Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 4565–4569, June 1991
Biochemistry

Photosynthetic electron transport controls nitrogen assimilation in cyanobacteria by means of posttranslational modification of the *glnB* gene product

(cloning/sequencing/uridylylation/photosystem/NH_4^+ concentration)

N. F. TSINOREMAS*†, A. M. CASTETS*, M. A. HARRISON†‡, J. F. ALLEN†§¶, AND N. TANDEAU DE MARSAC*

*Unité de Physiologie Microbienne, Département de Biochimie et Génétique Moléculaire, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France; and †Departments of Biochemistry and Molecular Biology and Pure and Applied Biology, The University of Leeds, Leeds LS2 9JT, England

Communicated by Charles J. Arntzen, January 22, 1991

ABSTRACT **A *glnB* gene is identified in the cyanobacterium *Synechococcus* sp. PCC 7942, and its gene product is found to be covalently modified as a result of imbalance in electron transfer in photosynthesis, where photosystem II is favored over photosystem I. The gene was cloned and sequenced and found to encode a polypeptide of 112 amino acid residues, whose sequence shows a high degree of similarity to the *Escherichia coli* regulatory protein, P_{II}. In *E. coli*, P_{II} is involved in signal transduction in transcriptional and posttranslational regulation of nitrogen assimilation. Increase in ammonium ion concentration is shown to decrease covalent modification of the *Synechococcus* P_{II} protein, as in enteric bacteria. We therefore propose that the photosynthetic electron transport chain may regulate the pathway of nitrogen assimilation in cyanobacteria by means of posttranslational, covalent modification of the *glnB* gene product. The existence of the *glnB* gene in different strains of cyanobacteria is demonstrated and its implications are discussed.**

Cyanobacteria comprise a large and diverse group of prokaryotes performing oxygenic photosynthesis. Several kinds of adaptation to changing light regime have been demonstrated in these organisms. Changes in spectral composition can cause alterations in dynamics of the thylakoid membrane (short-term adaptation) (1) and in expression of genes for components of the photosynthetic apparatus (long-term adaptation) (2–3). Such processes serve to optimize the efficiency of photosynthetic electron transport by changing the relative rates of light absorption by the photosystems (4, 5). Three modes of long-term light adaptation have also been distinguished in cyanobacteria: adaptation to intensity, inverse chromatic adaptation, and complementary chromatic adaptation (3). It has been suggested that covalent protein modification, particularly protein phosphorylation (6, 7), is involved in short-term and long-term adaptation (8). Growth of cells of the cyanobacterium *Synechococcus* sp. PCC 6301 under light preferentially absorbed by the phycobilisome and therefore by photosystem II has been shown to result in phosphorylation of a thylakoid membrane protein of 15 kDa and of soluble proteins at 13 kDa and 19 kDa (6–8). Phosphorylation is decreased under light preferentially absorbed by chlorophyll a and therefore by photosystem I. It has been proposed that the 15-kDa and 19-kDa phosphoproteins are involved in short-term regulation of excitation energy distribution (6) and the 13-kDa protein is involved in long-term regulation (8). This proposal is consistent with the observation (8) of *in vivo* labeling of the 13-kDa polypeptide under relatively long-term illumination specifically favoring photosystem II.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

The 13-kDa polypeptide implicated in long-term adaptation in cyanobacteria shows N-terminal sequence similarity with P_{II}, the *glnB* gene product from *Escherichia coli* (9, 10). P_{II} is reversibly uridylylated in *E. coli* by a uridylyltransferase, encoded by the *glnD* gene product. Uridylylation of P_{II} promotes activation of glutamine synthetase by initiating its deadenylylation (10). P_{II} also plays a role in the transcriptional control mediated by the Ntr system (11). Transcriptional activation of Ntr-system-regulated genes occurs under nitrogen-limiting conditions when the activator protein, NR_I (NtrC), becomes phosphorylated. Conversely, in nitrogen excess NR_I is inactivated by dephosphorylation. The phosphorylation–dephosphorylation of NR_I is controlled by another bifunctional protein, NR_{II} (NtrB). Modification of NR_I by NR_{II} requires an interaction between NR_{II} and P_{II} (12). Therefore P_{II} transmits information concerning the nitrogen status of the cell, sensed by the *glnD* gene product, to glutamine synthetase adenylyltransferase and NR_{II}. Glutamine synthetase from *Anabaena* sp. PCC 7120 is not adenylylated in *E. coli* (13), an observation consistent with the suggestion, here shown to be incorrect, that *glnB* is absent from cyanobacteria (14).

