Role of the *Synechococcus* PCC 7942 nitrogen regulator protein PipX on NtcA controlled processes

Javier Espinosa¹, Karl Forchhammer², and Asunción Contreras¹*

¹ División de Genética, Universidad de Alicante, Apartado 99, E-03080 Alicante, Spain
² Institut für Mikrobiologie und Molekularbiologie, Universität Giessen, Heinrich-Buff-Ring 26-32, D 35392 Giessen, Germany

*Corresponding author. Mailing address: División de Genética, Facultad de Ciencias, Universidad de Alicante, Apartado 99, E-03080 Alicante, Spain. Phone: 34 96 590 3957. Fax: 34 96 590 9569. E-mail: contrera@ua.es
ABSTRACT

The *Synechococcus* sp. PCC 7942 nitrogen regulator PipX interacts in a 2-oxoglutarate dependent manner with the global nitrogen transcription factor NtcA and the signal transduction protein P II. *In vivo*, PipX is involved in the NtcA-dependent induction of *glnB* and *glnN* genes. To further investigate the extent to which PipX is involved in global nitrogen control, the effect of *pipX* inactivation on various nitrogen regulated processes was determined. The PipX deficient mutant was able to use nitrate as a nitrogen source and to efficiently inhibit the nitrate transport upon ammonium addition but showed reduced nitrate and nitrite reductase activities and a delay in the induction of nitrate utilization after transfer of cultures from ammonium to nitrate containing media. In contrast to the wild-type, glutamine synthetase activity was not upregulated upon depletion of combined-nitrogen from cultures of the mutant strain. Inactivation of *pipX* impaired induction of *nblA* and delayed phycobilisome degradation, but did not affect recovery of nitrogen-deprived cultures. Taken together, the results obtained in this work indicate that PipX interacts with NtcA to facilitate efficient acclimation of cyanobacteria to conditions of nitrogen limitation.
INTRODUCTION

Cyanobacteria are phototrophic organisms that perform oxygenic photosynthesis. Autotrophic growth requires the constant assimilation of ammonium via the glutamine synthetase-glutamate synthase (GS-GOGAT) cycle (Muro-Pastor et al., 2005), resulting in consumption of the carbon-skeleton of 2-oxoglutarate to yield glutamate. GS, which catalyses the incorporation of ammonium into glutamate to yield glutamine is the key enzyme of nitrogen assimilation. Two types of GS are produced by the non-diazotrophic cyanobacterium *Synechococcus sp.* PCC 7942 (hereafter called *Synechococcus*): GSI, a typical eubacterial GS encoded by *glnA*, and GSIII, encoded by *glnN*. The GSIII class is strongly induced in nitrogen-depleted cultures and appears to play a role in acclimation to conditions of nitrogen starvation (Sauer et al., 2000). Due to the lack of 2-oxoglutarate dehydrogenase in cyanobacteria, synthesis of 2-oxoglutarate represents the final step in the oxidative branch of the TCA cycle and directly links 2-oxoglutarate levels to nitrogen assimilation (Herrero et al., 2001). Therefore, the cellular 2-oxoglutarate concentration is an excellent indicator of the cell carbon to nitrogen balance. 2-oxoglutarate modulates the activity and/or binding properties of three key cyanobacterial nitrogen regulators: the signal transduction protein PII, the transcriptional activator NtcA, and the regulatory factor PipX (Forchhammer, 2004; Espinosa et al., 2006).

