Identification of PamA as a PII-binding Membrane Protein Important in Nitrogen-related and Sugar-catabolic Gene Expression in *Synechocystis* sp. PCC 6803*

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The PII signaling protein plays a pivotal role in the coordination of carbon and nitrogen metabolism in a wide variety of bacteria, Archaea, and plant chloroplasts. By using a yeast two-hybrid screening system, we identified a transmembrane protein, designated PamA (encoded by *slt0985*), as a PII-binding protein in *Synechocystis* sp. PCC 6803. The interaction between PII and PamA was confirmed *in vitro*, and the interaction was inhibited in the presence of ATP and 2-oxoglutarate, whereas the interaction was not influenced by the phosphorylation status of PII. Northern blot analyses revealed that the transcripts of a set of nitrogen-related genes, including *nblA*, *nrtABCD*, and *ureG*, were decreased in a *pamA* deletion mutant. The mRNA and protein levels of a group 2 or factor *SigE* were also reduced by the *pamA* mutation, and transcripts for sugar catabolic genes, such as *gap1*, *zwf*, and *gnd* that are under the control of *SigE*, were consequently decreased in the *pamA* mutant. In addition, the *pamA* mutant was found to be unable to grow in glucose-containing media. These results indicate that PamA has a role in the transcript control of genes for nitrogen and sugar metabolism in *Synechocystis* sp. PCC 6803.

Cyanobacteria are prokaryotes that perform oxygenic photosynthesis similar to that in higher plants and algae. These bacteria coordinately regulate various aspects of cellular metabolism in response to changes in their environment. Carbon and nitrogen are important for cell growth, and the complex metabolism of each of these elements is regulated in synchrony under all conditions. The internal balance of carbon and nitrogen in unicellular cyanobacteria is monitored by the PII sensor protein, which is highly conserved among bacteria, Archaea, and plant chloroplasts (1–3).

It has been shown that PII protein regulates nitrogen metabolism in unicellular cyanobacteria (4). As in *Escherichia coli* and related bacteria, the PII protein of *Synechococcus* sp. PCC 7942 binds to ATP and 2-OG in a synergetic manner (5). The intracellular 2-OG level is assumed to reflect not only the carbon status but also the nitrogen status in cyanobacteria because of the lack of canonical 2-OG dehydrogenase (6). Therefore, PII is considered to be able to integrate energy, carbon, and nitrogen signals by monitoring ATP and 2-OG levels (4). In addition to ATP and 2-OG binding, *Synechococcus* and *Synechocystis* PII proteins are phosphorylated at a serine residue under nitrogen starvation (7, 8). This phosphorylation level is also affected by carbon status (9), and recent analyses revealed that PII dephosphorylation specifically responded to intracellular 2-OG concentrations (10).

A PII-deficient mutant (MP2) of *Synechococcus* sp. PCC 7942 does not inhibit the nitrate/nitrite transporter in the presence of ammonium, which is the preferred nitrogen source for unicellular cyanobacteria; however, it inhibits the nitrate/nitrite transporter in the wild-type strain (11). High affinity bicarbonate transporters are constitutively activated regardless of the ambient carbon status in a PII-deficient mutant of *Synechocystis* sp. PCC 6803 (8). Thus, it was suggested that carbon metabolism is also regulated by PII in this cyanobacterium. However, the underlying molecular mechanisms by which the nitrate/nitrite and bicarbonate transporters are regulated by PII remain unknown.

The expression of nitrogen-related genes in cyanobacteria is activated by the global nitrogen regulator NtcA, a transcription factor that belongs to the CRP family (12, 13). It was recently shown that 2-OG binds directly to NtcA and thereby promotes its interaction with target promoters (14–16). Thus, both PII and NtcA are two independent 2-OG sensors among cyanobacteria. With regard to the potential role of PII in transcriptional regulation, it was shown that the level of expression of NtcA-regulated genes, such as *ntcA*, *nirA*, *glnA*, and *amt1*, was reduced in MP2 cells, implicating PII in transcriptional activation of *NtcA* regulons (17). Aldehni et al. (18) also revealed that PII was involved in regulating the *in vivo* activity of the *NtcA* regulons in *Synechococcus* sp. PCC 7942 (18). However, the underlying mechanism for this PII function also remains unclear.