Here we describe the isolation, molecular cloning, and nucleotide sequence‖ of the *glnB* gene from *Synechococcus* sp. PCC 7942, a cyanobacterium that does not fix molecular nitrogen. We chose this strain for molecular cloning and *Synechococcus* sp. PCC 6301 to study the role of the gene product in the nitrogen status of the cell. The two strains are genetically very closely related (15) but *Synechococcus* sp. PCC 7942 has superior transformation efficiencies and a small plasmid-cured derivative (strain spc) is available (16). Molecular cloning in a highly transformable strain that is cured of the resident small plasmid pUH24 that served to construct cloning vectors (17) will be useful for future work concerning the functional roles of the *glnB* gene in cyanobacteria. In addition, we report the existence of the *glnB* gene in various cyanobacteria and examine its function in *Synechococcus* sp. PCC 6301.

MATERIALS AND METHODS

Materials. Restriction enzymes, calf intestinal alkaline phosphatase, T4 DNA ligase, and T4 polynucleotide kinase were purchased from either Boehringer Mannheim or Genofit (Geneva), and snake venom phosphodiesterase was from

Abbreviation: ORF, open reading frame.

‡Present address: Department of Plant Biology, 411 Genetics and Plant Biology Building, University of California, Berkeley, CA 94720.
§Present address: Department of Biology, University of Oslo, Box 1045, Blindern, N-0316 Oslo 3, Norway.
¶To whom reprint requests should be addressed.

‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. M62447).

Sigma. The Klenow fragment of DNA polymerase I, [α-^{32}P]dCTP (3000 Ci/mmol; 110 TBq/mmol), dATP[α-^{35}S] (1000 Ci/mmol; 37 TBq/mmol), [γ-^{32}P]ATP (3000 Ci/mmol; 110 TBq/mmol), random priming, and sequencing kit were from Amersham. Nucleotides and dideoxynucleotides were purchased from Pharmacia. All enzymes were used according to the manufacturer's instructions. All chemicals were reagent grade.

Culture Conditions and DNA Purification. Cyanobacterial strains from the Pasteur Culture Collection were grown photoautotrophically at 25°C in medium BG 11 as described (18). Chromosomal DNA was extracted as described (19).

Library Construction, Transformation, and Screening. A *Hin*dIII size-directed library from *Synechococcus* sp. PCC 7942 strain spc was constructed as follows. Total DNA was digested with *Hin*dIII and fractionated by electrophoresis on a 0.7% agarose gel. DNA fragments of appropriate size (see *Results*) were purified by electroelution and inserted into the *Hin*dIII site of pTZ18R (20). Transformation of *E. coli* was carried out by electroporation (21). Standard methods were used for screening transformants by *in situ* colony hybridization (20).

Synthesis of Oligonucleotides. A mixture of synthetic oligonucleotides was used to probe the *glnB* gene:

```
5'-CCGTTCAAACTGGACGAAGTCAAAATCGC-3'
      G  C  T  G  G
```

The sequence corresponded to a portion of the chemically derived N-terminal sequence of P_{II} (9). The mixture of 29-mers and the three 17-mers used as sequencing primers were synthesized as described (22) in the Institut Pasteur, Paris (the 29-mers in the Unité de Chimie Organique and the 17-mers in the Unité de Biochimie Cellulaire).

Hybridization with ^{32}P-Labeled Probes. The 29-mer oligonucleotide probe was labeled at the 5' end with [γ-^{32}P]ATP using T4 polynucleotide kinase. All other DNA probes were labeled by random priming according to the protocol provided by Amersham. Southern hybridization experiments were performed as described (19) except that the DNA hybridizations were performed at 52°C with the 29-mers and at 55°C with the *glnB* gene as probes.

DNA Sequencing Strategy and Analysis. Nucleotide sequencing analysis in pTZ18R was performed by the chain-termination method (23) on single-stranded DNA templates according to the protocol provided by Amersham. Initially the 29-mers were used to probe the *glnB* gene. The 17-mer primers were subsequently synthesized as required for completing the sequencing on both strands. Computer analysis of the DNA sequence data was performed using a program developed by the Unité d'Informatique Scientifique of the Institut Pasteur. Alignment and comparison of the amino acid sequence data were performed using the Sooty and Sweep program of the Leeds-Birkbeck ISIS data resource (24).