In cyanobacteria, multiple metabolic and developmental processes are induced by nitrogen starvation. NtcA, the global regulator for nitrogen control, activates genes involved in nitrogen assimilation, heterocyst differentiation and acclimation to nitrogen starvation (Herrero et al., 2001; Luque et al., 2001; Sauer et al., 2000). NtcA belongs to the CAP/CRP (the catabolite activator protein or cyclic AMP receptor protein) family of transcriptional activators. 2-oxoglutarate stimulates complex formation between PipX...
and NtcA (Espinosa et al., 2006), binding of NtcA to target sites (Vazquez-Bermudez et al., 2002) and transcription activation in vitro (Tanigawa et al., 2002). NtcA plays a key role in cyanobacterial nitrogen assimilation, being required for the expression of multiple genes repressed by ammonium, the preferred nitrogen source (Herrero et al., 2001). Genes or operons directly regulated by NtcA in Synechococcus include the nirA operon, encoding the nirA-nrtABCD-narB genes for nitrate assimilation, the nirB operon, encoding genes for maximum nitrate assimilation, the glnA and glnN genes for GSI and GSII, respectively, the glnB for P II, the nblA gene required for phycobilisome degradation (Luque et al., 2001) and the ntcA gene itself. In agreement with this, Synechococcus NtcA deficient mutants are highly pleiotropic, being unable to assimilate nitrate, upregulate GS (Vega-Palas et al., 1990), or acclimate appropriately to nitrogen starvation (Sauer et al., 1999).

The P II signal transduction protein is one of the most conserved and widespread signal transduction proteins in nature and plays key roles in nitrogen assimilatory processes (Arcondeguy et al., 2001; Ninfa & Jiang, 2005). Signal transduction by P II (glnB product) in cyanobacteria displays unique features both with respect to covalent modification as well as to downstream signalling (Forchhammer, 2004). Physiological studies showed that P II mediates the short term ammonium inhibition of nitrate transport (Lee et al., 2000) and controls nitrate uptake in response to light availability (Kloft & Forchhammer, 2005). Furthermore, nitrate reductase in Synechococcus may be directly regulated by P II (Takatani et al., 2006). However, in these cases, no physical interaction with P II was reported. Using yeast two-hybrid approaches, three cyanobacterial P II receptors could recently be identified: The enzyme N-acetyl-glutamate kinase (NAGK) (Burillo et al., 2004; Heinrich et al., 2004) and the regulatory factor PipX (Burillo et al., 2004; Espinosa et al., 2006) in Synechococcus, and the non conserved membrane
protein PamA in *Synechocystis* sp. PCC 6803 (Osanai *et al*., 2005). The physiological role of the PII interaction with these protein receptors is only understood in the case of NAGK. The PII-NAGK regulatory interaction has been conserved across domains of life during the evolution of oxygenic photosynthetic organisms (Burillo *et al*., 2004; Chen *et al*., 2006; Sugiyama *et al*., 2004). PII stimulates NAGK activity and relieves arginine inhibition to different extents in *Synechococcus* and *Arabidopsis thaliana* (Chen *et al*., 2006; Heinrich *et al*., 2004; Maheswaran *et al*., 2004).

Binding of PipX to PII and NtcA is inversely affected by 2-oxoglutarate (Espinosa *et al*., 2006). Complex formation between PipX and PII was impaired by 2-oxoglutarate in the presence of ATP, while the binding of PipX to NtcA could only be observed in the presence of 2-oxoglutarate. *In vivo*, PipX activated NtcA-dependent promoters glnB and glnN under conditions of nitrogen deficiency, corresponding to high intracellular levels of 2-oxoglutarate, supporting a role for PipX in the activation of NtcA-dependent promoters under nitrogen starvation. In order to investigate the involvement of PipX on nitrogen assimilation and acclimation to nitrogen deficiency, we now analyze the impact of the genetic inactivation of *pipX* on previously characterized NtcA-controlled processes.
METHODS

Molecular genetic techniques and growth conditions

The strains and plasmids used in this work are listed in Table 1. Cloning procedures were carried out with *E. coli* DH5α, using standard techniques. *Synechococcus* strains were grown photoautotrophically at 30°C while shaking under constant illumination (75 µE m⁻² s⁻¹) provided by cool white fluorescent lights in BG11 medium (nitrate-containing), BG11₀ (no added nitrogen) or BG11-NH₄ (BG11₀ supplemented with 10 mM NH₄Cl, 10 mM Hepes/NaOH pH 7.8). For growth on plates, the medium was solidified by addition of 1% (w/v) agar. Plates were incubated at 30°C under constant illumination. For initiation of nitrogen deprivation, mid exponential cultures (OD₇₅₀nm of 0.5) were harvested by centrifugation, washed twice with BG11₀ and finally resuspended in BG11₀. Whenever appropriate, cultures contained kanamycin (10 µg ml⁻¹) or chloramphenicol (5 µg ml⁻¹).

