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The Global Nitrogen Regulator NtcA Regulates Transcription of the Signal Transducer P_{II} (GlnB) and Influences Its Phosphorylation Level in Response to Nitrogen and Carbon Supplies in the Cyanobacterium *Synechococcus* sp. Strain PCC 7942

HYUN-MI LEE,[1] MARÍA FÉLIX VÁZQUEZ-BERMÚDEZ,[2] AND NICOLE TANDEAU DE MARSAC[1]*

Département de Biochimie et Génétique Moléculaire, Unité de Physiologie Microbienne, Institut Pasteur, 75724 Paris Cedex 15, France,[1] and Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla, Centro de Investigaciones Científicas Isla de la Cartuja, E-41092, Seville, Spain[2]

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The P_{II} protein is encoded by a unique *glnB* gene in *Synechococcus* sp. strain PCC 7942. Its expression has been analyzed in the wild type and in NtcA-null mutant cells grown under different conditions of nitrogen and carbon supply. RNA-DNA hybridization experiments revealed the presence of one transcript species 680 nucleotides long, whatever the nutrient conditions tested. A second transcript species, 620 nucleotides long, absent in the NtcA null mutant, was observed in wild-type cells that were nitrogen starved for 2 h under both high and low CO_2 and in the presence of nitrate under a high CO_2 concentration. Primer extension analysis indicated that the two transcript species are generated from two tandem promoters, a σ^{70} *Escherichia coli*-type promoter and an NtcA-dependent promoter, located 120 and 53 nucleotides, respectively, from the *glnB* initiation codon. The NtcA-dependent promoter is up-regulated under the conditions mentioned above, while the σ^{70} *E. coli*-type promoter displays constitutive levels of transcripts in the NtcA null mutant and slightly different levels in the wild-type cells, depending on the nitrogen and carbon supplies. In general, a good correlation between the amounts of the two transcript species and that of the P_{II} protein was observed, as revealed by immunodetection with specific antibodies. The phosphorylation level of P_{II} in the wild type is inversely correlated with nitrogen availability and directly correlated with higher CO_2 concentration. This regulation is correspondingly less stringent in the NtcA null mutant cells. In contrast, the dephosphorylation of P_{II} is NtcA independent.

In cyanobacteria, nitrogen assimilation is a genuine photosynthetic process that requires ATP and reducing equivalents generated in the light. Both nitrate and nitrite are reduced to ammonium in the presence of photosynthetically reduced ferredoxin as the physiological electron donor. Ammonium is incorporated, through the glutamine synthetase-glutamate synthase pathway, into glutamate to yield glutamine by an ATP-dependent ligation reaction catalyzed by glutamine synthetase, and glutamate synthase transfers the amido group of glutamine to 2-oxoglutarate to regenerate glutamate in the presence of reduced ferredoxin. Nitrogen assimilation is tightly regulated in response to environmental cues. Nitrate and nitrite are taken up and reduced only in the absence of ammonium and under CO_2 fixation conditions, and the level of glutamine synthetase protein is severely reduced in the presence of ammonium (12).

In the unicellular *Synechococcus* sp. strain PCC 7942, which does not fix molecular nitrogen, the signal transduction protein P_{II} (the *glnB* gene product) is a key element in the coordination of nitrogen and carbon metabolism (15). This protein is a homotrimer of 36 kDa whose isomeric forms carry either zero, one, two, or three phosphorylated seryl residues (Ser49), depending on the carbon and nitrogen supply of the cells (13, 14), in contrast to the *Escherichia coli* P_{II}, which is uridylylated at a tyrosyl residue (Tyr51) (41). The highest degree of P_{II} phosphorylation is observed in cells incubated under a high CO_2 concentration in the presence of nitrate or under nitrogen-limiting conditions, while the protein is mainly dephosphorylated under low CO_2 in the presence of ammonium (14, 15). In vitro phosphorylation experiments revealed that both the P_{II} kinase and phosphatase activities depend on 2-oxoglutarate and ATP but not on glutamine or glutamate (16, 23). The modification of the *Synechococcus* sp. strain PCC 7942 P_{II} protein is facilitated by the binding of ATP and 2-oxoglutarate (13, 16). Since the intracellular concentration of ATP is high under physiological conditions, it has been proposed that P_{II} primarily functions as a sensor of 2-oxoglutarate (13). This metabolite not only serves as a source of carbon skeleton for nitrogen assimilation but would also be of particular importance as a small-molecule effector in the control of this metabolic process in *Synechococcus* sp. strain PCC 7942.