***In Vivo* Protein Labeling.** Cells of *Synechococcus* sp. PCC 6301 were incubated with [^{32}P]orthophosphate as in ref. 9. Cells were removed immediately to an orange "light 2" (fluorescent strip lights filtered through Cinemoid orange 5 filter giving 50% transmittance at 580 and 630 nm), absorbed preferentially by photosystem II, or to a red "light 1" (tungsten filament lamps filtered through Chromoid ruby 114 filter giving 50% transmittance at 640 nm), absorbed preferentially by photosystem I (8). The incident light intensity in each case was $\approx$30 μE $m^{-2}\cdot s^{-1}$ [E = einstein (1 mol of photons)]. The spectral distribution, giving equal rates of cell growth, was as described (8). Filters were from Strand Lighting (London). When present, ammonium sulfate was at 2 mM final concentration. After 30 min of incubation whole cell proteins were extracted and prepared for SDS/PAGE as in ref. 9. Treatment with snake venom phosphodiesterase was performed on whole cell protein extract as described (25). Radioactivity was quantified by densitometry of autoradiographs after SDS/PAGE as in ref. 7.

RESULTS

Isolation and Nucleotide Sequence Analysis of the *Synechococcus* sp. PCC 7942 *glnB* Gene. The recently described N-terminal sequence of the 13-kDa protein from *Synechococcus* sp. PCC 6301 showed similarity to that of P_{II}, the *glnB* gene product of *E. coli* (9). We therefore tried to isolate the *glnB* gene from cyanobacteria using heterologous hybridization. Initially, a probe consisting of a 1.4-kb *Eco*RI–*Pvu* II DNA fragment from *E. coli* (11) was used to probe total *Synechococcus* sp. PCC 6301 and *Synechococcus* sp. PCC 7942 genomic DNA. The hybridization signal was neither strong nor specific enough to allow us to isolate the gene (not shown). Subsequently we used a *Sac* I–*Bgl* II DNA fragment carrying the *glnB* gene from *Azospirillum brasilense* (26). This probe showed a stronger signal, but it was not sufficiently strong for cloning the gene. Therefore using the N-terminal sequence of the 13-kDa protein from *Synechococcus* sp. PCC 6301 (9), a mixture of 29-mer oligonucleotides was synthesized to probe the corresponding gene from *Synechococcus* sp. PCC 6301 and PCC 7942. The DNA from the two cyanobacterial species gave identical signals (results not shown) and for the reasons we have discussed previously we selected the transformable species *Synechococcus* sp. PCC 7942 to clone the *glnB* gene. On the basis of the results of the Southern blot hybridizations obtained with the three different probes, *Hin*dIII fragments of $\approx$4.5 kilobases (kb) were isolated from partial libraries constructed into pTZ18R and analyzed further. The physical map of the *Hin*dIII fragment containing the gene, the sequencing strategy employed, and the nucleotide sequence are shown in Fig. 1. An open reading frame (ORF) of 336 bp was deduced, encoding a polypeptide of 112 amino acids. A UUG codon was taken as the start codon of the ORF. A sequence 5'-GGAG-3' occurs 7 nucleotides upstream from this putative translational start codon, showing good homology to Shine–Dalgarno sequences (27) and most likely acting as a ribosome-binding site (28, 29).

Analysis of Amino Acid Sequence. The protein encoded by the above ORF has sequence similarity with the *glnB* gene products of *Rhodobacter capsulatus* (30), *Klebsiella pneumoniae* (14), *E. coli* (10), *Bradyrhizobium japonicum* (31), and *Rhizobium leguminosarum* (32). The first three proteins have 112 amino acid residues, that of *E. coli* has 103, and those of *B. japonicum* and *R. leguminosarum* have 111. The deduced molecular mass of the protein encoded by the *Synechococcus* sp. PCC 7942 ORF is 12,359 daltons, slightly higher than those of the others. The *Synechococcus* sp. PCC 7942 *glnB* gene product has 66%, 65%, 64%, 63%, and 62% sequence identity with the *glnB* gene products of *Rb. capsulatus*, *K. pneumoniae*, *E. coli*, *B. japonicum*, and *R. leguminosarum*, respectively, and 93% sequence identity with the 30-amino acid residue N-terminal fragment from *Synechococcus* sp. PCC 6301 (Fig. 2). The amino acid at position 25 in the protein from *Synechococcus* sp. PCC 7942 is isoleucine instead of the leucine determined for PCC 6301 (Fig. 2). In the GlnB from *E. coli* the amino acid that is uridylylated by GlnD is the tyrosine at position 51 (12). A tyrosine also occurs at position 51 in the ORF of *Synechococcus* sp. PCC 7942 (Fig. 2). This tyrosine and a preceding residue are conserved (Fig. 2). We propose that the 336-bp *Synechococcus* sp. PCC 7942 ORF encodes a polypeptide that functions in the same way as GlnB in enteric bacteria. From now on we refer to this ORF as *glnB*.