At present, there are two proteins known to interact with PII in unicellular cyanobacteria. Irmler and Forchhammer (19) identified a PP2C-type protein phosphatase PphA of *Synechocystis* sp. PCC 6803 that dephosphorylates phosphorylated PII proteins *in vivo* and *in vitro* (19). A second PII interacting protein was recently discovered when it was found that PII formed a complex with N-acetyl glutamate kinase, a key enzyme of arginine biosynthesis, and controlled the activity in *Synechococcus* sp. PCC 7942 (20, 21). Despite these findings, it remains to be elucidated how PII is involved in transcriptional regulation, and no interacting protein involved in transcriptional regulation has been discovered so far.

In this study we used the yeast two-hybrid approach and identified a putative membrane protein PamA that binds PII in *Synechocystis* sp. PCC 6803. Transcripts for a set of nitrogen-related and sugar catabolic genes were reduced in a *pamA* mutant, suggesting that PamA might play a role in the control of their transcript abundances.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Culture Conditions**—The glucose-tolerant strain of *Synechocystis* sp. PCC 6803 (GT), isolated by Williams (22), and

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2 The abbreviations used are: 2-OG, 2-oxoglutarate; GT, glucose tolerant; CBD, cellulose binding domain; MSC, mechanosensitive channel; GST, glutathione S-transferase; PBS, phosphate-buffered saline.
its pamA deletion mutant were grown on BG-11 plates or in BG-11 liquid medium (5 mM NH4Cl as the nitrogen source) at 30 °C under continuous white light (≈70 μmol of photons m−2 s−1) (23). For photomixotrophic growth, BG-11 plates were supplemented with 5 mM glucose.

**Yeast Two-hybrid Library Screening** — The full-length PII gene, glnB (encoded by open reading frame ssl0707), of Synechocystis sp. PCC 6803 was amplified by PCR with specific primers (see TABLE ONE) and subcloned into the pAS2–1-AscI bait vector, consisting of the pAS2–1 vector (Clontech) with an introduced AscI restriction site. Details of the two-hybrid screening procedure will be described elsewhere.3

**Affinity Purification of GST-PamA-(475–680) and Cellulose Binding Domain (CBD)-PII Fusion Proteins** — A region of the Synechocystis sp. PCC 6803 genome encoding a COOH-terminal portion of PamA (amino acids 475–680) was amplified by PCR with specific primers (see TABLE ONE), digested with ScaI and XhoI, and inserted into the SmaI-XhoI sites of pGEX5X-1 (Amersham Biosciences). Full-length ssl0707 was amplified with specific primers (see TABLE ONE), digested with StuI and SacI, and inserted into the ScaI-SacI sites of pET35b (Novagen). The constructed plasmids encoding GST-PamA-(475–680) or CBD-PII were introduced separately into E. coli BL21 Codon Plus (Novagen) by transformation, expression of the encoded proteins was induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside (Wako) to 1 liter of LB medium, and the cells were subsequently cultured overnight at 37 °C. The cells were then collected by centrifugation and lysed in a solution containing 40 mM Tris-HCl (pH 8.0), 5% glycerol, 5 mM EDTA, and 4.5% Triton X-100, and an insoluble fraction containing the recombinant protein was isolated by centrifugation of the cell
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**FIGURE 2. Interaction of PII with the COOH-terminal region of PamA in vitro.** A, purification of recombinant PII and PamA. CBD-PII and GST-PamA-(475–680) fusion proteins were expressed in E. coli, purified by affinity chromatography, resolved by SDS-PAGE on a 12% gel, and stained with Coomassie Brilliant Blue. B, GST pull-down assay with purified recombinant PII and PamA fusion proteins. GST or GST-PamA-(475–680) bound to glutathione-Sepharose 4B beads was incubated with CBD-PII in the absence or presence of 1 mM ATP or 1 mM 2-OG as indicated, after which bead-bound proteins were subjected to immunoblot analysis with antibodies to the CBD tag. M, molecular mass markers.