Construction of plasmids and *luxAB* reporter strains

To construct plasmid pUAGC103, a 206 bp fragment corresponding to the upstream region of the *nblA* gene was PCR amplified using primers 5′-GGCGCTGCCTGGGAAAGTCACGCC-3′ and 5′-GGAGCCTCCGGCACTGCAGATG-3′ and cloned into the neutral site II targeting vector pAM1580. Plasmids pRK2013 and pRL443 were used, respectively, as a helper and conjugative plasmid to transfer pUAGC103 into *Synechococcus* strains (Elhai *et al.*, 1997). Transformants were selected on chloramphenicol containing BG11 or BG11-NH₄ plates.

Enzymatic activities

Nitrate uptake activity was assayed by measuring nitrate depletion from the external medium as described previously (Lee *et al.*, 1998). BG11 cultures grown to mid
exponential phase were used to determine nitrate and nitrite reductase activities at 30 °C using dithionite-reduced methyl viologen as the electron donor. Nitrate reductase activity (Herrero et al., 1985) was measured in permeabilized cells by including mixed alkyltrimethylammonium bromide (MTA) in the reaction mixture at a final concentration of 50 µg·ml⁻¹. Nitrite reductase activity (Herrero & Guerrero, 1986) was also assayed in cells made permeable with MTA (250 µg·ml⁻¹). Production (nitrate reductase) or disappearance (nitrite reductase) of nitrite during the reactions was measured as described previously (Snell & Snell, 1949). One unit of enzymatic activity corresponded, respectively, to 1 µmol of nitrite formed or disappeared per min. Glutamine synthetase activity (transferase assay) from mid-exponential cultures was measured in permeabilized cells as described (Bender et al., 1977).

**Determination of chlorophyll a**

Chlorophyll a concentration was determined from methanolic extracts as described (Mackinney, 1941).

**Determination of luciferase activity**

To determine bioluminescence, 1 ml of cultures was adjusted with fresh medium to an OD₇₅₀nm of 0.5, supplemented with decanal to a final concentration of 0.25 mM from a 50 mM stock solution made up in 10% dimethyl sulfoxide. Light emission was recorded in a Berthold LB9509 luminometer. Bioluminescence was recorded every 20 seconds for 10 minutes. Light emission increased to a maximum and then declined. Maximum luminescence at the peak, presented as RLU (Relative light Units) by the instrument, is the value used at each selected time point.

**Determination of pigments contents spectrophotometrically**

Wild type and PipX⁻ strains were grown in BG11 or BG11-NH₄ until they reached mid exponential phase (OD₇₅₀nm 0.4-0.5) and then cultures were centrifuged, washed and
finally resuspended in BG110. A whole-cell absorbance spectrum was carried out in order to estimate pigment contents. 1ml of cultures was taken at indicated times, diluted with fresh medium to an optical density at 750nm of 0.5 and absorbance spectra (550-750nm) was recorded on a UV/Visible Ultrospec 3100 pro (Amersham). Pigment content was calculated based on absorbance maxima at 631nm for phycocyanin and 684nm for chlorophyll $a$. 
RESULTS

Nitrate assimilation in the PipX⁻ strain

The expression of genes required for nitrate assimilation in *Synechococcus* depends on NtcA. Therefore, NtcA-deficient strains do not grow on nitrate, have undetectable nitrite reductase activity and low levels of nitrate reductase (Vega-Palas *et al.*, 1990). In addition to the NtcA-dependent induction in response to the absence of ammonium, the *nirA* operon is also subjected to activation by the nitrite-dependent activator NtcB (Aichi *et al.*, 2004; Maeda *et al.*, 1998). The fact that the PipX⁻ strain grows well in nitrate-containing medium, where it was originally selected, appeared at odds with our previous suggestions of PipX cooperating with NtcA-dependent activation of target promoters (Espinosa *et al.*, 2006). To investigate the involvement of PipX in the regulation of the nitrate assimilation genes, we analyzed the functions encoded by the *nirA* operon in a PipX-deficient mutant.