Identification of the *glnB* Gene in Different Cyanobacterial Species. *Synechococcus* sp. PCC 7942 is an obligate autotroph that cannot fix nitrogen (18). We have screened genomic DNA of various other representive species in order

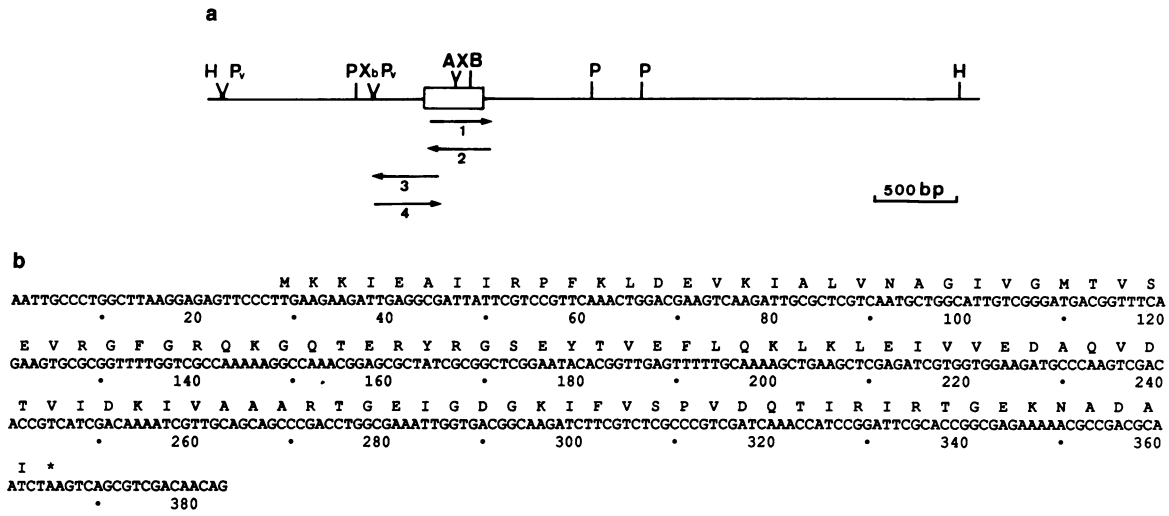

FIG. 1. Restriction map of the 4.5-kb *Hin*dIII–*Hin*dIII DNA fragment that contains the *glnB* gene from *Synechococcus* sp. PCC 7942 (*a*) and complete nucleotide sequence of the gene with the predicted amino acid sequence (*b*). H, *Hin*dIII; Pv, *Pvu* II; P, *Pst* I; Xb, *Xba* I; A, *Ava* I; X, *Xho* I; B, *Bgl* II. Horizontal arrows indicate the sequencing strategy: 1 is the mixture of 29-mers; 2, 3, and 4 are the different 17-mers. bp, Base pairs.

to investigate the occurrence of the *glnB* gene in cyanobacteria. A 0.7-kbp *Bgl* II–*Pvu* II fragment containing the *glnB* gene from *Synechococcus* sp. PCC 7942 was used as a probe. As shown in Fig. 3, hybridization bands were detected in all species under conditions that allowed ≈45% mismatching. We conclude that the *glnB* gene is likely to be present in all cyanobacteria.

Function of the *glnB* Gene Product in *Synechococcus* sp. PCC 6301. Fig. 4 shows comparative protein phosphorylation *in vivo* in *Synechococcus* sp. PCC 6301 cells incubated under light 2 or light 1 in the presence of [^{32}P]orthophosphate. Two polypeptides of apparent molecular masses 19 kDa and 13 kDa were found to develop high levels of phosphorylation. A third phosphoprotein has been reported at 15 kDa (6) and is not visualized in Fig. 4, being enriched in the membrane fraction and constituting a minor component of whole cell extract (9). Incubation under light 2 increased the labeling of the 13-kDa protein, as previously observed (8, 9). The 13-kDa protein has been shown to be closely related to GlnB (9). From the present study (Fig. 2) it is clear that this protein is a cyanobacterial *glnB* gene product with a predicted true molecular mass of 12.4 kDa. Fig. 4 also shows the effect of ammonium concentration on the labeling of the two polypeptides. Incubation for 30 min with ammonium sulfate at 2 mM final concentration decreased the labeling of the 13-kDa polypeptide, whereas that of the 19-kDa polypeptide was less affected. The effect of ammonium on labeling of the 13-kDa polypeptide is similar to its effect on uridylylation of *E. coli*