lysate at 17,400 g for 20 min at 4 °C. The insoluble fraction was washed with cell lysis buffer, suspended in sterilized water, and solubilized by the addition of half a volume of a solution containing 8 M urea, 50 mM Tris-HCl (pH 8.0), and 10 mM dithiothreitol and incubated for 1 h at 37 °C. The solubilized GST-PamA-(475–680) and CBD-PII proteins were dialedyzed against PBS or CBinD buffer (20 mM Tris-HCl (pH 7.5), 50 mM NaCl) and purified with glutathione-Sepharose 4B (Amersham Biosciences) or CBinD 100 resin (Novagen), respectively. The total yields of GST-PamA-(475–680) and CBD-PII were ~4 and 3.5 mg, respectively. Protein concentration was determined with the Bio-Rad protein assay.

**GST Precipitation Assay—**Purified GST or GST-PamA-(475–680) (2.5 μg) was bound to 30 μl of glutathione-Sepharose 4B, mixed with 3.0 μg of CBD-PII in 400 μl of binding buffer (50 mM Hepes-KOH (pH 7.9), 2 mM MgCl2, 10% glycerol, 0.1% Triton X-100), and incubated overnight at 4 °C. The resin was washed 3 times with 300 μl of binding buffer, suspended in an equal volume of SDS sample buffer (1 M Tris-HCl (pH 6.8), 20% glycerol, 12% 2-mercaptoethanol, 0.04% bromphenol blue), and heated for 5 min at 95 °C. The released proteins were then subjected to SDS-PAGE and detected by immunoblotting with antibodies specific to the CBD tag (Novagen).

**Production of Anti-PII and PamA Antiserum and Immunoblotting—**For the production of anti-PII and PamA antiserum, fusion proteins were purified as described above. The protein concentrations and purities were examined by Bio-Rad protein assay and SDS-PAGE with Coomassie Brilliant staining, respectively. Each 2 μg of purified PII and PamA protein was injected into rabbits, and the antiserum production was performed by Qagen (Tokyo, Japan). Immunoblotting was performed as described previously (24).

**FIGURE 3. Far Western analysis with the recombinant or endogenous PamA proteins.** A, 3.0 μg of GST or GST-PamA-(475–680) was electrophoresed with a 12% SDS-PAGE gel and blotted onto Immobilon-P membrane. Cell extracts containing nonphosphorylated PII or full-phosphorylated PII were incubated with the membranes, and the associated PII proteins were detected by anti-PII antiserum. Signals were visualized by reaction with alkaline phosphatase with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium as a substrate. As a control reaction, 1.5 μg of GST and GST-PamA-(475–680) was resolved in the same gel and detected by anti-GST antibody (Molecular Probe). B, Far Western analysis with the endogenous PamA protein. 3.0 μg of purified CBD-PII protein was resolved with a 12% SDS-PAGE gel. GT cell extracts containing the endogenous PamA protein was incubated with the membrane, and the PamA protein was detected with anti-PamA antiserum. Anti-SigE antiserum was used as a negative control. Detection of 0.6 μg of the recombinant PII protein with the anti-PII antiserum is shown in the right panel for comparison.