NtcA is a global nitrogen regulator that is widespread and highly conserved in cyanobacteria (12, 20). This DNA-binding protein, which belongs to the CRP family of bacterial transcriptional effectors, activates the expression of a number of genes in the absence of ammonium by recognizing the target consensus nucleotide sequence $GTAN_8TAC$ in their promoter regions (30). In *Synechococcus* sp. strain PCC 7942, NtcA positively regulates its own expression and activates the transcription of the *nir* operon (encoding nitrite reductase, the ABC-type permease complex, and nitrate reductase) (12, 30, 39), the *nirB ntcB* gene cluster (which encodes, respectively, a protein required for expression of nitrite reductase activity and a transcriptional effector of the bacterial LysR-type family that activates the *nir* operon in the presence of nitrite) (1, 26, 44), the *glnA* gene (encoding glutamine synthetase) (6, 7, 30), and the

* Corresponding author. Mailing address: Département de Biochimie et Génétique Moléculaire, Unité de Physiologie Microbienne, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France. Phone: 33 (0)1 45 68 8415. Fax: 33 (0)1 40 61 3042. E-mail: ntmarsac@pasteur.fr.

cynBDS operon (encoding two proteins likely to be involved in the active transport of cyanate and cyanase) (22). In the N_2-fixing filamentous heterocystous cyanobacterium *Anabaena* sp. strain PCC 7120, NtcA acts as an activator for the expression of genes for the assimilation of nitrogen sources alternative to ammonium and as an activator for heterocyst development (18, 19, 49). It has also been proposed that it could behave as a repressor for the *rbcL* gene (encoding the large ribulose-1,5-bisphosphate carboxylase–oxygenase subunit) and the *gor* gene (encoding glutathione reductase) (24, 40). In the facultative photoheterotroph *Synechocystis* sp. strain PCC 6803, *glnB* appears to be regulated by both electron transport and nitrogen availability, and the increased level of *glnB* under nitrogen starvation might be under the control of NtcA (21). In the marine unicellular N_2-fixing *Cyanothece* sp. strain BH68K, NtcA is involved in nitrogen assimilation rather than nitrogen fixation, and the expression of the *ntcA* gene may be under the control of the circadian rhythm (4).

Analysis of the phenotype of MP2, a P_{II} null mutant of the obligate photoautotroph *Synechococcus* sp. strain PCC 7942, revealed that nitrate utilization no longer depended on CO_2 fixation (15). Moreover, in contrast to the wild-type cells, in which ammonium exerts a rapid and reversible inhibition of nitrate and nitrite uptake, no inhibition was observed in this mutant. It was thus concluded that the unphosphorylated form of P_{II} is involved in the short-term inhibition by ammonium of nitrate and nitrite uptake (27). In this mutant, the synthesis of nitrate and nitrite reductases and glutamine synthetase was still subject to control by ammonium, suggesting that there is no direct interaction between P_{II} and the activity of NtcA in the regulation of nitrogen assimilation (15). However, other relationships between P_{II} and NtcA were not excluded.

Here we present results demonstrating that NtcA regulates P_{II} synthesis at the transcriptional level and is required for a full control of the phosphorylation state of P_{II} in response to nitrogen and carbon availability in *Synechococcus* sp. strain PCC 7942.

