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NrrA, a Nitrogen-regulated Response Regulator Protein, Controls Glycogen Catabolism in the Nitrogen-fixing Cyanobacterium *Anabaena* sp. Strain PCC 7120*[S]

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Background: Carbohydrate catabolism provides the energy for nitrogen fixation in filamentous cyanobacteria.
Results: A nitrogen-regulated response regulator NrrA controlled glycogen catabolism in the filamentous cyanobacterium *Anabaena* sp. PCC 7120.
Conclusion: NrrA controls not only cellular differentiation but also carbohydrate metabolism.
Significance: A molecular network regulating glycogen metabolism in the nitrogen-fixing cyanobacteria is proposed.

***Anabaena* sp. strain PCC 7120 is a filamentous cyanobacterium in which certain vegetative cells differentiate into heterocysts that are specialized cells for nitrogen fixation. Heterocysts are unable to carry out photosynthesis and depend on vegetative cells for carbohydrate to generate ATP and reductants required for nitrogen fixation. Thus, carbohydrate metabolism is very important for nitrogen fixation in the filamentous cyanobacteria; however, its regulatory mechanism remains unknown. In the present study, a nitrogen-regulated response regulator NrrA, which is a transcriptional regulator involved in heterocyst differentiation, was shown to control glycogen catabolism. The transcript levels of genes involved in glycogen catabolism, such as *glgP1* and *xfp-gap1-pyk1-talB* operon, were decreased by the *nrrA* disruption. Moreover, glycogen accumulation and depression of nitrogenase activities were observed in this disruptant. NrrA bound specifically to the promoter region of *glgP1*, encoding a glycogen phosphorylase, and to the promoter region of *sigE*, encoding a group 2 σ factor of RNA polymerase. SigE activated expression of the *xfp* operon, encoding enzymes of glycolysis and the pentose phosphate pathway. It is concluded that NrrA controls not only heterocyst differentiation but also glycogen catabolism in *Anabaena* sp. strain PCC 7120.**

Cyanobacteria are a large group of eubacteria characterized by oxygen-evolving photosynthesis. Heterocysts are terminally differentiated cells of filamentous cyanobacteria specialized for nitrogen fixation. Upon limitation of combined nitrogen in the medium, particular vegetative cells differentiate into heterocysts with a regular spacing of 10–15 cells (1, 2). Differentiating cells undergo drastic metabolic and morphological change, namely O_2-evolving photosystem II is inactivated, a rate of respiration is increased, and a thick envelope is formed outside of the cell wall, which limits oxygen diffusion into cells. Because heterocysts are unable to fix CO_2 photosynthetically, vegetative cells supply carbohydrate to heterocysts, probably in the form of sucrose (3), and, in return, vegetative cells receive nitrogen fixation products from heterocysts (4). Thus, the heterocyst-forming cyanobacteria represent a unique case of a truly multicellular bacterium with two different cell types, which depend on each other and contribute to the operation of the filament as the organismic unit.

Cyanobacteria store carbon assimilated by photosynthesis in the form of glycogen. Glycogen is utilized as carbon and energy reserves to cope with transient starvation and stress conditions (5, 6). Moreover, glycogen is catabolized to provide reductants and ATP required for nitrogen fixation, particularly in the dark (7). Glycogen content in heterocysts is higher than that of vegetative cells (8). Interconnection between glycogen and sucrose metabolism has been observed in the nitrogen-fixing filaments (3). Inhibition of sucrose degradation in heterocysts alters the accumulation of glycogen and sucrose and impairs diazotrophic growth (9, 10). Although regulation of carbohydrate metabolism is very important for the nitrogen-fixing cyanobacteria, little is known about its molecular mechanisms.

In the filamentous cyanobacterium *Anabaena* sp. strain PCC 7120 (hereafter *Anabaena* PCC 7120), we found that the *nrrA* gene encoding a response regulator of the OmpR family is involved in the regulation of heterocyst differentiation (11). Expression of *nrrA* is up-regulated by nitrogen deprivation under the control of NtcA (12), which globally controls nitrogen metabolism in cyanobacteria (13). NrrA is required for the full induction of the *hetR* gene (11, 14), encoding a master regulator of heterocyst differentiation (15, 16). The *sigE* gene (alr4249; previously called *sigF*), encoding a group 2 σ factor of RNA polymerase (17), is up-regulated in heterocysts after nitrogen deprivation (18) and is required for normal expression of heterocyst-specific genes including *nifH*, *fdxH*, and *hglE2* (19). NtcA binds to the upstream region of *sigE*, but it plays a partial role in the regulation of *sigE* expression (19). Another

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Table S1.
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transcriptional regulator in the transcriptional regulation of *sigE* is suggested.