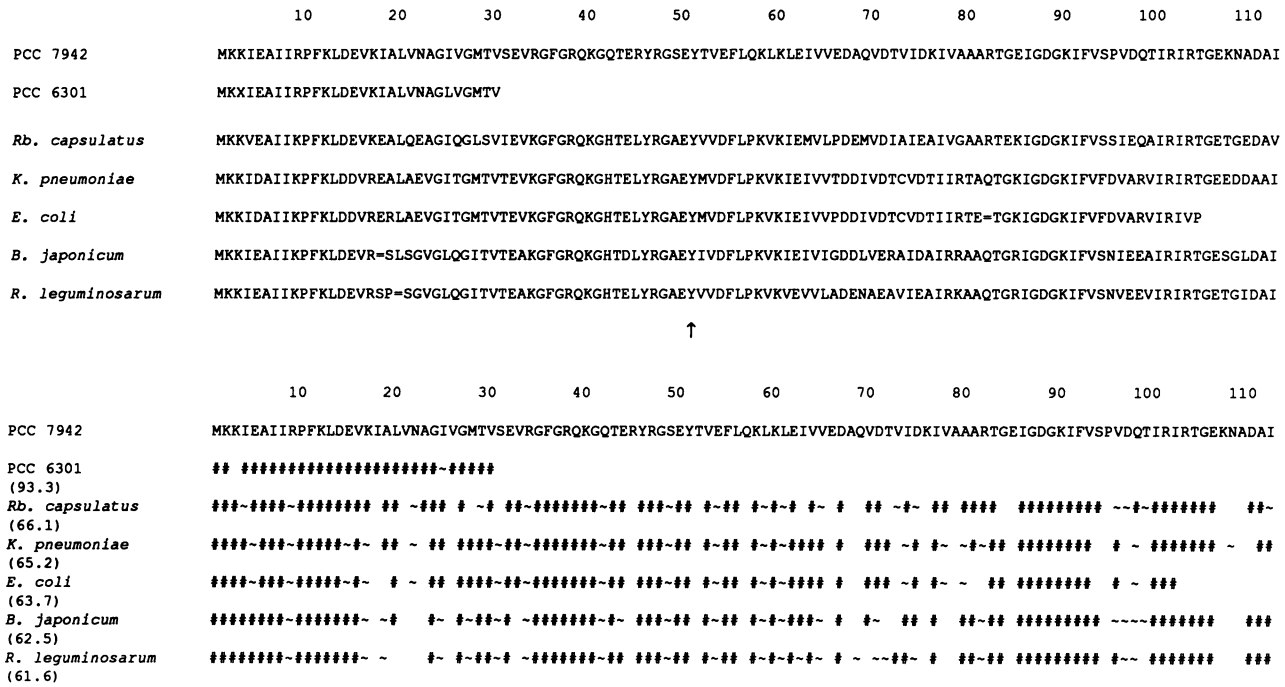

FIG. 2. Amino acid sequence alignments between the *glnB* gene products from *Synechococcus* sp. PCC 7942 (PCC 7942), *Rh. capsulatus* (30), *K. pneumoniae* (14), *E. coli* (10), *B. japonicum* (31), *R. leguminosarum* (32) and the N-terminal sequence of the 13-kDa protein (9) from *Synechococcus* sp. PCC 6301 (PCC 6301). #, Conserved amino acid residue; ~, conservative substitution; =, space inserted for the purpose of alignment; ↑, site of uridylylation of the *E. coli glnB* gene product, a tyrosyl residue in position 51.

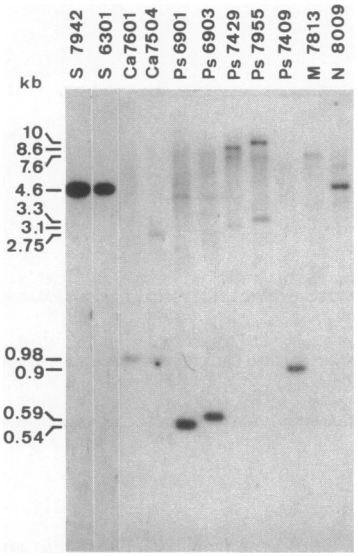

FIG. 3. Autoradiogram of a Southern blot of *Hin*dIII-digested total DNA from various cyanobacterial strains after hybridization with the ^{32}P-labeled DNA probe carrying the *Synechococcus* PCC 7942 *glnB* gene. Fragment sizes are given in kb. Hybridization was performed at 55°C. Genera are abbreviated as follows: S, *Synechococcus*; Ca, *Calothrix*; Ps, *Pseudanabena*; M, *Microcystis*; N, *Nostoc*.

P_{II} (10). Fig. 5 shows that the radiolabel of the 13-kDa polypeptide was rapidly removed by the action of phosphodiesterase, whereas that of the 19-kDa polypeptide was not. This indicates that the covalent modification of the 13-kDa polypeptide probably occurs by uridylylation of tyrosine-51, as in *E. coli*. It is therefore clear that P_{II} exists in cyanobacteria and may be modified there in the same way as in enteric bacteria, though in cyanobacteria photosynthetic electron transport also plays an important role (Fig. 4).