MATERIALS AND METHODS

Strains and culture conditions. Wild-type and mutant cells of *Synechococcus* sp. strain PCC 7942 were grown in liquid $BG11_0$ medium (43) containing 0.4 mM Na_2CO_3 and supplemented with 10 mM HEPES, pH 8.0. Either $NaNO_3$ (17.6 mM) or NH_4Cl (5 mM) was used as the N source. The $NtcA^-$ mutant, constructed by using plasmid pMAV58 according to the method of Vega-Palas et al. (48), was grown in ammonium-containing $BG11_0$ medium with chloramphenicol (7 μg ml^{-1}). Precultures grown in the presence of ammonium were incubated for 3 to 4 days (optical density at 750 nm [OD_{750}], approximately 0.6) at 30°C in air and illuminated with fluorescent lamps (OSRAM L18W/25 universal white) providing a photosynthetic photon flux density of 50 μmol m^{-2} s^{-1} measured with a LI-COR LI-185B quantum radiometer-photometer equipped with an LI-190SB quantum sensor. Experimental cultures were incubated at 35°C under the same photosynthetic photon flux density in a culture medium containing ammonium and supplemented with $NaHCO_3$ (10 mM) and with constant bubbling with air–3% (vol/vol) CO_2. Cells from mid-exponential-phase cultures (OD_{750}, approximately 0.4) were collected by centrifugation at 5,000 × *g* for 10 min at 25°C. The cell pellets were washed twice with $BG11_0$ and resuspended at the same cell density in $BG11_0$ medium containing either ammonium, nitrate, or no nitrogen source. After 2 h of incubation of the cells either in air without bubbling or bubbled with air–3% (vol/vol) CO_2, samples were collected for analysis.

Nucleic acid methods. Standard methods were used for *E. coli* plasmid DNA isolation. Restriction endonucleases (New England Biolabs or Pharmacia) and other DNA-modifying enzymes (New England Biolabs or Amersham) were used according to the manufacturers' recommendations.

Extraction of total RNA, gel electrophoreses, blottings, and hybridizations were performed as described previously (27). DNA probes were labelled with [α-^{32}P]dATP (110 TBq $mmol^{-1}$) by using a Megaprime random-labelling kit (Amersham). A probe internal to *glnB* (241 bp) was obtained by PCR amplification of the corresponding fragment from plasmid pPM119 (45) with specific primers to which an *Eco*RI site was added at the 5′ extremity of the coding strand (5′ CGGAATTCCGTTCAAACTGGAC 3′) and a *Bam*HI site was added at the 5′ extremity of the complementary strand (5′ CGGGATCCCGTCACCAATTT CGC 3′). The PCR product was cloned in the vector pTZ18R for further use in the DNA-binding assay. Plasmid DNA was extracted with the Nucleobond AX kit (Macherey-Nagel, Düren, Germany). A 0.6-kb *Kpn*I-*Xho*I fragment containing the *mpB* gene, encoding the RNA subunit of RNase P from *Synechococcus* sp. strain PCC 7942 (a gift from A. Vioque [3]), was used as a probe to quantify the amount of RNA loaded and transferred to the filters and to standardize the measurements.

Primer extension was performed as described by Liotenberg et al. (28) with 60 μg of total RNA and the 21-nucleotide-long primer 5′ GACTTCGTCCAGTT TGAACGG 3′. DNA sequencing was performed by using the sequencing dideoxynucleotide chain termination method (T7 sequencing kit; Pharmacia) with ^{35}S-dATP (37 TBq $mmol^{-1}$; Amersham) as the labelled nucleotide and the same primer mentioned above.

The relative transcript levels were quantified by scanning photoactivatable screens on a Molecular Dynamics 445SI PhosphoImager. All quantifications, data display, and analysis were performed with Molecular Dynamics Image Quant software.