Several genes up-regulated by nitrogen deprivation in *Anabaena* PCC 7120 are involved in carbon metabolism, such as glycogen degradation, *glgP1* (all1272); sucrose metabolism, *invB* (alr0819) and *spsB* (all4376); the oxidative pentose phosphate pathway, *zwf* (all4019), *gnd* (alr5275), *talA* (all4020), and *talB* (all2563); and glycolysis, *gap1* (all2566) and *pyk1* (all2564) (11). Expression of the *spsB* gene is shown to be regulated by NtcA (20). However, transcriptional regulation of the other genes has not been revealed. In this study, we investigated the regulation of genes involved in carbon metabolism that were up-regulated by nitrogen deprivation. It was found that NrrA and SigE were transcriptional regulators of glycogen catabolic genes. NrrA directly regulates expression of *glgP1* and *sigE*. The *sigE* gene is required for the regulation of *gap1*, *talB*, and *pyk1*. It is concluded that NrrA controls not only heterocyst differentiation but also glycogen catabolism in collaboration with SigE.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Culture Conditions—*Anabaena* sp. strain PCC 7120 and its derivatives were grown in the BG-11 medium (containing $NaNO_3$ as a nitrogen source) as described previously (11). For nitrogen deprivation experiments, cells grown in the BG-11 medium until they reached an OD_{750} of 0.4–0.5 were washed with the nitrogen-free medium ($BG\text{-}11_0$) and then resuspended in the $BG\text{-}11_0$ medium. Neomycin or spectinomycin was added to the medium at a final concentration of 30 μg/ml or 10 μg/ml, respectively, when required.

Mutant Construction—The *sigE* gene was inactivated as follows. DNA fragments upstream and downstream of the *sigE* gene were amplified by PCR using the primer pairs of sigE-5F and sigE-5R, and sigE-3F and sigE-3R, respectively (supplemental Table S1). The upstream fragment was cloned between the SacI and BamHI sites of pBluescript II KS^+ (Agilent Technologies), and then the downstream fragment was cloned between the BamHI and XhoI sites. A neomycin resistance cassette excised by digestion with BamHI from the plasmid pRL161 (21) was inserted into the BamHI site between the upstream and downstream fragments. The SacI-XhoI fragment was excised from the resultant plasmid and cloned between the SacI and XhoI sites of pRL271 (22) to construct pRsigEK. pRsigEK was transferred by conjugation into *Anabaena* PCC 7120 or DR4312S (11) according to Elhai and Wolk (23) to construct the *sigE* mutant DRsigEK or the *nrrA*/*sigE* double mutant DR4312/sigEK, respectively.

Real-time Quantitative Reverse Transcription-PCR—Total RNA was extracted from whole filaments according to Pinto *et al.* (24) and was treated with DNase I (Takara Bio, Shiga, Japan). cDNAs were synthesized from 1 μg of total RNA with 1 μl of *Anabaena*-specific primer mix (11) and 1 pmol of a 16 S rRNA-specific reverse primer, RTrrn16SR2 (supplemental Table S1), using PrimeScript first strand cDNA synthesis kit (Takara Bio). qRT-PCR[2] was performed with Thermal Cycler Dice Real Time System (Takara Bio) in a 10-μl reaction mixture containing 5 μl of SYBR Premix Ex Taq (Takara Bio), 0.2 μM each gene-specific forward and reverse primers (supplemental Table S1), and cDNA. Relative ratios were normalized with the value for 16 S rRNA and are represented as means of triplicate experiments.

Mapping of Transcription Initiation Sites by Rapid Amplification of cDNA Ends (RACE)-PCR—The transcription initiation sites of *glgP1* were determined using the SMARTer RACE cDNA Amplification kit (Takara Bio). 5′-RACE-PCR was carried out with 1 μg of total RNA and gene-specific primers as recommended by the supplier (supplemental Table S1). A resulting PCR product was cloned into a pGEM-T Easy vector (Promega), and nine clones were sequenced.