Nitrate and nitrite activities were assayed in cells from the wild type and PipX⁻ strains adapted to nitrate. As shown in Fig. 1, nitrate and nitrite reductase activities were significantly reduced in the PipX⁻ mutant, thus supporting the implication of PipX in positive regulation of the *narB* and *nirA* genes. Nitrate consumption by PipX⁻ strains grown in nitrate-containing media was significantly slower than nitrate consumption by the wild-type strain (compare Fig. 2A and B), thus indicating that PipX deficiency has a negative effect on nitrate assimilation. As it is the case with wild-type cells, addition of ammonium to PipX⁻ cells caused an immediate inhibition of nitrate utilization (Fig. 2 A and B) indicating that posttranslational regulation of nitrate assimilation by PII does not require PipX.

Since ammonium-repressed cultures of *Synechococcus* can not utilize nitrate, the rate of nitrate assimilation after transfer of ammonium-grown cultures to medium
containing nitrate was compared between wild type and PipX\(^{-}\) cultures. Nitrate consumption was determined three hours after shifting ammonium-repressed cultures to nitrate-containing medium. The NtcA\(^{-}\) strain, which is unable to utilize nitrate, was included as a negative control. As shown in Fig. 2C, the PipX\(^{-}\) culture removed nitrate from the medium at a rate significantly lower than the wild type strain, thus strongly suggesting that PipX has a role on the induction of the \textit{nirA} operon.

Taken together, the results indicate that the \textit{Synechococcus} PipX\(^{-}\) mutant assimilates nitrate with less efficiency than its wild type counterpart. The defect is more pronounced in cultures previously grown with ammonium, suggesting that PipX is required for rapid acclimation to ammonium withdrawal. On the other hand, the finding that the PipX\(^{-}\) mutant retains the ability to tune down nitrate uptake efficiently when the nitrate-adapted cultures are suddenly faced with ammonium, suggests that PipX is not required for the regulation of P\(_{II}\) functions under conditions of nitrogen sufficiency (low levels of 2-oxoglutarate).

**Glutamine synthetase activity is positively regulated by PipX**

Nitrogen assimilation by \textit{Synechococcus} cells requires glutamine synthetase activity, provided mainly by GSI, and under nitrogen deficiency conditions, also by GSIII. To determine the impact of \textit{pipX} inactivation on global glutamine synthetase activity, we compared the ability of wild type and PipX deficient strains of \textit{Synechococcus} to induce glutamine synthetase activity upon transfer of cultures from ammonium or nitrate supplemented media to media lacking a combined nitrogen source. Comparison of wild type and PipX\(^{-}\) strains indicated that PipX is required for appropriated induction of glutamine synthetase activity (Fig. 3). Glutamine synthetase levels were significantly lower in the mutant strain, an effect observed when the nitrogen source of cultures was either nitrate (compare time 0 in Fig. 3A) or ammonium.
(compare time 0 in Fig. 3B). In addition, no induction of glutamine synthetase activity was observed in the PipX deficient strain when cultures were deprived of nitrogen, thus indicating an important deficiency of glutamine synthetase activity in the PipX\textsuperscript{−} mutant. The basal levels of glutamine synthetase activity found in the PipX\textsuperscript{−} strain were indistinguishable from those of the NtcA\textsuperscript{−} strain grown with ammonium or in cells starved for nitrogen up to 2 days (data not shown).