**Far Western Blotting—**3.0 μg of GST or GST-PamA-(475–680) protein was subjected to SDS-PAGE with a 12% gel, and blotted onto Immobilon-P transfer membrane (Millipore). Cyanobacterial cell extracts containing nonphosphorylated or full-phosphorylated PII protein were prepared from an ammonia-grown GT cell culture or a GT cell culture deprived of nitrogen for 4 h. Cells were disrupted by sonication (30 s 5 times) in ice-cold PBS-T (PBS containing 0.1% Tween 20 (Sigma)), and debris was removed by centrifugation (17,400 g X 10 min). In previous experiments we found that the amount of PII protein was increased 2.1 times under this nitrogen deprivation condition (data not shown). Subsequently, 100 μg (for ammonia grown cells) or 48 μg (for nitrogen-deprived cells) of protein was used for the reaction with the membrane in 10 ml of PBS-T 3 h at 4 °C. After washes with PBS-T (10 min 5 times), binding proteins were cross-linked by 0.5% formaldehyde in PBS-T at room temperature for 30 min. The reactions were quenched by incubation with 2% glycine in PBS-T for 10 min and then washed with PBS-T (10 min 2 times). The membranes were incubated in 3% bovine serum albumin, and PII associated with GST or GST-PamA-(475–680) was detected by the anti-PII antiserum. For Far Western analysis with endogenous PamA, 3.0 μg of CBD-PII protein was similarly electrophoresed by SDS-PAGE and blotted onto a membrane as described above. To prepare cell extracts, cells were disrupted by sonication (30 s 5 times), and PamA proteins were solubilized with 1% Triton X-100 (Sigma-Aldrich) in PBS and incubated for 90 min at 4 °C. Debris was removed after centrifugation at 17,400 g for 10 min. 1.6
mg of protein was incubated with the membrane in 10 ml of ice-cold PBS containing 1% Triton X-100 for 3 h at 4 °C followed by washing with the same buffer (10 min 3 times). The subsequent steps were performed as mentioned above.

**Construction of a pamA Deletion Mutant**—A 5.5-kbp HpaI fragment of cosmid cs0120 (25) containing pamA was subcloned into pTZ18R (Amersham Biosciences) that had been digested with HinClI, ensuring that the orientation of pamA was the same as that of the lacZ gene of the vector. The resulting plasmid, p0985, was digested with Smal and Xhol, rendered blunt-ended with a DNA Blunting Kit (TaKaRa), and self-ligated to eliminate the BamHI site in the polylinker region. The new plasmid was digested with BamHI, resulting in the deletion of a ~2.4-kbp fragment (from the +165 position of the pamA-coding region to the 3′ end of the downstream transposase gene sll0986), and the deleted portion was replaced with a kanamycin resistance cassette (26) to yield p0985B. *Synechocystis* sp. PCC 6803 cells were transformed with p0985B and selected on BG-11 plates containing kanamycin (10 μg/ml).

**Isolation of RNA and Northern Blot Analysis**—Cells of mid-exponential phase cultures of *Synechocystis* sp. PCC 6803 (A750, 0.5–0.7) grown in BG-11 medium were collected by filtration and resuspended in BG-11 medium (BG-11 without NH4Cl). After culturing for 0 or 4 h, the cells were collected and subjected to RNA extraction by the acid phenol-chloroform method as previously described (27). Northern blot analysis of the isolated RNA was performed as described previously (28). For the construction of gene-specific probes, we amplified the corresponding coding region by PCR with specific primers (see TABLE ONE) and labeled the PCR products with digoxigenin with the use of a DIG DNA Labeling Kit (Roche Applied Science).

**Complementation of the pamA Mutant**—A DNA fragment including the pamA gene was obtained by digestion of the p0985B plasmid with SacI and EcoRV. The resulting 3.3-kbp fragment was blunt-ended with a DNA blunting kit (TaKaRa) and inserted into the cyanobacterial autonomous replication plasmid pVZ322 (29) digested with Smal. The resultant complementation vectors, namely, pVZ322:pamAC, were introduced into GN10 cells by triparental gene transfer (29).