Gel Mobility Shift Assay—The promoter region of *glgP1* (P1272), corresponding to the region from −293 to +12 with respect to the translation start site of the *glgP1* gene, was amplified by PCR using the primer pair P1272-F and P1272-R (supplemental Table S1) and was cloned into the HincII site of the pBluescript II SK^+ (Agilent Technologies). A digoxigenin-labeled T7promoter primer (supplemental Table S1) was used to prepare a digoxigenin-labeled probe in combination with P1272-R. DNA fragments, PhetR1 and PhetR2, corresponding to the region from −876 to −659 and from −440 to −182 with respect to the translation start site of the *hetR* gene, respectively, were prepared as described previously (14). Fragments P1272D and PhetR1D were generated by deleting the conserved sequence AA(A/T)GTCA using PrimeSTAR mutagenesis basal kit (Takara Bio) with primer pairs P1272D2-F and P1272D2-R, and PhetRD-F and PhetRD-R, respectively (supplemental Table S1). Probes PsigE, corresponding to the region from −258 to +34 with respect to the translation start site of the *sigE* gene, and P2806, corresponding to the region from −394 to −10 with respect to the translation start site of all2806, were prepared by PCR using the primer pair sigE-F and sigE-5R, and P2806-F and P2806-R (supplemental Table S1).

His-NrrA protein was overproduced and purified from *Escherichia coli* by Ni-affinity chromatography as described previously (14). Gel mobility shift assays were carried out using 1 fmol of the digoxigenin-labeled probes as described previously (14).

Glycogen Determination—The glycogen content of whole filaments was determined according to Forchhammer and Tandeau de Marsac (25). Filaments were suspended in 200 μl of 2.5% (v/v) sulfuric acid solution and boiled for 40 min. Glucose in the hydrolysate was quantified by a colorimetric assay using the *o*-toluidine solution (Sigma). Protein concentrations were determined with a protein assay kit (Bio-Rad) by using bovine serum albumin as the standard.

Acetylene Reduction Assays—The nitrogenase activity was determined by the acetylene reduction assays. Portions (1 ml) of the cell suspension (approximately 3–7 μg of Chl *a*/ml) were transferred to 7.8-ml vials, and acetylene was added to a final concentration of 12% (v/v) in air. After 0.5–1 h of incubation under illumination, the concentration of ethylene in the headspace was measured by gas chromatography (GC-8A; Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a Porapak T-packed column (80/100 mesh, 3 mm × 1 m). The rates of acetylene reduction to ethylene were normalized to OD_{750}.

[2] The abbreviations used are: qRT-PCR, quantitative reverse transcription PCR; RACE, rapid amplification of cDNA ends.

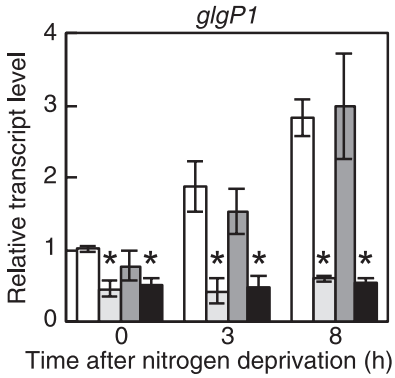

FIGURE 1. **Changes in the transcript level of *glgP1* after nitrogen deprivation.** The relative transcript levels of *glgP1* before (0 h) and 3 or 8 h after nitrogen deprivation were determined by qRT-PCR in the wild-type strain (*white bars*), the *nrrA* disruptant (*light gray bars*), the *sigE* disruptant (*dark gray bars*), and the *nrrA*/*sigE* double mutant (*black bars*). The transcript levels were determined in duplicate using three independently grown cultures. The transcript level at 0 h of the wild-type strain was taken as 1. Data that represent a significant difference ($p < 0.05$) by applying the *t* test between the wild-type strain and each mutant strain are marked with *asterisks*.

RESULTS

NrrA Positively Regulates Expression of the glgP1 Gene—In the previous study (11), effects of the *nrrA* gene disruption on whole gene expression of *Anabaena* PCC 7120 under nitrogen-deprived conditions were investigated using a DNA microarray. Expression of the *glgP1* gene, encoding a glycogen phosphorylase, was affected by the *nrrA* disruption (11). Differential expression of the *glgP1* gene was confirmed by qRT-PCR analysis. In the wild-type strain, the *glgP1* gene was up-regulated within 3 h after nitrogen deprivation, with increases in the transcript level of about 3-fold after 8 h (Fig. 1). In the *nrrA* disruptant, the *glgP1* transcript level was reduced to a half of the wild-type level in the presence of nitrate, and no induction of *glgP1* by nitrogen deprivation was observed (Fig. 1). It is indicated that NrrA positively regulates the expression of *glgP1*.