DISCUSSION

For cyanobacteria there is no evidence so far that adenylylation regulates glutamine synthetase activity (13, 33) nor was any radiolabeling at the molecular mass for glutamine synthetase subunits observed during this work. Instead of an

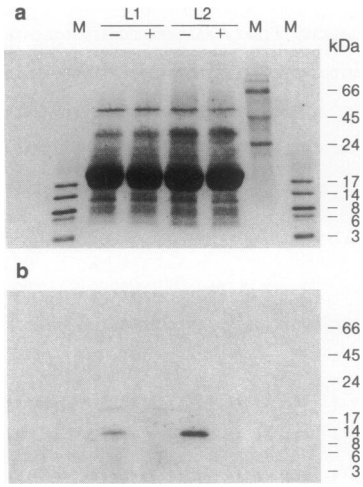

FIG. 4. SDS/PAGE analysis using Coomassie blue (*a*) and autoradiography (*b*) of proteins from whole cells of *Synechococcus* sp. PCC 6301, radiolabeled and prepared as described in the text, under light 1 (L1) or light 2 (L2) and in the presence (+) or absence (−) of ammonium sulfate at 2 mM final concentration. Ammonium sulfate at 2 mM had no effect on the pH of the growth medium. Positions of size markers are indicated (M) and molecular mass is expressed in kDa.

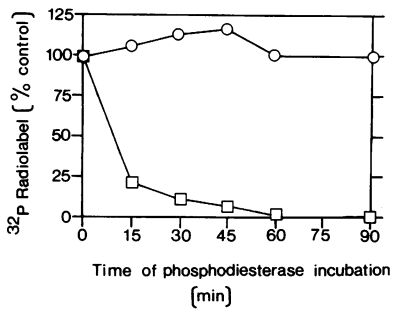

FIG. 5. Action of phosphodiesterase on the 19-kDa (○) and 13-kDa (P_{II}) (□) polypeptides from *Synechococcus* sp. PCC 6301. Radioactivity was quantified by densitometry of the autoradiograph obtained after SDS/PAGE of [^{32}P]orthophosphate-labeled whole cell proteins and is expressed as a percentage of ^{32}P activity remaining in a phosphodiesterase-free control.

adenylylation-mediated cascade system (10), it has been suggested that regulation of glutamine synthetase activity occurs in cyanobacteria by feedback inhibition by several amino acids (33, 34). Nucleotide sequence analysis of the *glnA* gene encoding glutamine synthetase in *Anabaena* sp. PCC 7120 (35) shows a tyrosine in position 402, which corresponds exactly to the adenylylation site in *E. coli*, as proposed by Tumer *et al.* (35). The inability of the cyanobacterial glutamine synthetase to become adenylylated in *E. coli* may therefore be due to structural differences between the two proteins and not to the absence of the modifiable amino acid as has been proposed (13). All of the above observations, in addition to there being no evidence for the existence of an Ntr-like system in cyanobacteria, have led to the view that the *glnB* gene is absent from these organisms. To our knowledge, the cloning and nucleotide sequence of a *glnB* gene in cyanobacteria have not been reported previously.

We chose to clone this gene in a *Synechococcus* species that is not able to fix molecular nitrogen but that can assimilate nitrate. The general reactions for nitrate assimilation are:

$$NO_3^- + 2Fd_{red} + 2H^+ \xrightarrow{\text{Nitrate reductase}} NO_2^- + 2Fd_{ox} + H_2O$$

$$NO_2^- + 6Fd_{red} + 8H^+ \xrightarrow{\text{Nitrite reductase}} NH_4^+ + 6Fd_{ox} + 2H_2O.$$

Both reactions require reducing equivalents from photosystem I as reduced ferredoxin (Fd_{red}). Reduction of ferredoxin by photosystem I is therefore required to produce ammonia, which is then incorporated into glutamine by glutamine synthetase. One hypothesis that may explain uridylylation of P_{II} in light 2 but not in light 1 is as follows. Under light 1, photosystem I turnover is fast and supported by an efficient donor system such as respiration. Photosystem I turnover maintains a high cellular ammonia concentration; therefore P_{II} is not uridylylated (Fig. 4). Under light 2, photosystem I turnover is slower than under light 1, and P_{II} becomes uridylylated because intracellular ammonia concentration is low. This assumption is supported by the effect of ammonium ions shown in Fig. 4. Alternatively, a second hypothesis can be made that P_{II} is modified by the redox state of plastoquinone, in the manner of phosphorylation of chloroplast thylakoid light-harvesting chlorophyll a/b protein (36). Reduced plastoquinone (light 2) would then trigger GlnB uridylylation, whereas the oxidized plastoquinone (light 1) would cause its deuridylylation. Further experiments are necessary to distinguish between these possibilities. Whatever the biochemical pathway leading to the modification of P_{II}, it appears that

nitrogen metabolism will be controlled by imbalance in the relative rates of photosynthetic electron transport through photosystem I and photosystem II. A comparable system may also operate in eukaryotic organisms, where expression of a nuclear gene for chloroplast glutamine synthetase may be switched on by light-induced changes in chloroplast metabolism (37).