Gel retardation assays. Preparation of cell extracts from *E. coli* DH5α containing the expression vector pTrc99A and from isopropyl-β-D-thiogalactopyranoside (IPTG)-induced cells of the NtcA-overproducing strain DH5α(pCSI26), gel retardation assays, and labelling of the probes were performed as described previously (30). The DNA probes were a 440-bp *Nhe*I-*Ava*I fragment of the *glnB* promoter region from plasmid pPM119; the PCR product corresponding to a fragment internal to the *glnB* gene, obtained as described above in "Nucleic acid methods"; and a 350-bp *Eco*RI-*Xho*I fragment of the *glnA* promoter region from pCSI38 (30). These probes were labelled with [α-^{32}P]dCTP (110 TBq/mmol; Amersham). The protein-DNA complex was visualized with an Instant Imager (Packard).

Quantification of the P_{II} protein and determination of its modification state. Cell extracts were prepared from cultures grown to an OD_{750} of 0.4, and aliquots corresponding to 10 μg of total protein were separated by polyacrylamide gel electrophoresis under either denaturing or nondenaturing conditions as described by Forchhammer and Tandeau de Marsac (14). The protein content of cell extracts was estimated by the method of Lowry modified as described previously (32), with bovine serum albumin as a standard. The P_{II} protein was revealed by immunoblotting with a P_{II}-specific antiserum in an enhanced chemoluminescence detection system (ECL kit; Amersham). Quantification was done with the National Institutes of Health Image program.

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Consensus NNNNGTA...N8...TAC.........N22-23........TANNNT.N4-9.  tsp
   glnB   TGCTGTAGCAGTAACTACAACTGTGGTCTAGTCAGCGGTGTTACCAAAGAGTC  tsp
   glnA   TTATGTATCAGCTGTTACAAAAGTGCCGTTTCGGGCTACC.TAGGATGAAAG  tsp
   nirA   AGTTGTAGTTTCTGTTACCAATTGCGAATCGAGAACTGCC.TAATCTGCCGA  tsp
   nirB   TTTAGTAGCAATTGCTACAAGCCTTGACTCTGAAGCCCGC.TTAGGTGGAGCCATT  tsp
   ntcA   AAAAGTAGCAGTTGCTACAAGCAGCAGCTAGGCTAGGCCG.TACGGTAACG  tsp
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FIG. 1. Alignment of NtcA recognition sequences of different genes from *Synechococcus* sp. strain PCC 7942 (30).

RESULTS

Control of *glnB* expression by NtcA. Examination of the nucleotide sequence of the *glnB* gene of *Synechococcus* sp. strain PCC 7942 revealed the presence of a consensus DNA-binding site for the transcriptional effector NtcA (Fig. 1) located upstream of the *glnB* initiation codon between nucleotides 88 and 102. Experiments were designed to analyze the conditions under which the expression of the *glnB* gene of *Synechococcus* sp. strain PCC 7942 was regulated and to determine the corresponding start sites for transcription. RNA-DNA hybridizations, using a probe internal to the *glnB* gene, and primer extension analysis were performed. Total RNA was extracted from ammonium-grown cultures of the wild type and the NtcA null mutant after transfer of the cells for 2 h to a medium containing either ammonium, nitrate, or no combined nitrogen and under either air or air enriched with 3% (vol/vol) CO_2. One or two transcript species of slightly different sizes (0.68 and 0.62 kb) were observed, depending on the nutrient conditions and the strain (Fig. 2). A particularly high level of transcripts was found in the wild-type cells incubated under nitrogen limitation and a high CO_2 concentration; their abundance was low under each of the other conditions tested.

Primer extension analysis revealed four extension products

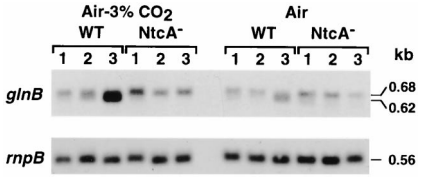

FIG. 2. RNA-DNA hybridization of total RNA from cells of wild-type *Synechococcus* sp. strain PCC 7942 (WT) and the NtcA-deficient mutant ($NtcA^-$) in response to the nature of the nitrogen source and the availability of CO_2. Ammonium-grown cells were transferred for 2 h to $BG\text{-}11_0$ medium containing ammonium (lanes 1), nitrate (lanes 2), or no nitrogen source (lanes 3), under either air or air–3% (vol/vol) CO_2. The same RNA blots were hybridized with a DNA probe internal to the *glnB* gene encoding the P_{II} protein and with a DNA probe of the *rnpB* gene encoding the RNA subunit of RNase P to provide an estimate of the RNA loading.