NrrA Binds to the Promoter Region of the glgP1 Gene—Gel mobility shift assays were carried out with purified His-NrrA and DNA probe for the upstream region of the *glgP1* gene (Fig. 2*A*). His-NrrA reduced the electrophoretic mobility of probe P1272 including the *glgP1* promoter, and the amount of the NrrA-P1272 complex increased in proportion to the concentration of His-NrrA (Fig. 2*A*, *lanes 1–4*). The band intensity of the NrrA-P1272 complex was decreased upon addition of a nonlabeled P1272 fragment (Fig. 2, *lanes 5* and *6*). The addition of a fragment PhetR2, which does not interact with His-NrrA (14), did not affect the amount of the NrrA-P1272 complex (Fig. 2*A*, *lanes 7* and *8*). These results show that His-NrrA binds to the region upstream of the *glgP1* gene in a sequence-specific manner.

The transcription initiation sites of *glgP1* were determined by RACE-PCR experiments. Two 5′ ends of the *glgP1* transcripts were detected corresponding to nucleotides −26 and −27 with respect to the translation start site (Fig. 3*A*). Putative −10 and −35 promoter regions were found upstream of the transcription initiation sites (Fig. 3*A*). Sequence analyses of upstream regions of the *glgP1* orthologs from *Anabaena* spp. and *Nostoc* spp. identified a conserved sequence (AAAGTCA) (Fig. 3*B*). This conserved sequence was also found within the promoter region of the *glgP* gene (slr1367) of *Synechocystis* sp. PCC 6803 (Fig. 3*B*), which is up-regulated by nitrogen deprivation (26). The NrrA binding region of the *hetR* promoter has been determined by DNase I footprinting assays (14), and a similar sequence (AATGTCA) was found within the region (Fig. 3*B*). Interaction between NrrA and the conserved sequence was elucidated by competition assays (Fig. 2). A fragment P1272D, in which the conserved sequence was deleted, did not compete

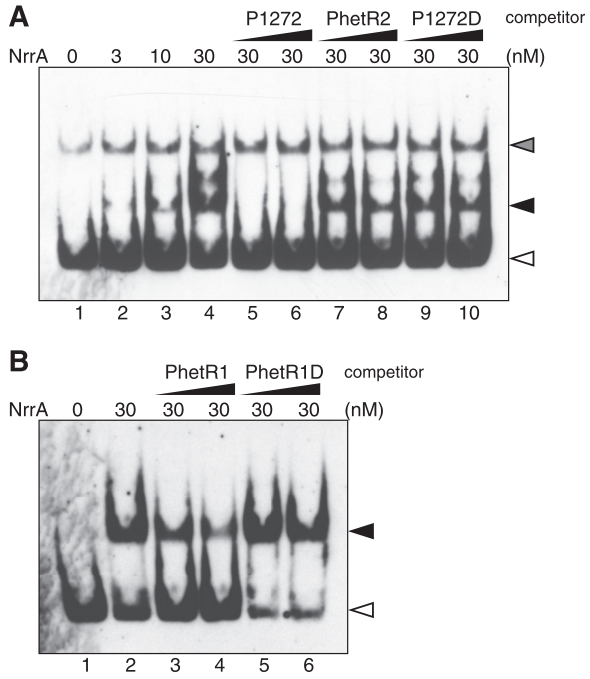

FIGURE 2. **Gel mobility shift assays with His-NrrA and the promoter regions of the *glgP1* and *hetR* genes.** *A*, digoxigenin-labeled probe P1272 (50 pM) including the *glgP1* promoter region was mixed with His-NrrA in the amounts indicated above each *lane*, and then the mixtures were subjected to electrophoresis. Nonlabeled fragments of P1272 (*lanes 5* and *6*), PhetR2 (*lanes 7* and *8*), and P1272D (*lanes 9* and *10*) were added at a final concentration of 0.5 nM (*lanes 5*, *7*, and *9*) or 1.5 nM (*lanes 6*, *8*, and *10*). *B*, binding of His-NrrA to probe PhetR1 (50 pM) was examined. His-NrrA was added in the amount indicated above each *lane*. Nonlabeled fragments of PhetR1 (*lanes 3* and *4*) and PhetR1D (*lanes 5* and *6*) were added at a final concentration of 5 nM (*lanes 3* and *5*) and 15 nM (*lanes 4* and *6*). *Filled arrows*, complexes of His-NrrA and P1272 or PhetR1; *open arrows*, probe alone; *gray arrow*, a nonspecific fragment amplified by PCR.

