PII Is Important in Regulation of Nitrogen Metabolism but Not Required for Heterocyst Formation in the Cyanobacterium Anabaena sp. PCC 7120

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PII is an important signal protein for regulation of nitrogen metabolism in bacteria and plants. We constructed a mutant of glnB, encoding PII, in a heterocystous cyanobacterium, Anabaena sp. PCC 7120, with a cre-loxP system. The mutant (MP2α) grew more slowly than the wild type under all nitrogen regimens. It excreted a large amount of ammonium when grown on nitrate due to altered activities of glutamine synthetase and nitrate reductase. MP2α had a low nitrogenase activity but was able to form heterocysts under diazotrophic conditions, suggesting that PII is not required for heterocyst differentiation.

Analysis of the PII with mass spectroscopy found tyrosine nitration at Tyr-51 under diazotrophic conditions while no phosphorylation at Ser-49 was detected. The strains 51F and 49A, which have PII with mutations of Y51F and S49A, respectively, were constructed to analyze the functions of the two key residues on the T-loop. Like MP2α, they had low nitrogenase activity and grew slowly under diazotrophic conditions. 49A was also impaired in nitrate uptake and formed heterocysts in the presence of nitrate. The up-regulation of ntcA after nitrogen step-down, which was present in the wild type, was not observed in 51F and 49A. While our results showed that the Ser-49 residue is important to the function of PII in Anabaena sp. PCC 7120, evidence from the PII pattern of the wild type and 49A in non-denaturing gel electrophoresis suggested that Ser-49 is not modified. The possible physiological roles of tyrosine nitration of PII are discussed.

Cyanobacteria are a large group of prokaryotes that carry out oxygenic photosynthetic electron transport that provides reducing power for carbon and nitrogen metabolism. Many cyanobacteria can assimilate different forms of combined nitrogen from their environment. Certain cyanobacteria can also carry out nitrogen fixation. The nitrogen metabolism in cyanobacteria is transcriptionally controlled by NtcA, a transcription factor that belongs to the Crp transcription factor superfamily (1–3). Another important regulator of nitrogen metabolism in cyanobacteria is PII (4–5). PII proteins are widespread in prokaryotes and plants, and they interact with a number of target proteins involved in nitrogen metabolism (6). PII proteins function as a sensor of cellular concentration of 2-oxoglutarate (2-OG)2 (7, 8), which reflects the status of cellular nitrogen level in many bacteria. In proteobacteria, PII proteins are encoded by glnB and glnK (9, 10), and the posttranslational modification through uridylylation of a tyrosine residue is critical to their nitrogen metabolism (11). Cyanobacterial PII, encoded by glnB, has been studied extensively in unicellular cyanobacteria (4, 5). It has been shown that cyanobacterial PII also functions as a sensor of the intracellular level of 2-OG and binds 2-OG and ATP in a synergistic fashion (12). In unicellular cyanobacteria, except for Prochlorococcus marina (13), evidence from radioisotope labeling and site-directed mutagenesis reveals that PII could be phosphorylated at the position of Ser-49 (14, 15). Immunoblotting after non-denaturing gel electrophoresis of cellular proteins shows that the level of phosphorylation of PII proteins correlates with the status of nitrogen limitation (14). Recent evidence suggests that the Ser-49, which is located at the T-loop of PII, is critical to the regulation of the interaction of PII with its targeting proteins (16, 17).

Some filamentous cyanobacteria form special cells called heterocysts for nitrogen fixation (18–19). Differentiation from a vegetative cell to a heterocyst is a complex process that is regulated by several important genes, such as hetR (20–22) and ntcA (2, 23). Although PII functions as a sensor of the cellular level of 2-OG, which is a signal of heterocyst differentiation (24), the role of PII in heterocyst formation remains unanswered because it has not been possible to construct a glnB null mutant in heterocystous cyanobacteria so far (25, 26). The nature of PII modification in heterocystous cyanobacteria is not certain either. The PII pattern of immunoblotting after non-denaturing gel electrophoresis, which could indicate a covalent modification, did not change according to the nitrogen regimens (25, 27). Although PII phosphorylation has been suggested in Anabaena sp. PCC 7120 (26), biochemical evidence of PII modification in heterocystous cyanobacteria has not been reported.