**RESULTS AND DISCUSSION**

**Identification of a PII-binding Protein by Yeast Two-Hybrid Screening**—Yeast two-hybrid screening of 3.8 × 107 independent clones of a *Synechocystis* genomic library with *Synechocystis* PII as bait yielded four positive clones. One of these four clones contained the PII gene (gIIb) itself, consistent with the trimeric nature of PII, and the other three clones were found to harbor the 3′ terminal region of a predicted gene sll0985. The potential protein encoded by sll0985 comprised 680 amino acids and contained seven membrane-spanning segments, as predicted by the SOSUI program (30) (Fig. 1A). Analysis of sequence similarity with the BLAST program indicated that this protein belonged to the protein family defined by the MSC mechanosensitive ion chan-
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Figure 6. Complementation of the pamA mutant. A, schematic representation of complementation vectors. A DNA fragment containing pamA and up-stream unknown gene sll0984 were inserted into pVZ322 vector and transformed into GN10 cells. B, the plasmid maintenance in GN10 was confirmed by PCR with the specific primers (Table ONE). C, Northern blot analysis (10 μg of RNA per lane) with probes specific for gap1. The positions of molecular size standards are indicated in kilobases.

Table One

| Gene     | Purpose | Forward or reverse | Sequence          |
|----------|---------|--------------------|-------------------|
| ss10707  | Yeast   | F                  | 5’-GAGGGCGGCCGCAATGACGTATTTC-TGAAGTT-3’ |
| gnd      | Yeast   | R                  | 5’-CTCAAAATTTAAGCTTC-GG-3’ |
| pamA     | Recombinant protein production | F                 | 5’-GCAGCCCTT7GAAAAGATGAA-AAGG-3’ |
| pamA     | Recombinant protein production | R                 | 5’-AAAGACTCTTAAATAGCTCGG-TATC-3’ |
| gnd      | Cp      | R                  | 5’-CAGGGCGGCCGCAATGACGTATTTC-TGAAGTT-3’ |
| gnd      | Cp      | R                  | 5’-CAGGGCGGCCGCAATGACGTATTTC-TGAAGTT-3’ |
| gnd      | Cp      | R                  | 5’-CAGGGCGGCCGCAATGACGTATTTC-TGAAGTT-3’ |
| gnd      | Cp      | R                  | 5’-CAGGGCGGCCGCAATGACGTATTTC-TGAAGTT-3’ |
| nblA     | Pr      | F                  | 5’-GAGGGCGGCCGCAATGACGTATTTC-TGAAGTT-3’ |
| nblA     | Pr      | R                  | 5’-TTTACAATATACCCATTTTGG-3’ |
| nblA     | Pr      | R                  | 5’-TTTACAATATACCCATTTTGG-3’ |
| glnA     | Pr      | F                  | 5’-GAGGGCGGCCGCAATGACGTATTTC-TGAAGTT-3’ |
| glnA     | Pr      | R                  | 5’-TTTACAATATACCCATTTTGG-3’ |
| glnA     | Pr      | R                  | 5’-TTTACAATATACCCATTTTGG-3’ |
| glnA     | Pr      | R                  | 5’-TTTACAATATACCCATTTTGG-3’ |
| gnd      | Pr      | F                  | 5’-GAGGGCGGCCGCAATGACGTATTTC-TGAAGTT-3’ |
| gnd      | Pr      | F                  | 5’-GAGGGCGGCCGCAATGACGTATTTC-TGAAGTT-3’ |

Note: The sequences are given for PCR primers used in the experiment.

Additional information: To further analyze the interaction between PII and PamA, we performed Far Western blotting analyses. GST or GST-PamA-(475–680) protein was resolved in a 12% SDS-PAGE gel and blotted onto a membrane. To examine the effect of PII phosphorylation on the PII-PamA interaction, cell extracts were prepared from ammonia-grown or nitrogen-starved GT cells and incubated with the membrane, and bound PII was detected with PII-specific antiserum. Both nonphosphorylated (ammonia-grown) and fully phosphorylated (nitrogen-starved) PII could interact with GST-PamA-(475–680) but not with GST (Fig. 3A). This indicates that phosphorylation states do not affect the PII-PamA interaction. 2-OG was increased immediately after nitrogen starvation (increased ~2-fold, 15 min after nitrogen depletion), whereas phosphorylation of PII proteins only took place 1 h after nitrogen depletion (14). Thus, these results imply that PII and PamA proteins may be promptly dissociated under nitrogen starvation by 2-OG accumulation.