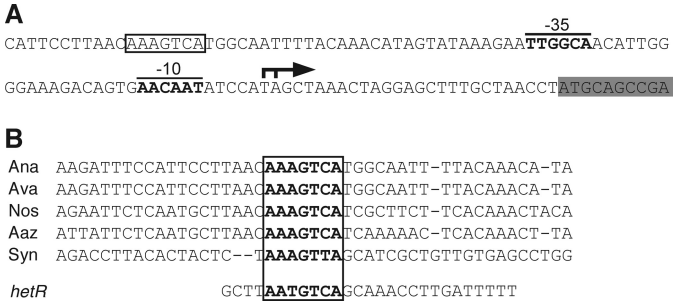

FIGURE 3. **Nucleotide sequence of the *glgP1* promoter region.** *A*, The coding region of the *glgP1* gene is *shaded* in *gray*. A *bent arrow* and *boldface letters* indicate the identified transcription initiation sites and the −10 and −35 promoter regions, respectively. A NrrA-binding site is enclosed in a *box*. *B*, alignment of the regions upstream of *glgP1* orthologs from *Anabaena* PCC 7120 (*Ana*), *Anabaena variabilis* ATCC 29413 (*Ava*), *Nostoc punctiforme* PCC 73102 (*Nos*), *Anabaena azollae* 0708 (*Aaz*), and *Synechocystis* PCC 6803 (*Syn*). The NrrA-binding region within the *hetR* promoter (14) is shown under the alignment. The conserved sequences are shown by *boldface letters*.

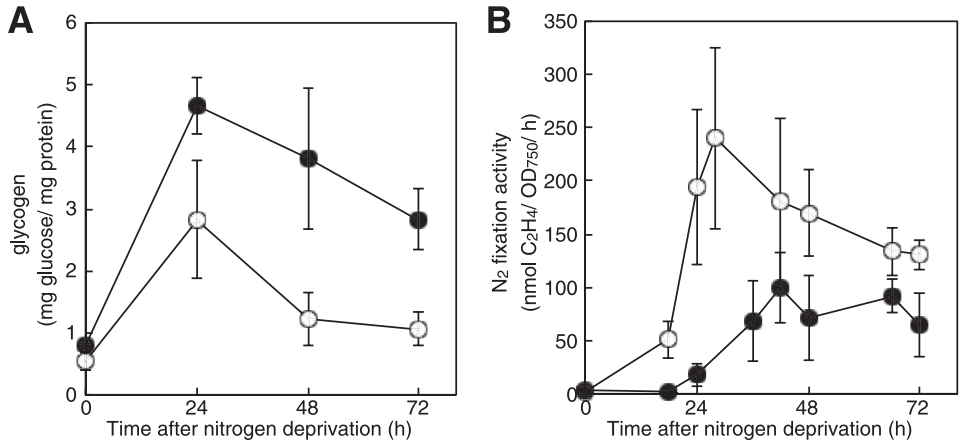

FIGURE 4. **Glycogen content (*A*) and nitrogenase activities (*B*) of the wild-type strain (*open circles*) and the *nrrA* disruptant (*closed circles*) during diazotrophic growth.** Filaments grown with nitrate were transferred to the nitrogen-free medium at time 0. Amounts of glycogen and nitrogenase activities were determined at the indicated times. Means ± S.D. (*error bars*) of three independent experiments are shown.

with P1272 (Fig. 2*A*, *lanes 9* and *10*), indicating that the conserved sequence is indispensable for the NrrA-P1272 interaction. NrrA bound to the *hetR* promoter region, including the conserved sequence (Fig. 2*B*, *lanes 1–4*), as reported previously (14). Deletion of the conserved sequence from PhetR1 (PhetR1D) abolished the interaction between NrrA and PhetR1 (Fig. 2*B*, *lanes 5* and *6*).