(Fig. 3). Two of them, which might correspond to the transcription start points designated *tsp1* and *tsp2*, varied in abundance with the conditions of nitrogen and carbon availability in both the wild-type and the NtcA null mutant cells. The two additional small extension products most likely result from earlier pauses caused by a GC-rich stretch starting immediately downstream from *tsp2*. The transcription start point *tsp1*, situated 120 nucleotides from the *glnB* initiation codon, was found with RNA from both the wild-type and NtcA null mutant cells, whatever the conditions tested. The transcript species corresponding to *tsp1* was preceded on the DNA sequence by a σ^{70}-like promoter (−10 TAAAAT; −35 TTGCCT). The second transcription start point, *tsp2*, localized 53 nucleotides from *glnB*, corresponded to extension products whose abundance increased under nitrogen limitation and high CO_2 concentration in the wild-type cells. This was accompanied by a decreased intensity of the extension products corresponding to *tsp1*. The transcription start point *tsp2* was preceded on the DNA sequence by a −10 box, TACCAA, and a perfect consensus NtcA-binding sequence, $GTAN_8TAC$ (30), between nucleotides −35 and −50. No extension products corresponding to *tsp2* were detectable in the RNA from the $NtcA^-$ mutant cells, whatever the conditions tested. These results indicated that NtcA controls the expression of the *glnB* gene at *tsp2*.

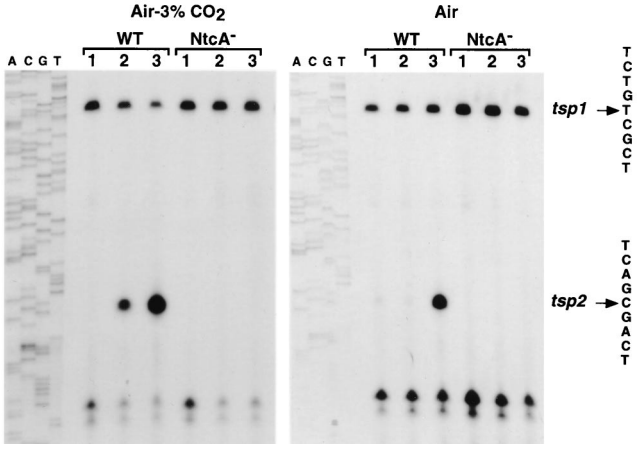

FIG. 3. Primer extension with the *glnB* gene. Total RNA (60 μg) from wild-type *Synechococcus* sp. strain PCC 7942 (WT) and NtcA-deficient mutant ($NtcA^-$) cells was annealed with an oligonucleotide specific to the *glnB* gene and extended with avian myeloblastosis virus reverse transcriptase as indicated in Materials and Methods. The cells were incubated as described in the legend to Fig. 2. Lanes A, C, G, and T contain a dideoxy sequencing ladder of the same DNA region used as a size control of the extension products. The sequences around the 5′ ends are listed on the right. *tsp1* and *tsp2* are putative transcription start points.