We report here that a glnB mutant has been successfully constructed in Anabaena sp. PCC 7120 through a genetic system
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based on cre-loxP (28). We demonstrate that PII from *Synechocystis* sp. PCC 6803 is phosphorylated under nitrogen-limiting conditions. However, our evidence shows that PII from *Anabaena* sp. PCC 7120 is not modified at Ser-49, whereas a tyrosine nitration at Tyr-51 was identified. The significance of PII and its modification in *Anabaena* sp. PCC 7120 is discussed.

**EXPERIMENTAL PROCEDURES**

**Strains and Growth Conditions—** *Anabaena* sp. PCC 7120 (*Anabaena* 7120 hereinafter) and *Synechocystis* sp. PCC 6803 (*Synechocystis* 6803 hereinafter) were grown in BG11 medium (29) with or without combined nitrogen. Measurement of growth rate was performed by monitoring optical density at 730 nm.

**DNA Manipulation and Mutant Construction—** Isolation of total cyanobacterial genomic DNA was carried out as described by Zhao *et al.* (30). The procedure for *glnB* deletion with the cre-loxP (28) system is as follows. A fragment upstream of the *glnB* gene was amplified with PCR using primers PUU and PUL (the sequences of these and other primers used in this study are provided in supplemental Table S1) that contain the loxP sequence. The fragment was inserted into pET3d (31), generating plasmid pET3dx. An erythromycin cartridge was then inserted into pET-3dx, generating pET3dGLnBup, which was transformed into the wild type of *Anabaena* 7120. The transformants were allowed to grow until all chromosomes contained the loxP sequence upstream of the *glnB* gene, and the strain was named MI-up. A fragment downstream of the *glnB* gene was amplified with PCR using primers PDD and PDL and digested with BglII and XbaI. The fragment was then inserted into pET3dx, which was digested with the same enzymes. The resultant plasmid was digested with BglII and BamHI, and the generated fragment was inserted into plasmid pBBR-1 (32) that contained the loxP sequence upstream of the *glnB* gene, and the resultant plasmid, generating pBBRGLnBdown, which was then transformed into the strain MI-up. The transformants were treated as above. The resultant strain (MI-ud) contained two loxP sequences in the same direction flanking the *glnB* gene. The homozygous nature of the loxP was confirmed by PCR. The strain MI-ud was then transformed with pRL-25C containing a cre gene under control of the petE promoter (33, 34). The resultant strain was incubated with 2 μM copper in BG11 medium to induce cre expression, which led to deletion of the sequence between the two loxP sequences (28), including *glnB*. The complete absence of *glnB* was confirmed by Southern hybridization performed according to Huang *et al.* (35) to confirm the deletion of *glnB*. The templates for probes used in Southern hybridization were synthesized with PCR. Probe 1 was obtained with primers 1U and 1L, and probe 2 was obtained with primers 2U and 2L. The probes were labeled with random primer extension method. Complement of MP2α with *glnB* was achieved by transformation of MP2α with pAM505 (36) containing different *glnB* genes. Site-specific mutagenesis of the *glnB* gene for construction of mutant *glnB* encoding PII with S49A, Y51F mutations was performed according to Huang *et al.* (35) using the primers provided in supplemental Table S1. The mutations were confirmed by DNA sequencing.

**Electrophoresis and Mass Spectroscopy—** In two-dimensional electrophoretical (2-DE) separation of total soluble proteins from *Anabaena* 7120, the first dimension for isoelectric focusing was carried out with pre-made gel strips (pH 3–10) from GE Healthcare. The second-dimensional electrophoresis was performed with gels that were 25 cm (width) by 20 cm (length). Proteins after the second-dimensional electrophoresis were either transferred to a polyvinylidene difluoride membrane for immunoblotting according to Zhao *et al.* (30) or stained with Coomassie blue. The pl value of a protein spot was estimated by its position in gel. Calculation of pl of a protein according to its amino acid composition was performed using DNAstis (Hitachi, Beijing, China). The protein spots of interest were extracted from the gels after staining and digested in-gel with trypsin. The generated tryptic peptides were analyzed first with matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MS) to determine their identities. The tryptic peptides from identified PII digestion were then separated with a nano-liquid chromatography system (Micro-Tech Scientific, Vista, CA). The column was 0.15 × 15 mm packed with C18 resin. Buffer A was 0.1% trifluoroacetic acid, and buffer B was 95% acetonitrile in 0.1% trifluoroacetic acid. The peptides were eluted during a period of 120 min with a linear gradient from 1 to 50% buffer B at a flow-rate of 0.4 μL.min⁻¹ before they were injected into a coupled Fourier transformation ion cyclotron resonance mass spectrometer (FT-ICR-MS) (Bruker Daltonics, Leipzig, Germany). The instrument was set to acquire a 0.2-s MS scan from 100 to 2000 Da and a 2-s MS/MS scan of selected precursor in a cycle. MS and MS/MS peaks were picked using the FT-MS algorithm with a signal to noise ratio above 4. The generated MS data were analyzed with the software Mascot (Matrix Science Ltd., London) using the data base of NCBI-nr (060604). Non-denaturing gel electrophoresis was performed according to Forchhammer and Tandeau de Marsac (14), followed by electrophoretical transfer of the proteins to a polyvinylidene difluoride membrane before detection of PII with anti-PII antibodies.