NrrA Activates Glycogen Catabolism—Fig. 4*A* shows glycogen contents in the wild-type strain and the *nrrA* disruptant after nitrogen deprivation. The glycogen content in the wild-type strain increased to about 5-fold during the first 24 h and then decreased to a level twice as much as that in the nitrate-grown filaments. In the *nrrA* disruptant, the glycogen content drastically increased by nitrogen deprivation and then decreased to a level approximately 3-fold higher than that in the wild type 72 h after nitrogen deprivation. It is suggested that NrrA activates glycogen degradation.

To see the correlation between glycogen catabolism and nitrogen fixation, nitrogenase activities were determined (Fig. 4*B*). Nitrogenase activity was highest 28 h after nitrogen deprivation in the wild-type strain, whereas that in the *nrrA* disruptant reached a maximum level 42 h after nitrogen deprivation, which was consistent with the delay in heterocyst differentiation of the *nrrA* disruptant (11). In addition, nitrogenase activities in the *nrrA* disruptant were lower than that in the wild type even after maturation of heterocysts (Fig. 4*B*). These results support the idea that NrrA positively regulates nitrogenase activity by stimulating glycogen catabolism.

SigE Is a Positive Regulator of the xfp Operon—The *rre37* gene, an ortholog of *nrrA* in *Synechocystis* sp. PCC 6803, is involved in regulation of glycolytic genes, *gap1* and *pfkA*, under nitrogen-depleted conditions (27). In *Anabaena* PCC 7120, glycolytic genes, *gap1* (all2566) encoding a glyceraldehyde-3-phosphate dehydrogenase and *pyk1* (all2564) encoding a pyruvate kinase, were up-regulated by nitrogen deprivation (11). These genes were transcribed with the *xfp* gene (all2567) encoding a phosphoketolase and the *talB* gene (all2563) encoding a transaldolase as an operon, and the putative promoters were found upstream of *xfp* and *talB* (data not shown). Disruption of *nrrA* prevented the induction of these genes by nitrogen deprivation (Fig. 5). However, gel mobility shift assays have failed to show any interaction between NrrA and fragments upstream of *xfp* and *talB* (data not shown). Thus, effects of the *nrrA* disruption on expression of the *xfp* operon may be indirect and mediated by other proteins whose activities or expression are affected by *nrrA*.

Using DNA microarrays, we found that the *xfp* operon was down-regulated by disruption of the *sigE* gene.[3] The effect of the *sigE* disruption on expression of genes involved in glycogen catabolism was determined by qRT-PCR. Expression of *glgP1* was not affected by disruption of *sigE* (Fig. 1), whereas induction of the *xfp* operon by nitrogen deprivation was abolished in the *sigE* disruptant (Fig. 5). Thus, SigE is a transcriptional activator of the *xfp* operon. The genetic relationship between NrrA and SigE was investigated with an *nrrA/sigE* double mutant. Disruption of the *nrrA* gene again reduced the *glgP1* transcript level and abolished the induction of *glgP1* by nitrogen deprivation in the *sigE* mutant background (Fig. 1). In contrast, expression patterns of the *xfp* operon in the double mutant were comparable with the single mutants (Fig. 5). Because NrrA does not bind to the promoter regions of the *xfp* operon, NrrA is likely to control the expression of the *xfp* operon via SigE.

NrrA Directly Regulates the sigE Expression—The *sigE* transcript is shown to increase 12 h after nitrogen deprivation in *Anabaena* PCC 7120 (19). Changes in the transcript level of *sigE* were examined at earlier stages of nitrogen deprivation. Expression of *sigE* was increased 8 h after nitrogen deprivation (Fig. 6). The transcript level of *sigE* at 8 h was decreased by disruption of *nrrA* (Fig. 6). Interaction of NrrA with the region upstream of the *sigE* gene (PsigE), in which the promoter region necessary for the *sigE* expression was included (19), was determined by gel mobility shift assays (Fig. 7). In the presence of both PsigE and PhetR2, NrrA specifically bound to PsigE (*lanes 1–3*). The specific binding of NrrA to PsigE was investigated further using P2806 as a competitor. NrrA did not interact with P2806 (*lanes 4–6*). The specific interaction between NrrA and PsigE was observed in the presence of P2806 (*lanes 7–9*). These results indicate that NrrA directly regulates the *sigE* expression.