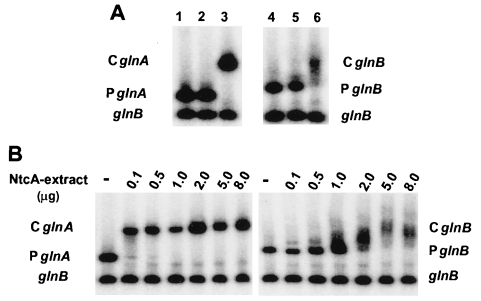

FIG. 4. Gel retardation of DNA fragments from the *glnB* and *glnA* promoter regions by cell extracts of an NtcA-overproducing *E. coli* strain. (A) *glnA* promoter region, P_{glnA} (lanes 1 to 3), and *glnB* promoter region, P_{glnB} (lanes 4 to 6), incubated with a DNA fragment internal to the *glnB* gene as the competitor DNA. Lanes 1 and 4, no NtcA-containing extract added; lanes 2 and 5, 5 μg of extract from cells of *E. coli* DH5α(pTrc99A) added; lanes 3 and 6, 5 μg of NtcA-containing extract from IPTG-induced cells of *E. coli* DH5α(pCSI26) added. C_{glnA} and C_{glnB} are complexes formed after incubation of the DNA fragments carrying P_{glnA} and P_{glnB}, respectively, with the NtcA-containing extracts. (B) Same conditions as in panel A, lanes 1 and 4, with various amounts (0 [−] to 8.0 μg) of extract from IPTG-induced cells of *E. coli* DH5α(pCSI26) added.

Binding of NtcA to the *glnB* promoter region. Mobility shift assays of electrophoretically resolved DNA fragments carrying the upstream region of *glnB* were performed with cell extracts of an NtcA-overproducing *E. coli* strain. This strain harbors plasmid pCSI26, which carries the *ntcA* gene downstream from the synthetic IPTG-inducible promoter *trc* and thus overexpresses NtcA after IPTG induction (30). As a positive control, experiments were performed in parallel with the promoter region of the *glnA* gene (P_{glnA}). The DNA fragment containing the promoter region of the *glnB* gene (P_{glnB}) was retarded by the NtcA-containing extract from *E. coli* carrying pCSI26 but not by the extract from *E. coli* cells harboring pTrc99A, the vector used to construct pCSI26 (Fig. 4A). The DNA fragment corresponding to an internal part of the *glnB* gene did not display any mobility shift and did not compete with either P_{glnB} or P_{glnA} (Fig. 4A). These results confirmed that the NtcA protein might bind specifically to DNA upstream from the *glnB* gene. At least a 50-fold-higher concentration of the cell extract was required, however, to obtain an NtcA-promoted shift of P_{glnB} than to obtain a shift of P_{glnA} (Fig. 4B), and the retarded band was fuzzier, whatever the concentration of the cell extracts tested (Fig. 4A and B).

Immunological detection of P_{II} in the wild type and in an NtcA null mutant. The amount of P_{II} protein was estimated by immunoblotting with specific P_{II} antibodies. In general, the total amount of the protein was found to be in good correlation with the levels of the transcripts in both the wild-type and the $NtcA^-$ cells incubated under the different nutrient conditions tested (Fig. 5). This indicated that, in addition to a basal level of expression of the *glnB* gene, which is NtcA independent, there is control by NtcA that occurs at a transcriptional level.

Modulation of phosphorylation levels of P_{II} isoforms by NtcA. In the wild-type cells, the relative abundance of the four

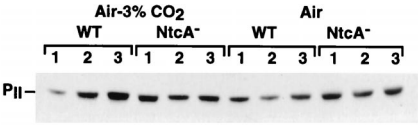

FIG. 5. Immunoblot analysis of the P_{II} protein in cells of wild-type *Synechococcus* sp. strain PCC 7942 (WT) and of the NtcA-deficient mutant ($NtcA^-$) in response to the nature of the nitrogen source and CO_2 availability. The cells were incubated as described in the legend to Fig. 2.

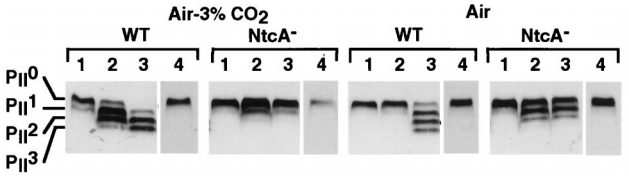

FIG. 6. In vivo phosphorylation of the P_{II} protein in cells of wild-type *Synechococcus* sp. strain PCC 7942 (WT) and the NtcA-deficient mutant (NtcA$^-$) in response to the nature of the nitrogen source and CO_2 availability. Lanes 1 to 3, the cells were incubated as described in the legend to Fig. 2. Lanes 4, cells starved for nitrogen as in lanes 3 were further incubated for 2 h following addition of ammonium to the culture medium. P_{II}^0, P_{II}^1, P_{II}^2, and P_{II}^3 correspond to the four isoforms of the trimeric P_{II} protein containing zero, one, two, or three phosphorylated monomers.