**Biochemical Assays—** Nitrogenase activity was performed according to Zhao *et al.* (37). Nitrate uptake was measured with a nitrate electrode (Fisher Scientific) that has a sensitivity range of 7.0 μM to 1.0 M. Nitrate reductase activity was measured according to Herrero *et al.* (38). Glutamine synthetase activity was measured according to Orr *et al.* (39). Excretion of ammonium by *Anabaena* 7120 was assayed according to the method of Solorzano (40). Quantitative PCR of *ntcA* was performed using primers PntcaU and PntcaL according to Huang *et al.* (35) with 16 S rRNA as a control.

**RESULTS**

**Construction of glnB Mutant of Anabaena 7120—** In an attempt to construct a *glnB* mutant in *Anabaena* 7120, we first used the SacB-based method (41) but only obtained a meridiploid.3 Because of the success of using the cre-loxP system in constructing conditional mutants in various organisms (42), we

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3 Y. Zhang and J. Zhao, unpublished results.
A physical map of a DNA fragment containing glnB and its adjacent area of Anabaena 7120. Physical map of the same chromosomal area in MP2a is shown below the physical map of WT. The glnB was deleted and a copy of loxP, a gene for spectinomycin resistance, and a fragment from plasmid pBRRmc were inserted. The probes used for Southern hybridization are indicated. H, HindII; M, MluI and E, EcoRV. B, total DNA was isolated from the wild type (W) and MP2a (M) and digested with HindII. DNA fragments were separated with agarose gel and transferred to a nitrocellulose paper before being hybridized with probe 1, which was obtained by PCR using primers 1U and 1L. The size is shown on the left (in kb). C, same as panel B except that DNA was digested with EcoRV and MluI. The probe 2 for hybridization was obtained by PCR using primers 2U and 2L. The size is shown on the left (in kb). D, immunoblotting analysis of MP2a. Total cellular extracts from MP2a (M) and the wild type (W) were separated with SDS-PAGE and transferred to a polyvinylidene difluoride membrane before detection of PII (indicated by an arrow). A recombinant PII was used as control (lane C).

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PII Modification in Anabaena 7120—Because different results of PII modification were reported in different heterocystous cyanobacteria, we investigated the modification of PII in Anabaena 7120. Immunoblotting was performed after 2-DE, and the results are shown in Fig. 3. Three protein forms were detected in the wild type Anabaena 7120 grown with ammonium (Fig. 3A), nitrate (Fig. 3B), or under diazotrophic condition (Fig. 3C). The positions of these three protein forms in the two-dimensional gels were identical, and their pl values were 5.7, 7.4, and 9.0. The pl value of 9.0 was close to the calculated value (pl = 9.13) based on the amino acid composition of PII from Anabaena 7120. The spot with pl of 5.7 had a molecular mass that was ~2 kDa smaller than that of the other two protein genes in a genetic background without influence from the host glnB gene. To study whether Ser-49 was modified in Anabaena 7120, we transformed MP2a with glnB-S49A, which encodes a PII with a S49A mutation, on a low copy shuttle vector pAM505. The strain generated was named 49A. We then performed non-denaturing gel electrophoresis followed by immunoblotting to detect the PII pattern, which indicates the status of PII modification in response to different nitrogen regimens (14), in Anabaena 7120 and Synechocystis 6803. One major band was detected in Synechocystis 6803 cells grown with ammonium, whereas there were four bands when the cells were incubated in growth medium without combined nitrogen for 10 h (Fig. 2A). No such pattern change was detected in the wild type and 49A of Anabaena 7120 (Fig. 2, B and C) in response to different nitrogen regimens as immunoblotting showed that there were four bands in these strains under all nitrogen regimens. In heterocysts, there were a major band and a minor band of PII. The major band had a mobility similar to that of the most mobile band of the wild type (Fig. 2D).
forms. The protein form with a pI of 7.4 was a minor protein and it accounted for only ~10% of total PII. Heterocysts contained only one form of PII, and it had a pI of 9.0 and a molecular mass of intact PII (Fig. 3D). We also analyzed PII of *Synechocystis* 6803 by 2-DE. One PII with a pI of 7.3, which was close to the calculated pI value (7.20), was detected when the cells were grown in the presence of ammonium (Fig. 3E) while several protein spots with more acidic pI values and a spot with a more basic pI (9.1) were detected when the cells were deprived of combined nitrogen for 10 h (Fig. 3F).