[3] S. Ehira and M. Ohmori, unpublished data.

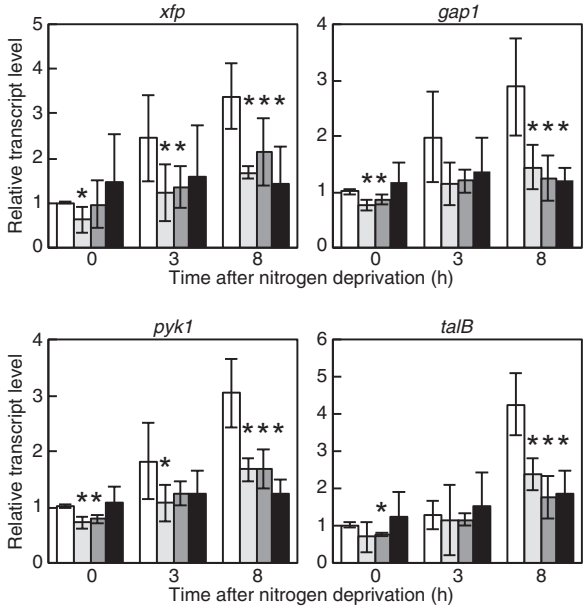

FIGURE 5. **Changes in the transcript levels of the *xfp* operon after nitrogen deprivation.** The relative transcript levels of *xfp*, *gap1*, *pyk1*, and *talB* were determined by qRT-PCR as described in Fig. 1.

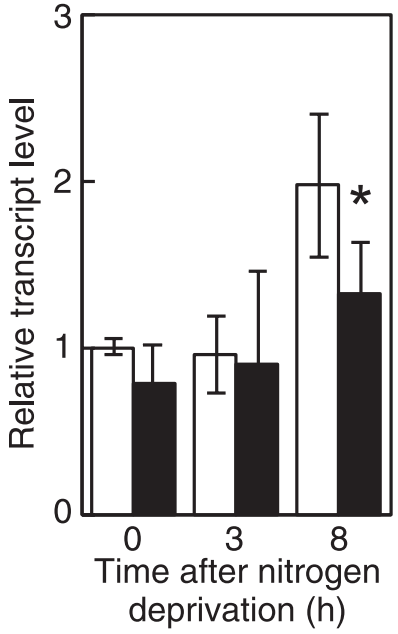

FIGURE 6. **Changes in the transcript levels of *sigE* after nitrogen deprivation.** The relative transcript levels of *sigE* were determined by qRT-PCR in the wild-type strain (*white bars*) and the *nrrA* disruptant (*black bars*) as described Fig. 1.

DISCUSSION

In the present study, we demonstrated that NrrA controlled glycogen catabolism in *Anabaena* PCC 7120 (Fig. 8). Disruption of the *nrrA* gene resulted in reduced expression of the genes involved in glycogen degradation and glycolysis (Figs. 1 and 5) and in accumulation of glycogen (Fig. 4). NrrA not only regulates the expression of the *glgP1* gene directly (Fig. 2), but also regulates the expression of the *xfp* operon indirectly via SigE (Figs. 1 and 5). NrrA plays a pivotal role in the regulation of carbon metabolism in collaboration with SigE.

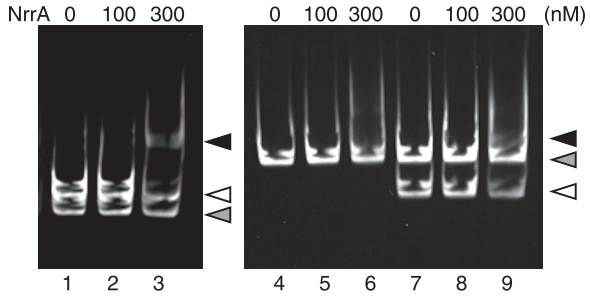

FIGURE 7. **Gel mobility shift assays with His-NrrA and the *sigE* promoter region.** His-NrrA was mixed with 10 nM PsigE (*lanes 1–3* and *7–9*) or 10 nM P2806 (*lanes 4–6*) in the amounts indicated above each *lane*. Probe PhetR2 (*lanes 1–3*) or P2806 (*lanes 7–9*) was added in a final concentration of 10 nM to demonstrate the specific interaction between NrrA and PsigE. *Black arrow*, complex with NrrA and PsigE; *open arrow*, PsigE alone; *gray arrow*, PhetR2 or P2806 alone.