P_{II} isoforms, P_{II}^0, P_{II}^1, P_{II}^2, and P_{II}^3, which possess either zero, one, two or three phosphorylated monomers, respectively, varied with both the nitrogen supply and the concentration of CO_2 (Fig. 6). In the presence of ammonium, whatever the CO_2 level, or in the presence of nitrate under low CO_2, P_{II} was mainly unphosphorylated. Three to four isoforms were observed in the presence of nitrate under high CO_2 and in nitrogen-limiting conditions under both high and low CO_2. In the NtcA null mutant, whatever the availability of CO_2, P_{II} remained unphosphorylated in cells incubated in the presence of ammonium, as in the wild-type cells. In contrast, only two (under high CO_2) to three (under low CO_2) isoforms were observed when the cells were incubated with nitrate or under nitrogen limitation. As expected, the same results were obtained under both nitrogen conditions, since the NtcA-deficient mutant is impaired in the expression of a number of genes involved in nitrogen assimilation (48). Addition of ammonium to the nitrogen-starved cells led to a complete dephosphorylation of P_{II} in the NtcA null mutant, as in the wild type. Therefore, NtcA is not required for the dephosphorylation of P_{II} but only for the accumulation of phosphorylated forms in response to both the nitrogen and carbon sources.

DISCUSSION

In this study, a role for the global nitrogen regulator NtcA in the control of the transcription of the *glnB* gene, which encodes the signal transduction P_{II} protein, in response to changes in the availability of nitrogen and carbon in the environment has been demonstrated in the unicellular cyanobacterium *Synechococcus* sp. strain PCC 7942.

At least two distinct promoter-like structures have been identified that control the expression of the unique *glnB* gene in *Synechococcus* sp. strain PCC 7942, depending on the nitrogen supply and CO_2 concentration. The first, located upstream from *tsp1*, is of the common σ^{70} *E. coli* type. It yields constitutive levels of *glnB* mRNA in both wild-type and NtcA null mutant cells. The second, located upstream from *tsp2*, is activated by the global nitrogen regulator NtcA. The putative NtcA-binding site from the *Synechococcus glnB* promoter exactly matches the consensus sequence ($GTAN_8TAC$), as is the case with other genes from this organism, such as *glnA*, *ntcA*, and the *nir* operon (Fig. 1). However, there is obviously a different binding affinity for the *glnB* and the *glnA* promoter regions (Fig. 4). Previous in vitro studies have shown that NtcA from the filamentous heterocystous cyanobacterium *Anabaena* sp. strain PCC 7120 displays different affinities, depending on the target genes (40). A similar type of control could occur in *Synechococcus* sp. strain PCC 7942.

The abundance of the NtcA-dependent transcripts is inversely correlated with the availability of nitrogen and increases with higher CO_2 concentrations (Fig. 3). This positive effect of CO_2 could be exerted by CO_2 itself or by a metabolite derived from its fixation. A corresponding decrease in the level of the transcripts that initiate at *tsp1* is particularly evident in cells incubated with high CO_2. This would result if the binding of NtcA to the *tsp2* transcript species prevents the RNA polymerase from initiating transcription from the more distal *tsp1* promoter region, and it is not due to a specific recognition of the *tsp1* promoter region by NtcA, since no NtcA consensus sequence is present in that region.