We further investigated PII modification with mass spectrometry. Total soluble proteins of *Anabaena* 7120 and *Synechocystis* 6803 were separated with 2-DE, and the PII spots according to the immunoblotting results in Fig. 3 were digested in-gel with trypsin and extracted. The tryptic peptides were separated with nano-liquid chromatography before analysis with FT-MS. A, the tandem MS spectrum of tryptic peptide GSEYTVEFLQK of *Anabaena* 7120 (from UniProt/Trembl: Q9L422_ANASP). The b, b-H2O (marked as b*) and y ions are indicated. The y7, y8, b*, b*+, b*+ ions indicate a NO2 (+44.9851 Da) modification at Tyr-51. As the mass error was below 0.01 Da, the possibility of other additions, including CH4NO (+45.0215 Da), was excluded. A more detailed analysis with MS/MS is presented as supplemental Fig. S2. B, a tandem MS spectrum of phosphopeptide from *Synechocystis* 6803 grown without combined nitrogen. The phosphopeptide was obtained from a spot after 2-DE corresponding to the spot 2 of Fig. 3F, and it had a sequence of YRGSP[Pi]EYTVEFLQK (from UniProt/Swissprot:GLNB_SYNY3). The ions with molecular masses indicate modifications.
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Characterization of MP2α and glnB Mutant Genes—To investigate the significance of Tyr-51 modification, we constructed glnB-y51f, which encodes a PII with an Y51F mutation. Complement of MP2α with a wild type glnB or glnB-y51f generated strains termed MP2c and 51F, respectively. An attempt to complement MP2α with a mutant glnB encoding the PII lacking the N-terminal 19 residues has not been successful.3

We measured growth rates of Anabaena 7120 strains under different nitrogen regimens, and the results are shown in Fig. 5. (also see supplemental Fig. S3). Under the three nitrogen regimens tested, the growth of MP2α was approximately half as fast as that of the wild type. Complement of MP2α with wild type glnB gene (MP2c) fully restored the growth rates, demonstrating that the slow growth of MP2α was due to the lack of glnB. Another phenotype of MP2α was that it excreted ammonium when grown with nitrate (Fig. 5D). Complement of MP2α with the wild type and mutant glnB genes completely stopped the ammonium excretion. The importance of Ser-49 was demonstrated by the growth curves since replacement of Ser-49 with Ala led to an incomplete complement of MP2α. Compared with MP2α, 49A grew more slowly under the nitrate regimen. Both 51F and 49A grew slowly under diazotrophic condition, and their growth rates were similar to that of MP2α under the same condition (Fig. 5C). Heterocyst differentiation in MP2α appeared normal 24 h after nitrogen step-down (Fig. 6A), demonstrating that heterocyst formation does not require PII. Examination of other strains showed that 49A formed heterocysts when grown with nitrate (Fig. 6B).