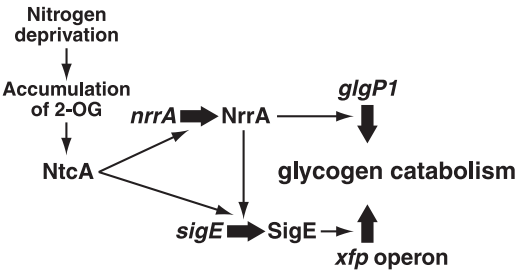

FIGURE 8. **Regulatory network model of genes involved in glycogen catabolism.** *Thick black arrows* represent up-regulation of gene expression. For details, see the text.

In the *nrrA* disruptant, the transcription of *glgP1* was suppressed, and its induction by nitrogen deprivation was abolished (Fig. 1). It was shown that NrrA bound to the *glgP1* promoter region, and the sequence AAAGTCA within the *glgP1* promoter was required for their interaction (Fig. 2*A*). A similar sequence (AATGTCA) found within the NrrA-binding site of the *hetR* promoter (14) was also indispensable for the binding of NrrA to the *hetR* promoter (Fig. 2*B*). Thus, the sequence AA(A/T)GTCA is a candidate for the NrrA recognition sequence.

It was noted that SigE was a transcriptional activator of the *xfp* operon (Fig. 5). Expression of *sigE* is shown to be up-regulated in differentiating cells at late stage of heterocyst differentiation using the *sigE-gfp* transcriptional reporter fusion (18), and SigE is involved in regulation of heterocyst-specific genes, such as *nifH*, *fdxH*, and *hglE2* (19). In the present study, we showed that the transcription of *sigE* was up-regulated within 8 h after nitrogen deprivation, and NrrA regulated the *sigE* expression (Fig. 6). Binding of NtcA to the region approximately 700 bp upstream of *sigE* has been reported; however, the NtcA-binding site is dispensable for the regulation of *sigE* expression (19). The 260-bp region upstream of the translation start site of *sigE* has been shown to be necessary for its transcriptional regulation (19). NrrA bound to the DNA fragment including the 260-bp upstream region of *sigE* (Fig. 7). A putative NrrA-binding site (AAAGACA) is found within the 260-bp upstream region of *sigE*. It is likely that the expression of the *sigE* gene is regulated by NrrA and partly by NtcA (Fig. 8).

Up-regulation of the *xfp* operon preceded that of the *sigE* gene (Figs. 5 and 6). The activity of SigE is regulated by the anti-σ factor ChlH in *Synechocystis* PCC 6803 (28). It is sup-

posed that SigE is post-translationally activated first, and then the transcription of the *sigE* gene is activated to further induce the *xfp* operon.

The *nrrA* disruptant exhibited a high abundance of glycogen during diazotrophic growth (Fig. 4*A*), indicating the reduced expression of the *glgP1* gene accompanied with decreased degradation of glycogen. Glycogen is utilized to provide reductants and ATP for nitrogen fixation (29). The depressed nitrogenase activity in the *nrrA* disruptant may be due to the decreased degradation of glycogen (Fig. 4*B*). Alternatively, down-regulation of the *sigE* gene in the *nrrA* disruptant might reduce expression of the *nifHDK* operon encoding the nitrogenase subunits (19). However, the transcript levels of *nifHDK* in heterocysts of the *nrrA* disruptant were higher than that in the wild-type strain (data not shown). Hence, it is unlikely that the down-regulation of *sigE* is responsible for the depressed nitrogenase activity.

In *Synechocystis* PCC 6803, *rre37*, an ortholog of *nrrA*, and *sigE* are involved in regulation of sugar catabolism (26, 27). Rre37 and SigE independently regulate sugar catabolism under the control of NtcA in contrast to *Anabaena* PCC 7120, and transcriptional regulation of *sigE* is exclusively dependent on NtcA (30). In *Anabaena* PCC 7120, expression of genes involved in sugar metabolism is subjected to developmental regulation. Because *ntcA*, *nrrA*, and *sigE* are up-regulated in differentiating cells (11, 18, 31), a regulatory cascade consisting of NtcA, NrrA, and SigE could regulate reorganization of the carbon metabolic system in the process of heterocyst development.

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