and the two pyruvate kinase genes of *Synechocystis* sp. PCC 6803 would be expressed at different levels on the basis of their codon usage (36), which may also imply the functional difference.

Global analysis of the circadian pattern of gene expression in *Synechocystis* sp. PCC 6803 (37) recently revealed that transcription of *sigE* manifests robust circadian oscillation, peaking around the end of the day (circadian time 9 to 10 h). The expression of sugar catabolic genes—including *pfkA* (*sll1196*), *gap1, zwf, opcA, gnd, tal*, and *glgX* (*slr0237*)—was also found to peak between the end of the day and the start of the night (circadian time 11 to 14 h). These findings are thus consistent with our results showing that expression of these sugar catabolic genes is dependent on SigE.

In this study, we additionally analyzed the regulatory position of Hik8 that was recently shown to be involved in the activation of common genes with SigE (32). Hik8 is an ortholog of SasA of *Synechococcus* sp. PCC7942 that is essential to sustain the robust circadian oscillation (32, 38). Considering the circadian oscillation of SigE and corresponding target genes, we suspected some regulatory interactions between SigE and Hik8. It was speculated that SigE regulates Hik8 or vice versa. However, this was not the case (Fig. 6B), and the relationship was summarized in Fig. 8. Further analysis should be required to clarify the regulatory circuits.

SigE was first recognized as a nitrogen-regulated sigma factor, and its expression was suggested to be under the control of the global nitrogen regulator NtcA (20). However, the present results suggest that SigE is unlikely to contribute directly to the regulation of nitrogen metabolism. A requirement for the OPP pathway during nitrogen deficiency has been previously
suggested in nitrogen-fixing cyanobacteria (39, 40), and the activities of G6PD and 6PGD are actually increased by nitrogen depletion in \textit{Synechocystis} sp. PCC 6803 (unpublished results). SigE might thus play a role in communication between carbon and nitrogen metabolism.

REFERENCES


FOOTNOTES

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1The abbreviations used are: GT, glucose tolerant; LAHG, light-activated heterotrophic growth; G6PD, glucose-6-phosphate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase; OPP, oxidative pentose phosphate.

FIGURE LEGENDS

Fig. 1. Disruption of sigE in Synechocystis sp. PCC 6803 and its effect on the transcriptome. A, The sigE gene was interrupted by insertion of a kanamycin resistance (KmR) cassette (~1.2 kbp) at a unique BglII site. The orientation of the kanamycin resistance gene was opposite to that of sigE. B and C, Disruption of sigE in the insertion mutant (G50) was confirmed by PCR with specific primers indicated in A and by immunoblot analysis with antiserum to SigE, respectively. The wild-type Arrows in B indicate the specific PCR products. (GT) strain was analyzed for comparison. D, Comparison of gene expression patterns of the sigE mutant and the wild-type strain by microarray analysis. Each point represents the G50/GT expression ratio and the signal intensity in G50 for an ORF fragment on the array. Experiments were performed for three times with biologically independent RNAs.

Fig. 2. Down-regulation of the expression of glycolytic and OPP pathway enzymes in G50 cells. A and B, Northern blot analysis of transcripts derived from genes that contribute to glycolysis [pfkA (sll1196), gap1,
pyk1] or to the OPP pathway (zwf, opcA, gnd, tal), respectively. GT or G50 cells were grown under the normal growth condition, and RNA was isolated either before or 1 or 4 h after a shift from light (L) to dark. Total RNA (10 μg) was then subjected to Northern analysis with probes specific for the indicated genes. The positions of molecular size markers (in kilobases) are indicated. The lower panels show rRNA stained with methylene blue as a loading control. C and D, Enzyme activities of G6PD and 6PGD, respectively. Cells treated as described above were assayed for the activities of G6PD and 6PGD. Data are expressed relative to the value for GT cells under the normal growth condition and are means ± SD of values from three independent experiments.

Fig. 3. Reduced rate of glucose uptake in G50 cells. GT or G50 cells in mid–logarithmic phase were suspended in BG-11 supplemented with 10 mM NH₄Cl and 2 mM glucose, and the decrease in the glucose concentration of the medium was determined at the indicated times thereafter. Data are means ± SD of values from three independent experiments.

Fig. 4. Down-regulation of the expression of glycogen catabolic enzymes in G50 cells. A, Northern blot analysis of transcripts derived from glycogen catabolic genes. GT or G50 cells were grown under the normal growth condition, and RNA was isolated either before or 1 or 4 h after a dark shift. Total RNA (10 μg) was subjected to Northern analysis with probes specific for the indicated genes. The positions of molecular size standards (in kilobases) are indicated. B, Analysis of intracellular glycogen abundance. The amount of glycogen in cells under the normal growth condition or 1, 4, or 6 h after a dark shift was determined. Data are expressed relative to the value for GT cells under the normal growth condition and are means ± SD of values from three independent experiments.