Heterocyst formation in 49A and the excretion of ammonium in MP2α under the nitrate regimen suggested that the nitrogen metabolism in these strains was disturbed. Because of the importance of ntcA in nitrogen control, we measured the expression of ntcA in these strains with quantitative PCR (Fig. 7A). The expression of ntcA in the wild type increased more than 2-fold when the cells were subjected to nitrogen step-down for 12 h, and this up-regulation was not observed in 51F and 49A. The glutamine synthetase activity in MP2α grown with nitrate was only 35% of that of the wild type whereas it was abnormally high when grown with ammonium (Fig. 7B). Because the levels of glnA mRNA were similar in all strains (Fig. 7C), the low glutamine synthetase activity of MP2α grown with nitrate was likely due to a decreased enzymatic activity rather than a reduced amount of glutamine synthetase under this condition. Under the nitrate regimen, nitrate reductase activity in MP2α was three times that in the wild type (Fig. 7D). The nitrate uptake activity in 49A was only half that of the wild type (Fig. 7E), suggesting that nitrogen supply could be a limiting factor in 49A when grown with nitrate. We also measured nitrogenase activities with filaments 40 h after nitrogen step-down and found that the nitrogenase activities in MP2α, 49A, and 51F were only 38, 37, and 34% of that in the wild type, respectively (Fig. 7F).
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The glnB mutant of *Anabaena* 7120 makes it possible to study the physiological significance of PII and PII modification. The phenotypes of MP2α and several other mutants support that PII interacts with a variety of proteins and regulates their functions (5–8). For example, PII in *Anabaena* 7120 probably regulates both glutamine synthetase and nitrate reductase, and the low glutamine synthetase activity and high nitrate reductase activity are likely responsible for the excretion of ammonium in MP2α grown with nitrate. The mutation of S49A of PII leads to a partial inhibition of nitrate uptake, which in turn results in heterocyst formation in 49A in the presence of nitrate (Fig. 6). The nitrogense activity is low in 49A and 51F, suggesting that both Ser-49 and Tyr-51 are important residues at the T-loop of the PII from *Anabaena* 7120. These two residues are also important to the up-regulation of ntcA expression when combined nitrogen is deprived (Fig. 7), implying that they could participate in regulation of gene expression controlled by NtcA (44–46).

![Image](https://example.com/image.png)

**FIGURE 7. Requirement of PII for regulation of nitrogen metabolism in *Anabaena* 7120.** The following parameters in the wild type (WT), MP2α (MP), 49A, and 51F were measured. A, the abundance of ntcA mRNA. Cells grown with ammonium (red), with nitrate (blue), and subjected to nitrogen deprivation for 12 h (green) were used for RNA extraction. Quantitative PCR was performed to determine the abundance of ntcA mRNA relative to 16 S rRNA. B, glutamine synthetase (GS) activity in cells grown with ammonium (red), nitrate (blue), and molecular nitrogen (green). C, the abundance of glnA mRNA with cells grown with nitrate. Other conditions were the same as in panel A. D, nitrate reductase (NR) activity in cells grown with nitrate. E, nitrate uptake in cells grown with nitrate. F, nitrogenase activity in whole filaments 40 h after nitrogen deprivation.

**DISCUSSION**

PII is the only component of the proteobacterial PII signaling system that is found in cyanobacteria (43). In some unicellular cyanobacteria, PII is phosphorylated at Ser-49 and not uridylylated at Tyr-51 as in proteobacteria (5). A previous attempt at construction of a glnB (encoding PII) mutant in heterocystous cyanobacteria was not successful (25, 26). The questions concerning the roles of PII in heterocyst differentiation and functions therefore remained unanswered. In this study, we constructed a glnB mutant (MP2α) of *Anabaena* 7120 by the cre-loxP system (28). One possible reason for us to be able to obtain a glnB mutant in *Anabaena* 7120 through the cre-loxP system is that the loxP sequence is first inserted on both sides of the target gene and induction of cre expression occurs only after the target gene with flanking loxP sequence becomes homozygous. Because the cre-loxP system is very efficient (28), it is possible that the target gene is deleted on all chromosomes within a short time and the chance for formation of a meridiploid is reduced. We found the cre-loxP system very useful based on our success in mutant construction, including deletion of a 22-kb-large fragment, in *Anabaena* 7120.