Therefore, PipX exerts a positive role in the nitrogen regulation of the glutamine synthetase activity in \textit{Synechococcus}. As it is the case with NtcA, PipX is required for both, basal levels of glutamine synthetase activity (as seen in ammonium-grown cultures) and for appropriated upregulation of glutamine synthetase under nitrogen limiting conditions.

\textbf{PipX is involved in induction of chlorosis but not in the recovery of cultures from nitrogen starvation}

When \textit{Synechococcus} cultures are subjected to nutrient limitation and other stress conditions, they modify the composition of the photosynthetic machinery by degrading their light-harvesting antennae, the phycobilisomes (Schwarz & Forchhammer, 2005). The loss of phycobilisomes and reduction of the chlorophyll \textit{a} content are responsible for the yellow appearance of the chlorotic cultures. This acclimation process, known as chlorosis or bleaching, requires strong expression of the \textit{nblA} gene, which is subject to positive control by NtcA (Luque \textit{et al.}, 2001) and NblR, a response regulator involved in general acclimation (Schwarz & Grossman, 1998). NblR-deficiency results in a strong \textit{non bleaching} phenotype, but the NtcA deficient mutant is still able to enter chlorosis although it shows a delay in phycobilisome degradation and a faster reduction of the chlorophyll \textit{a} content (Sauer \textit{et al.}, 1999).
As shown in Fig. 4A, nitrogen deprived cultures of the PipX null mutant showed the same phenotype than the NtcA\textsuperscript{−} strain, a weak and transient \textit{non-bleaching} appearance. Pigment analysis revealed the same pattern of pigment degradation that have been reported for the NtcA strain under nitrogen starvation, a delay in phycocyanin degradation and a faster loss of chlorophyll a content (Fig. 4B-4D). As expected for a protein specifically involved in 2-oxoglutarate signaling, induction of chlorosis by sulfur starvation was not affected in the PipX\textsuperscript{−} strain (data not shown).

NtcA is required for cell survival of chlorotic cultures, since NtcA\textsuperscript{−} cells subjected to nitrogen starvation rapidly loose the ability to reinitiate growth on nitrogen containing media (Sauer \textit{et al.}, 1999). To determine the involvement of PipX in cell survival after chlorosis, wild type, NtcA\textsuperscript{−} and PipX\textsuperscript{−}mutant strains were tested for their ability to survive up to 9 days in nitrogen-depleted media. In contrast with the NtcA\textsuperscript{−} strain, the ability of the PipX\textsuperscript{−} strain to resume growth was very similar to that of the wild type strain (Fig. 5), indicating that PipX is not required for the NtcA-dependent survival of nitrogen starved cultures.

**PipX activates the \textit{nblA} gene under nitrogen deficiency**

To confirm that the delayed phycocyanin degradation in cultures of the PipX\textsuperscript{−} strain during nitrogen induced chlorosis was related to defects in NtcA-dependent activation of the \textit{nblA} gene, promoter fusions to \textit{luxAB} reporter genes were used to analyze the consequences of \textit{pipX} inactivation on the nitrogen dependent induction of the \textit{nblA} gene. The P\textsubscript{\textit{nblA}}::\textit{luxAB} construct included the previously characterized regulatory region containing five transcription start sites, three NtcA binding sites, and putative NbIR binding sites (Luque \textit{et al.}, 2001). It also includes a recently reported putative light-responsive element (HLR1) involved in negative regulation (Kappell \textit{et al.}, 2006).
Reporter expression was determined by bioluminiscence measurements from cultures of wild type, NtcA\(^{-}\) and PipX\(^{-}\) mutant derivatives grown with ammonium and shifted to nitrogen depleted medium. As shown in Fig. 6, the nitrogen starvation response was impaired in both the NtcA\(^{-}\) and PipX\(^{-}\) mutants but the extent of the impairment was different. Consistent with previous results using \textit{luxAB} fusions to other NtcA dependent promoters (Espinosa \textit{et al.}, 2006), the PipX contribution to \textit{nblA} upregulation was very significant, but smaller than that of NtcA, indicating that PipX is also involved in the NtcA-dependent transcriptional induction of the \textit{nblA} gene.
DISCUSSION