The presence of P_{II} in cyanobacteria also suggests the existence of a cyanobacterial Ntr system. Vega-Palas *et al.* (38) have recently presented evidence for a cyanobacterial Ntr system by cloning a *Synechococcus* sp. PCC 7942 nitrogen regulatory gene, *ntcA*, implicated not only in the synthesis of the nitrate assimilatory system but also in that of glutamine synthetase and of the assimilatory permease.

Cyanobacteria are able to undergo inverse chromatic adaptation (39) by controlling expression of genes for components of the photosynthetic apparatus. Altered gene expression, reflected as adjustment of photosystem stoichiometry, may be coupled to the photosystem requirement of the cell and therefore to the light regime under which the cells are grown. Several hypotheses have been proposed to explain a coupling of gene expression to changes in light quality. Melis *et al.* (40) suggested that changes in the ratio of NADPH to ATP trigger the signaling mechanism. Fujita *et al.* (41) have proposed that adjustment of thylakoid membrane composition occurs in direct response to the redox state of plastoquinone. These two mechanisms are not inconsistent since the NADPH/ATP ratio affects the redox state of plastoquinone and vice versa. It has been proposed that the uridylylation of the 13-kDa protein, P_{II}, may be involved in long-term adaptation to light 1 and light 2 (9).

Here we have demonstrated an interaction between photosynthesis and nitrogen assimilation in *Synechococcus* strains in which uridylylation–deuridylylation of P_{II} may be expected to play a key role. We suggest that this mechanism may control gene expression at the transcriptional level in addition to the possibility of posttranslational control of protein function by covalent modification. In addition to its importance for photosynthesis and nitrogen assimilation, the discovery of a *glnB* gene in different cyanobacterial genera opens the way for investigation of the role of signal-transducing P_{II} proteins in strains that exhibit cell differentiation (29, 42), nitrogen fixation (42, 43), and complementary chromatic adaptation (3, 44).

We thank J. Claverie-Martin and B. Magasanik for the *glnB* probe from *E. coli*, C. Elmerich for the *Azospirillum glnB* probe, G. Rapoport and T. Msadek for providing facilities for oligonucleotide synthesis, and J. Houmard for helpful discussions. We thank the U. K. Science and Engineering Research Council for a Postgraduate Research Studentship (M.A.H.) and Research Grants (J.F.A.). This work was also supported by the Institut Pasteur and by the French Centre National de la Recherche Scientifique, U.R.A. 1129 (A.M.C. and N.T.d.M.).