Additionally, we examined the effect of PamA truncation on the PII interaction (Fig. 3B). In brief, purified CBD-PII was electrophoresed and blotted onto a membrane. Cells lysates prepared from GT cells were incubated with this filter, and endogenous PamA, interacting with the filter bound PII, was detected with the PamA antiserum. The results indicate that phosphorylation states do not affect the PII-PamA interaction.
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FIGURE 7. Glucose sensitivity in the pamA mutant. A, GT and GN10 strains of Synechocystis sp. PCC 6803 were spotted onto BG-11 ammonium plates supplemented (or not) with 5 mM glucose. Each spot consisted of 1 μl of culture diluted to an A750 of 0.1, 0.05, or 0.01 as indicated. The BG-11 plates were photographed after 5 days of culture. B. GT and GN10 cells were cultivated in BG-11 ammonium liquid medium and supplemented with 10 mM glucose at 48 h after start of cultivation (indicated by the arrows). Cell growth and density were determined by measurement of A750 with a spectrophotometer (Beckman model DU640).

FIGURE 8. Immunoblot analysis with antiserum to PII. GT and GN10 cells were grown in the presence of ammonium and then transferred to a medium lacking combined nitrogen. Cells were collected at indicated times and disrupted by sonication. A, the amount of PII proteins in GT and GN10. Total protein (7 μg) was subjected to electrophoresis with a 15% SDS-PAGE gel and immunoblotting with antiserum to PII. B, phosphorylation states of PII in GT and GN10. Total protein (5 μg) was subjected to nondeaturing polyacrylamide gel electrophoresis and immunoblotting with antiserum to PII. The differently modified PII proteins were separated according to their increased negative charge. P0 indicates nonphosphorylated PII trimers, and P1, P2, and P3 represent PII trimers containing one, two, and three phosphorylated PII proteins, respectively.

showed that native PamA could also interact with PII, denying an artificial interaction by the PamA truncation.

Transcripts of Nitrogen-related Genes Were Decreased by the pamA Mutation—To analyze the functions of PamA, we constructed a pamA deletion mutant (GN10) of Synechocystis by transforming cells with a targeting plasmid in which a genomic fragment from the 5’ region of pamA to the 3’ end of the downstream transposase gene sll0986 was replaced by a kanamycin resistance cassette (Fig. 4A). This pamA deletion mutant grew equally well as the parental GT strain (data not shown). However, Northern blot analysis of the RNA expression of a set of nitrogen-related genes revealed that the transcripts of nbla, nrtABCD, and ureG were greatly reduced in GN10 compared with the GT strain (Fig. 4B). nbla (whose product is responsible for phycobilisome degradation) and the nrtABCD operon (which encodes the nitrate/nitrite transporter) are known to be regulated by NtcA (32–35). On the other hand, the transcripts of other NtcA-regulated genes such as glnA (which encodes glutamine synthetase type I), glnB, amt1 (which encodes the ammonium transporter), and glnN (which encodes glutamine synthetase type III) were less affected by the pamA mutation (Fig. 4C). These genes, except for ureG, are known to be controlled by NtcA (36). The ureG gene, which encodes a urease accessory protein, was shown to be regulated by NtcA in Prochlorococcus marinus PCC 9511 (37) and, therefore, could also be regulated by NtcA in Synechocystis sp. PCC 6803. These results indicate that only a set of NtcA-regulated genes was affected by PamA disruption, suggesting that genes included in NtcA regulons might be sub-grouped by differential controls. Thus, the pamA function is involved in the expression of a part of the NtcA-regulated genes.