Fig. 5. Phenotypic effects of the sigE mutation. A, Cultures of GT or G50 cells grown to mid–exponential phase were spotted onto BG-11 plates containing both 10 mM NH₄Cl as a nitrogen source and 5 mM glucose. Each spot consisted of 1 μl of culture diluted to an OD₇₅₀ of 0.1, 0.05, or 0.01, as indicated. The plates were incubated in the dark with the exception of exposure to light for 15 min each day. Plates were photographed at the indicated times after inoculation. B, Cells were spotted onto plates as in A, incubated in continuous darkness for 4 days (no light stimulation), and then transferred to the light. Plates were photographed at the indicated times after the shift from dark to light.
Fig. 6. A, Northern blot analysis of transcripts derived from cph1-rcp1 genes. GT or G50 cells were grown under the normal growth condition, and RNA was isolated either before or 1 or 4 h after a dark shift. Total RNA (10 μg) was subjected to Northern analysis with probes for cph1. The positions of molecular size standards (in kilobases) are indicated. B, The amount of SigE proteins in GT and hik8 mutant. GT and hik8 mutant cells were grown at normal growth condition and proteins were obtained by disrupting the cells by sonication. Total protein (5 μg) was subjected to immunoblotting with antiserum to SigE.

Fig. 7. Glycolysis, gluconeogenesis, the OPP pathway, and glycogen metabolism in Synechocystis. The depicted scheme was predicted from the data in reference (35) and the KEGG database (http://www.genome.jp/kegg/pathway.html). Genes whose transcripts were reduced in abundance by the sigE mutation are shown in bold. The asterisk indicates that the effect of the sigE mutation on the expression of slr1367 was apparent only after a dark shift.

Fig. 8. Schematic model for the SigE function. SigE and Hik8 positively regulate gene expression for sugar catabolism (including glycolysis, the OPP pathway, and glycogen catabolism) and cph1-rcp1 probably under control of the diurnal rhythm. In addition Hik8 but not SigE positively regulates sugar anabolic genes. Activation by these components could be cooperative or independent.
Table I. Genes whose products contribute to sugar catabolism and whose expression was affected by the $\text{sigE}$ mutation.

<table>
<thead>
<tr>
<th>Gene (G50/GT)</th>
<th>ORF</th>
<th>Gene product</th>
<th>Expression ratio</th>
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<tr>
<td>$\text{pfkA}$</td>
<td>$\text{sll1196}$</td>
<td>Phosphofructokinase</td>
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<td>$\text{gap1}$</td>
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<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
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<td>$\text{sll0587}$</td>
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<td>Glucose-6-phosphate dehydrogenase</td>
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<td>$\text{opcA}$</td>
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<td>Allosteric activator of G6PD</td>
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<td>$\text{gnd}$</td>
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<td>6-Phosphogluconate dehydrogenase</td>
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Data represent the ratio of the transcript level in G50 to that in wild-type cells. The array included at least two spots for each ORF. Values shown are means ± SD of data from four to six spots, with the exception of that indicated by the asterisk, which is the average of only two spots (four spots were under the threshold value for detection).
Figure 1

A

Km<sup>r</sup>

Primer 1

Bgl II

Primer 2

sigE (sll1689)

B

GT G50

C

SigE

GT G50

D

Expression ratio (G50/GT)

Signal intensity
Figure 2

A and B: Western blot analysis showing expression levels of various genes under different conditions. The blot contains gels for different samples labeled as GT and G50, and time points of L, 1 h, and 4 h.

C and D: Bar graphs depicting relative GPD activity under different conditions and exposure times.
Figure 3
Figure 4

A

B
Figure 5

A

B

Downloaded from http://www.jbc.org/ by guest on November 19, 2017
Figure 6

A

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- cph1/rcp1
- rRNA

B

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Figure 7
Figure 8

Circadian control (Kai complex)

Gene expression peaking at the end of the day

SigE

Sugar Catabolism Cph1Rcp1

Hik8 (SasA)

Sugar Anabolism

+
Positive regulation of sugar catabolic pathways in the cyanobacterium Synechocystis sp. PCC 6803 by the group 2 sigma factor SigE
Takashi Osanai, Yu Kanesaki, Takayuki Nakano, Hiroyuki Takahashi, Minoru Kanehisa, Iwane Suzuki, Norio Murata and Kan Tanaka

J. Biol. Chem. published online June 8, 2005

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