We found no evidence for seryl phosphorylation in PII of *Anabaena* 7120 with mass spectroscopic study. Similar to *Nostoc punctiforme* (25), *Anabaena* 7120 does not show any PII pattern change in non-denaturing gel electrophoresis (Fig. 3). More evidence for the lack of phosphorylation of PII comes from the results of 2-DE that show that the patterns of protein spots in these gels are unchanged under different nitrogen regimens. Further evidence for the lack of phosphorylation of Ser-49 is that our S49 mutant shows the same PII pattern as that of the wild type, indicating that the pattern of PII could not be due to a modification of Ser-49. On the other hand, phosphorylation of Ser-49 in PII of *Synechocystis* 6803 is identified under nitrogen-limiting conditions (Fig. 4). This result provides biochemical evidence that PII in *Synechocystis* 6803 could be phosphorylated. It also suggests that it is unlikely that the lack of phosphopeptides in PII from *Anabaena* 7120 was due to loss of a phosphate group during the sample preparation procedure. The lack of Ser-49 modification in PII of *Anabaena* 7120 reported here is different from the results reported by Laurent et al. (26), who showed the PII pattern detected by immuno-blotting changed according to nitrogen regimens. The reason for the discrepancy is not known at the moment. It is noticed that Laurent et al. (26) used wild type *Anabaena* 7120 for construction of their mutant strain S49A whereas our 49A is constructed in a strain with a glnB-less background. The PII pattern of *Anabaena* 7120 in non-denaturing gel could be due to a different combination of the full-length PII and the truncated PII in PII trimer formation because tyrosine nitration does not change the pI value of the modified protein. The PII form with pI of 7.4 in *Anabaena* 7120 (Fig. 3) only accounted for less than 10% of total PII and would be unlikely to influence the PII pattern in non-denaturing gel significantly.

Tyrosine nitration at Tyr-51 of PII is detected in *Anabaena* 7120 under diazotrophic conditions. Usually, tyrosine nitration occurs through oxidation of tyrosine either by peroxynitrite or heme-based peroxidase (48). It has been noted that deprivation of combined nitrogen created an oxidative environment in heterocystous cyanobacteria (49, 50). This could lead to formation of nitrotyrosine at Tyr-51. Protein nitration has been shown to occur in animal tissues such as the cardiovascular system under...
oxidative stress, and its physiological significance has been recognized (51). Addition of a $-\text{NO}_2$ at tyrosine adds a bulky group to the phenolic ring and could lower the $pK_a$ of the OH group of the tyrosine. This could lead to a change of protein conformation (48). In analyzing proteins from isolated heterocysts by native gel electrophoresis, we observed a minor PII band and a major PII band. On the other hand, only one spot of PII was observed in isolated heterocysts in 2-DE. We speculate that the minor PII band in the native gel could be the PII with tyrosine nitration. Incubation with SDS results in loss of conformation of the two PII forms, and therefore only one spot was observed in 2-DE.

Both gain of function (52) and loss of function (53) have been reported in proteins due to tyrosine nitration. In Anabaena 7120, PII nitration is unlikely to be involved in loss of function because that would require all PII or nearly all PII to be nitrated. Another possibility is that tyrosine nitration of PII in heterocysts leads to a gain of function. The growth of 51F with nitrate is faster than MP2a, suggesting that the gene glnB-y51f is functional and able to complement the mutant under the nitrate regimen. However, the gene glnB-y51f is unable to complement MP2a under diazotrophic conditions, indicating Tyr-51 is important for nitrogen fixation. It is well established that PII in proteobacteria is uridylylated at Tyr-51 under a nitrogen-limiting condition (7, 8). Tyr-51 is located on the T-loop of PII, and this loop is critical to the binding of 2-OG (36). Modification of Tyr-51 by uridylylation has an effect of reinforcing the signal of no combined nitrogen (7, 8). Addition of an electrophilic $-\text{NO}_2$ group at Tyr-51 could have a similar effect as uridylylation except that the modification is irreversible. This kind of modification, however, might be suitable for heterocyst functions. Heterocysts are terminally differentiated cells, and there is plenty of combined nitrogen in heterocysts because of nitrogen fixation. Although glutamate synthase is absent in heterocysts (47, 55), aminotransferases are active in heterocysts (55), which could lead to less availability of 2-OG. A PII with a Tyr-51 nitration could amplify a signal of 2-OG so that heterocysts can carry out nitrogen fixation and ammonium assimilation in the presence of ammonium and glutamine. On the other hand, it is also possible that the nitrotyrosine formed under diazotrophic condition is a passive modification resulting from oxidative stress and senescence of heterocysts. The inability for glnB-y51f to complement MP2a could reflect a fact that the hydroxyl group of Try-51 is critical to PII function and therefore a Phe residue could not replace Tyr-51. Further study is needed to fully understand the physiological significance of tyrosine modification of PII of Anabaena 7120.

Although our study shows that PII is critical to heterocyst function, the formation of heterocysts and the presence of a normal pattern of heterocysts in MP2a demonstrate that heterocyst differentiation does not depend on PII. The process of cell differentiation and the function of the differentiated cells are therefore separated.

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