The results presented here provide insight into the role of PipX in nitrogen control. Synechococcus PipX- cells showed reduced activity of nitrogen assimilation enzymes (glutamine synthetase, nitrate reductase and nitrite reductase), retarded induction and slower rate of nitrate consumption, and, when subjected to nitrogen starvation, retarded phycobilisome degradation and a faster reduction of the chlorophyll content. Therefore, the PipX-deficient mutant shows a pleiotropic phenotype reminiscent of that of the NtcA- strains. For some traits the PipX- and NtcA- phenotypes were very similar, while for others the PipX- phenotype was less severe than the NtcA-.

Assays for glutamine synthetase activity (Fig. 3 and data not shown) and induction of chlorosis (Fig. 4A and data not shown) gave similar results with the two mutant strains. Nitrate assimilation, an ability lost in the NtcA- strain, was impaired but still operative in the PipX deficient mutant (Fig. 2). Given that, at promoters strictly dependent of NtcA for activation, PipX plays a positive but non essential role for transcription (Espinosa et al., 2006), it is not surprising that PipX- mutants display a less severe or leaky NtcA- phenotype. The auxiliary role of PipX at NtcA dependent promoters can also be inferred from induction experiments with the P_{nblA}::luxAB reporter fusion (Fig. 6). The nitrogen-specific role of PipX in chlorosis strongly suggests that PipX participates in the NtcA-dependent activation of the nblA gene. The finding that PipX is not required for survival of cultures after nitrogen starvation (Fig. 5) also supports the view that PipX is involved in the early induction, but not in the long-time maintenance, of NtcA activity.

Taken together, the results presented here strongly suggest that, in addition to the previously analyzed glnB and glnN genes (Espinosa et al., 2006) and the nblA promoter studied here (Fig. 6), operons or genes for nitrate assimilation (nirA and...
probably \textit{nirB}), glutamine synthesis (\textit{glnA}) and additional genes for pigment regulation
are also under PipX control. Thus, the relatively small but representative sample of
\textit{NtcA}-dependent genes analyzed so far at the level of transcriptional induction and/or
protein activity, demonstrate the importance of PipX in global nitrogen regulation. The
mechanism and molecular details involved in transcriptional activation by PipX at
\textit{NtcA}-dependent promoters is presently being addressed.

\textit{In vivo}, PipX-\textit{NtcA} and PipX-\textit{P}$_{\text{II}}$ complexes are likely to form, respectively,
under high and under low intracellular levels of 2-oxoglutarate. Consistent with this, the
genetic analyses performed so far show the implication of PipX in \textit{NtcA} activation
when the intracellular 2-oxoglutarate levels rise as a consequence of combined-nitrogen
depletion from cultures. On the other hand, complex formation between PipX and the
\textit{P}$_{\text{II}}$ protein should be expected when cultures are faced with ammonium. Since the \textit{P}$_{\text{II}}$-
dependent inhibition of the nitrate uptake exerted by ammonium remained functional in
the PipX- strain (Fig. 2B), the PipX protein does not play a role in the \textit{P}$_{\text{II}}$-mediated
regulation of NRT. Considering that \textit{Synechococcus} \textit{P}$_{\text{II}}$ proteins are very abundant, able
to interact with different partners, and sense and adopt different conformations and
modification status according to the nitrogen/carbon ratio, it seems more likely that
PipX-\textit{P}$_{\text{II}}$ complexes serve to modulate PipX availability and/or activity. In other words,
although the physiological significance of the PipX-\textit{P}$_{\text{II}}$ complexes remain to be
established, the experimental evidences gathered so far favor the idea of PipX being a
\textit{P}$_{\text{II}}$ target rather than a \textit{P}$_{\text{II}}$ regulator.
ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 1
Nitrate and nitrite reductase activities from *Synechococcus* cells. Mean values and standard deviations from five independent assays are represented. Nitrate reductase (grey bars), nitrite reductase (white bars). Wild type (WT), PipX null mutant (PipX-).