1. Williams, W. P. & Allen, J. F. (1987) *Photosynthesis Res.* **13,** 19–45.
2. Bryant, D. A. (1987) *Can. Bull. Fish. Aqua. Sci.* **214,** 423–500.
3. Tandeau de Marsac, N. (1990) in *The Molecular Biology of Plastids and Mitochondria*, eds. Bogorad, L. & Vasil, I. K. (Academic, New York), Vol. 7B, pp. 417–446.
4. Bonaventura, C. & Myers, J. (1969) *Biochim. Biophys. Acta* **189,** 366–383.
5. Murata, N. (1969) *Biochim. Biophys. Acta* **172,** 242–251.
6. Allen, J. F., Sanders, C. E. & Holmes, N. G. (1985) *FEBS Lett.* **193,** 271–275.
7. Sanders, C. E. & Allen, J. F. (1987) in *Progress in Photosynthesis Research*, ed. Biggins, J. (Nijhoff, Dordrecht, The Netherlands), Vol. 2, pp. 761–764.
8. Sanders, C. E., Melis, A. & Allen, J. F. (1989) *Biochim. Biophys. Acta* **976,** 168–172.
9. Harrison, M. A., Keen, J. N., Findlay, J. B. C. & Allen, J. F. (1990) *FEBS Lett.* **264,** 25–28.
10. Son, H. S. & Rhee, S. G. (1987) *J. Biol. Chem.* **262,** 8690–8695.
11. Bueno, R., Rahel, G. & Magasanik, B. (1985) *J. Bacteriol.* **164,** 816–822.
12. Ninfa, A. J. & Magasanik, B. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5909–5913.
13. Fisher, R., Tuli, R. & Haselkorn, R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3393–3397.
14. Holtel, A. & Merrick, M. (1988) *Mol. Gen. Genet.* **215,** 134–138.
15. Golden, S. S., Nalty, M. S. & Cho, D.-S. C. (1989) *J. Bacteriol.* **171,** 24–29.
16. Kuhlemeier, C. J., Thomas, A. A. M., Van der Ende, A., Van Leen, R. W., Borrias, W. E., Van den Hondel, C. A. M. J. J. & Van Arkel, G. A. (1983) *Plasmid* **10,** 156–163.
17. Tandeau de Marsac, N. & Houmard, J. (1987) in *The Cyanobacteria*, eds. Fay, P. & Van Baalen, C. (Elsevier, Amsterdam), pp. 251–302.
18. Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M. & Stanier, R. Y. (1979) *J. Gen. Microbiol.* **111,** 1–61.
19. Damerval, T., Castets, A. M., Guglielmi, G., Houmard, J. & Tandeau de Marsac, N. (1989) *J. Bacteriol.* **171,** 1445–1452.
20. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
21. Dower, W. J., Miller, J. F. & Ragsdale, C. W. (1988) *Nucleic Acids Res.* **16,** 6127–6145.
22. Mazel, D., Guglielmi, G., Houmard, J., Sidler, W., Bryant, D. A. & Tandeau de Marsac, N. (1986) *Nucleic Acids Res.* **14,** 8279–8290.
23. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
24. Akrigg, D., Bleasby, A. J., Dix, N. I. M., Findlay, J. B. C., North, A. C. T., Parry-Smith, D., Wooton, J. C., Blundell, T. L., Gardner, S. P., Hayes, F., Islam, S., Sternberg, M. J. E., Thornton, J. M. & Tickle, I. J. (1988) *Nature (London)* **335,** 745–746.
25. Laskowski, M., Sr. (1980) in *Methods Enzymol.* **65,** 276–284.
26. de Zamaroczy, M., Delorme, F. & Elmerich, C. (1990) *Mol. Gen. Genet.* **224,** 421–430.
27. Shine, J. & Dalgarno, L. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 1342–1346.
28. Tomioka, N. & Sugiura, M. (1983) *Mol. Gen. Genet.* **191,** 46–50.
29. Rippka, R. & Herdman, M. (1985) *Ann. Inst. Pasteur/Microbiol.* **136A,** 33–39.
30. Kranz, R. G., Pace, V. M. & Caldicott, I. M. (1990) *J. Bacteriol.* **172,** 53–62.
31. Martin, G. B., Thomashow, M. F. & Chelm, B. K. (1989) *J. Bacteriol.* **171,** 5638–5645.
32. Colonna-Romano, S., Riccio, A., Guida, M., Defez, R., Lamberti, A., Iaccarino, M., Arnold, W., Preifer, U. & Pühler, A. (1987) *Nucleic Acids Res.* **15,** 1951–1964.
33. Orr, J. & Haselkorn, R. (1982) *J. Bacteriol.* **152,** 626–635.
34. Orr, J. & Haselkorn, R. (1981) *J. Biol. Chem.* **256,** 13099–13104.
35. Tumer, N. E., Robinson, S. J. & Haselkorn, R. (1983) *Nature (London)* **306,** 337–342.
36. Allen, J. F., Bennett, J., Steinback, K. E. & Arntzen, C. J. (1981) *Nature (London)* **291,** 25–29.
37. Edwards, J. E. & Coruzzi, G. M. (1989) *Plant Cell* **1,** 241–148.
38. Vega-Palas, M. A., Madueno, F., Herrero, A. & Flores, E. (1990) *J. Bacteriol.* **172,** 643–647.
39. Jones, L. W. & Myers, J. (1965) *J. Phycol.* **1,** 6–13.
40. Melis, A., Manodori, A., Glick, R. E., Ghirardi, M. L., McCauley, S. W. & Neale, P. J. (1985) *Physiol. Veg.* **23,** 757–765.
41. Fujita, Y., Murakami, A. & Ohki, K. (1987) *Plant Cell Physiol.* **28,** 283–292.
42. Wolk, C. P. (1982) in *The Biology of Cyanobacteria*, eds. Carr, N. G. & Whitton, B. A. (Blackwell, Oxford, UK), pp. 359–386.
43. Haselkorn, R. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. M. (Am. Soc. Microbiol., Washington), pp. 735–742.
44. Tandeau de Marsac, N. (1983) *Bull. Inst. Pasteur (Paris)* **81,** 201–254.