The Expression of sigE and Sugar Catabolic Genes Was Decreased by the pamA Mutation—Because a group 2 σ factor sigE is regulated by NtcA and induced by nitrogen depletion (38), we examined the expression of the sigE gene in the pamA mutant. Northern analyses and immunoblotting revealed that the sigE transcript and the SigE protein levels were decreased by the pamA mutation (Fig. 5, A and B). Recently, we found that SigE activates transcription of sugar catabolic genes (39). Subsequently, we tested the transcript levels of gap1 (encoding glyceraldehyde-3-phosphate dehydrogenase), zwf (encoding glucose-6-phosphate dehydrogenase), and gnd (encoding 6-phosphogluconate dehydrogenase) and found that the mRNAs of gap1, zwf, and gnd were decreased by the pamA mutation (Fig. 5C). Consistent with the transcript levels, the enzyme activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were confirmed to be reduced by the pamA mutation (data not shown).

Complementation of the pamA Mutant—To genetically confirm that the observed characteristics of GN10 actually resulted from the pamA
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deficiency but not from a secondary mutation, we performed a complementation test with a plasmid containing the wild-type pamA gene, pVZ322:pamAC (Fig. 6A). After the introduction, the plasmid maintenance was confirmed by PCR (Fig. 6B) with pamA-specific primers (TABLE ONE). Northern analysis revealed that the wild-type pamA gene restored the transcript levels of gap1 (Fig. 6C) and other genes (data not shown), indicating the decreased mRNAs of nitrogen-related and sugar catabolic genes were caused by the pamA mutation.

The pamA Mutant Exhibited Glucose-sensitive Phenotype—In search of phenotypes conferred by the pamA deletion, we found that GN10 cells were not able to grow on BG-11 plates supplemented with 5 mM glucose (Fig. 7A), whereas the parental glucose-tolerant strain could grow in the presence of glucose at concentrations of up to at least 30 mM (data not shown). The glucose-sensitive phenotype in GN10 was also observed in liquid cultures with 10 mM glucose (Fig. 7B). Under the same photomixotrophic condition, the sigE mutant did not show comparable sensitivity to glucose (data not shown), indicating that the phenotype was not caused only by the decreased amount of SigE protein in GN10. Koksharova et al. (40) demonstrated that a Gap1-deficient mutant of Synechocystis was unable to grow in glucose-containing medium (40), suggesting that the reduced transcription of glucose catabolic genes, including gap1, in GN10 cells might be responsible for their observed glucose sensitivity. The transcript levels of gap1 in the pamA mutant and the sigE mutant were decreased up to 20–30 or 60–70% of the parental GT strain, respectively. Thus, the lesser transcript accumulation of gap1 in the pamA mutant than in the sigE mutant could be a reason for the differential glucose sensitivity. Consistently, when glucose concentration in the medium was increased to 20 mM glucose in BG-11 liquid medium, the sigE mutant also showed glucose sensitivity (data not shown). We also confirmed that the complementation by the wild-type pamA gene with pVZ322:pamAC restored the glucose sensitivity in GN10 (data not shown).

The Hypothetical Function of PamA—At present we have no reasonable explanation on the exact role of PamA in nitrogen-related transcriptional regulation. Immunoblot analysis with antiserum to PII revealed that the amount of PII proteins was decreased in GN10 (Fig. 6B), suggesting that this protein may be a reason for the differential glucose sensitivity. Consistently, when glucose concentration in the medium was increased to 20 mM glucose in BG-11 liquid medium, the sigE mutant also showed glucose sensitivity (data not shown). We also confirmed that the complementation by the wild-type pamA gene with pVZ322:pamAC restored the glucose sensitivity in GN10 (data not shown).

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Identification of PamA as a PII-binding Membrane Protein Important in Nitrogen-related and Sugar-catabolic Gene Expression in *Synechocystis* sp. PCC 6803

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