Figure 2
Nitrate uptake from *Synechococcus* strains. Nitrate taken up (µmoles.mg⁻¹ Chl a) is plotted as a function of time. Uptake assays were performed on nitrate grown cells from (A) wild-type or (B) PipX- cultures incubated for 0–30 min in the presence of nitrate, with (open symbols) or without (closed symbols) addition of ammonium. Mean values and standard deviations from four independent assays are represented. (C) Induction of nitrate uptake from ammonium repressed cultures. Nitrate taken up was measured during 60 minutes on previously ammonium-grown cells, which were incubated in ammonium-free, nitrate supplemented BG11 medium for 3 hours. Mean values and standard deviations from 4 independent assays are represented. Wild type (WT), PipX null mutant (PipX-), NtcA null mutant (NtcA-).

Figure 3
Glutamine synthetase activity from *Synechococcus* strains. Cultures were grown in BG11 (A) and BG11-NH₄ (B), transferred to BG11₀ and assayed immediately (time 0) and at 16 and 24 hours. Glutamine synthetase (transferase) activity is given in Units (µmoles per minute) per mg of chlorophyll a. Mean values and the corresponding standard deviations from three independent experiments are reported. Wild type, (grey bars) PipX- (white bars)
Figure 4

Pigment degradation by *Synechococcus* strains. (A) Cultures of wild-type and mutant derivatives grown in BG11-NH$_4$ (N+) or incubated in BG11$_0$ (–N) for 48 and 96 h. (B) Absorbance spectra from wild type (orange) and PipX$^-$ (blue) strains in BG11-NH$_4$ (+) or incubated in BG11$_0$ (-) for 2, 4 and 10 days. The absorbance peak of phycocyanin (PC), and chlorophyll *a* (Chl) are indicated. Groups of spectra were shifted along the *y* axis for clarity. (C) Relative amount of phycocyanin as a function of time. (D) Relative amount of Chlorophyll *a* as a function of time. Wild type (WT); PipX null mutant (PipX$^-$).

Figure 5

Survival of *Synechococcus* strains after nitrogen starvation. Drops of cultures incubated for 4 to 9 days on BG11$_0$ medium were spotted onto BG11-NH$_4$ plates and incubated for 2 weeks. Wild type (WT), PipX null mutant (PipX$^-$), NtcA null mutant (NtcA$^-$).

Figure 6

Time course of *P*$_{nblA}$::*luxAB* induction in *Synechococcus* strains. Cells were grown in the presence of ammonia and when they reached mid exponential phase, they were shifted to combined-nitrogen free medium. After the initiation of nitrogen deprivation at time point 0, bioluminescence from the reporter strains was recorded. A representative time course experiment is shown from 3 independent experiments that yielded similar results. Wild type (WT), PipX null mutant (PipX$^-$), NtcA null mutant (NtcA$^-$).
BIBLIOGRAPHY


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<td><em>E. coli</em> DH5α</td>
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<td>pAM1580 derivative with ( P_{\text{nblC}}::\text{luxAB} ) fusion</td>
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Fig. 1

WT PipX

NarB, NirA

Fig. 2

A

WT

Nitrate taken up

0 2 4 6

0 5 10 15 20 25 30

Nitrate taken up

0 5 10 15 20 25 30

B

PipX

Nitrate taken up

0 2 4 6

0 1 0 2 0 3 0 4 0 5 0 6

C

NO₃⁻+NH₄⁺

NO₃⁻

WT

PipX

NtcA⁻
Fig. 5

WT

PipX^-

NtcA^-

Fig. 6

$\text{NH}_4^+ \rightarrow \text{N}$

$10^3 \text{RLU}$

Time (hours)