In *Synechocystis* sp. strain PCC 6803, nitrogen availability and electron transport have been shown to control the expression of the *glnA* and *glnB* genes (21). In this cyanobacterium, one putative promoter of the *glnB* gene is constitutively expressed at a very low level in the absence of nitrogen and in the presence of ammonium and/or nitrate. The other promoter is functional only in cells starved for nitrogen (21). Therefore, the *glnB* control system in *Synechocystis* sp. strain PCC 6803, as well as those of other genes (e.g., *icd*, encoding $NADP^+$-isocitrate dehydrogenase, and *glnA* and *glnN*, encoding glutamine synthetases I and III) (38, 42), differs from the one that operates in *Synechococcus* sp. strain PCC 7942 for *glnA* (6, 30) and *glnB* (Fig. 3), in which the inducible promoter is active in cells grown both in the absence of nitrogen and in the presence of nitrate. Moreover, the physical organizations of the promoter regions in these two strains differ. In contrast to *Synechocystis* sp. strain PCC 6803, in *Synechococcus* sp. strain PCC 7942 the two promoter sequences do not overlap and the constitutively expressed promoter is upstream of the nitrogen-regulated one (Fig. 7).

Two types of promoters for the genes encoding P_{II} and P_{II}-like proteins that recognize either σ^{70} or a nitrogen-regulated RNA polymerase are commonly found in bacteria. Their physical organization and their levels of expression may vary, depending on the organism, but in most cases nitrogen control is exerted from a σ^{54}-dependent promoter through the NtrC protein (2, 5, 9–11, 17, 25, 29, 34, 36, 37, 46, 47, 50, 51).

In this study, we did not look at the kinetics of phosphorylation and dephosphorylation of P_{II} in the wild-type and mutant strains in response to nitrogen shifts. Therefore, we do not know whether the accumulation of the phosphorylated forms of P_{II} depends on a kinase or a phosphatase activity. Nevertheless, the dephosphorylation of P_{II} in the presence of ammonium appears to be NtcA independent, while the accumulation of the phosphorylated forms is at least in part NtcA dependent in *Synechococcus* sp. strain PCC 7942 (Fig. 6, compare lanes 3). The transcriptional activator NtcA might therefore modulate the phosphorylation state of P_{II} in response to

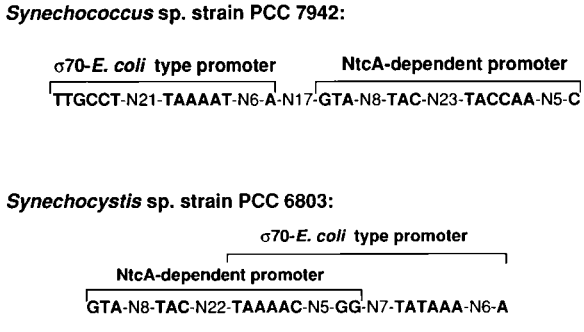

FIG. 7. Comparison of the physical organization of the promoter regions of the *glnB* genes from *Synechococcus* sp. strain PCC 7942 and *Synechocystis* sp. strain PCC 6803.

the intracellular N-C balance by directly or indirectly controlling the synthesis, activity, and/or stability of the serine kinase-phosphatase enzyme system, which posttranslationally modifies P_{II}.

At present, in *Synechococcus* sp. strain PCC 7942, we do not know whether NtcA is a single protein or a complex of one or more proteins or if it needs to be liganded to some effector(s) or posttranslationally modified to be active or to modulate its activity, depending on environmental conditions. The effect of NtcA on the activation of transcription of the *glnB* gene in *Synechococcus* sp. strain PCC 7942 and on the increased phosphorylation state of the protein could result from a higher level of expression of the corresponding *ntcA* gene and/or from a greater affinity of this regulator for its target sites under a high CO_2 concentration. Whether this is due to an increased electron transport activity or to the presence of a specific CO_2 fixation product(s) remains to be elucidated. The fact that the concentration of 2-oxoglutarate varies greatly in cyanobacterial cells, depending on the supply of carbon and nitrogen (8, 33, 35, 38), and that this is a very important metabolite involved in the regulation of nitrogen assimilation pathways in cyanobacteria and other prokaryotes (15, 35, 31) favors the hypothesis that this compound could play a key role in the regulatory system that coordinates the corresponding metabolic processes via P_{II} and NtcA.

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