**Microbiology**
0-mic.sgmjournals.org.fama.us.es

**Published online ahead of print January 24, 2014, doi:**
**10.1099/mic.0.076042-0**
Microbiology **April 2014** vol. 160 no. Pt 4 **789-794**

# NtcA is responsible for accumulation of the small isoform of ferredoxin:NADP oxidoreductase

**Armin Omairi-Nasser**[1],†‡, **Carla V. Galmozzi**[2],†§, **Amel Latifi**[3], **M. Isabel Muro-Pastor**[2] and **Ghada Ajlani**[1]

Author Affiliations

Author Notes

**Correspondence**
M. Isabel Muro-Pastor imuro@ibvf.csic.es Ghada Ajlani gajlani@cea.fr

Received 13 December 2013.
Accepted 22 January 2014.

## Abstract

In several cyanobacteria, *petH*, the gene encoding ferredoxin:NADP oxidoreductase (FNR), is transcribed from at least two promoters depending on growth conditions. Two transcripts (short and long) are translated from two different translation initiation sites, resulting in two isoforms (large and small, respectively). Here, we show that in *Synechocystis* PCC6803 the global transcriptional regulator NtcA activates transcription from the distal *petH* promoter. Modification of the NtcA-binding site prevents NtcA binding to the promoter *in vitro* and abolishes accumulation of the small isoform of FNR *in vivo*. We also demonstrate that a similar *petH* transcription and translation regime occurs in other cyanobacteria. The conditions under which this system operates provide hints for the function of each FNR isoform.

† These authors contributed equally to this work.

**Edited by:** C.-C. Zhang

## Introduction

The gene *petH* encodes ferredoxin:NADP oxidoreductase (FNR), an enzyme that is involved in the last step of oxygenic photosynthesis, in cyanobacteria as well as in chloroplasts. FNR provides NADPH for anabolic reactions and is also implicated in the oxidation of NADPH, produced by catabolism or accumulated due to an imbalance in photosynthetic reactions. Most phycobilisome-containing cyanobacteria possess a large FNR isoform ($FNR_L$) that contains a phycobilisome-linker domain. Fewer strains accumulate a small isoform ($FNR_S$), lacking the linker domain, in addition to $FNR_L$ or as the only FNR form (Thomas *et al.*, 2006).

We showed recently that in *Synechocystis* sp. PCC6803 (hereafter *Synechocystis*), following nitrogen starvation, *petH* produces a transcript bearing a 5′-untranslated region that is longer than the one transcribed under standard conditions. The longer transcript folds into a secondary structure that inhibits $FNR_L$ translation initiation and promotes that of $FNR_S$ (Omairi-Nasser *et al.*, 2011). Although FNR isoform accumulation was not examined in the cyanobacterium *Anabaena* sp. strain PCC7120 (hereafter *Anabaena*), its transcript organization is similar to that of *Synechocystis*. Two transcripts were found for the *Anabaena petH*; the shorter is constitutive, whilst the longer is NtcA-dependent and mainly located in heterocysts (Valladares *et al.*, 1999).

NtcA is a global transcriptional regulator that belongs to the cAMP receptor protein (Crp)/Fnr bacterial superfamily, and regulates nitrogen and carbon assimilation genes in cyanobacteria in response to 2-oxoglutarate. 2-Oxoglutarate is an intermediate of the TCA cycle that provides the carbon skeleton for nitrogen incorporation into amino acids. In cyanobacteria, 2-oxoglutarate also functions as a regulatory effector for NtcA and PII, whose activities alter gene expression and metabolism (Muro-Pastor *et al.*, 2001; Körner *et al.*, 2003; Osanai *et al.*, 2006; Luque & Forchhammer, 2008). In many cases, NtcA-activated promoters are similar to class II Crp-dependent promoters, in which the transcription factor binds to a consensus sequence centred at ~40 bases upstream from the transcription start point (Luque & Forchhammer, 2008). A consensus sequence for NtcA binding has been defined as GTAN8TAC (Herrero *et al.*, 2001; Mitschke *et al.*, 2011b). The recently solved

NtcA structure shows that NtcA is a dimeric protein with a very similar overall structure to that of Crp (Llácer *et al.*, 2010; Zhao *et al.*, 2010).

Putative NtcA-binding sites are present upstream from the promoter that produces the longer *petH* transcript in *Synechocystis*. We identified the actual binding site, centred at −41.5 bases from the distal *petH* transcription start point, and showed that NtcA is required for $FNR_S$ synthesis in *Synechocystis*. Moreover, the *petH* transcription and translation mechanisms, leading to the accumulation of two FNR isoforms, are conserved in *Anabaena* and in *Synechococcus* sp. PCC7002.

An NtcA-binding site was identified upstream from the transcription start point producing a longer *petH* transcript in *Anabaena* (Valladares *et al.*, 1999). Here, we show that NtcA binding to this distal promoter is responsible for $FNR_S$ translation in heterocysts.

## Methods

### *Strains and growth conditions.*

WT and mutants of *Synechocystis* were grown photoautotrophically, at 33 °C, in a $CO_2$-enriched atmosphere and under continuous light (50 μE $m^{-2}$ $s^{-1}$), which are standard growth conditions in our laboratory. The medium composition is described in Ughy & Ajlani (2004). For nitrogen starvation, cells were harvested by centrifugation and resuspended in a medium where NaCl replaced $NaNO_3$. When appropriate, media were supplemented with 5 μg streptomycin $ml^{-1}$, 50 μg spectinomycin $ml^{-1}$ or 50 μg kanamycin $ml^{-1}$. *Synechococcus* sp. PCC7002 was grown under the same conditions used for *Synechocystis* except that A+ medium was used (Stevens & Porter, 1980). *Anabaena* sp. PCC7120 WT and CSE2 strains were grown photoautotrophically, at 30 °C, in $BG11_0C$, which is BG11 medium without $NaNO_3$ and with 10 mM $NaHCO_3$, supplemented with 6 mM $NH_4Cl$ plus 12 mM TES (pH 7.5) and bubbled with 1% $CO_2$ in air until the exponential phase. Then the ammonium was eliminated and the cells incubated for the time indicated in $BG11_0C$ (nitrogen free), with 1% $CO_2$ in air. In the case of the CSE2 strain, the medium was supplemented with 2 μg streptomycin $ml^{-1}$ and 2 μg spectinomycin $ml^{-1}$.

### *Mutagenesis and plasmid construction.*

Mutagenic PCRs were performed on a plasmid carrying a 232 bp *Spe*I/*Sna*BI fragment, containing the *petH* 5′-non-coding region from *Synechocystis*, using mutagenic primer pairs 5′-GGTAAATCTAGACATGGGTTAC-3′/5′-GTAACCCATGTCTAGATTTACC-3′ for the X mutation and 5′-CGGTTATAAGATACATGGGTTAC-3′/5′-GTAACCCATGTATCTTATAACCG-3′ for the P mutation. The resulting plasmids were sequenced to verify the fidelity of the PCR amplification. To create the cargo plasmids, the modified *Spe*I/*Sna*BI fragments were used to replace the corresponding WT fragment in pBX, a plasmid carrying a 900 bp fragment of *petH* with the Ω cassette inserted 70 bp upstream from the putative NtcA-binding site – an insertion shown to be neutral to *petH* expression (Omairi-Nasser *et al.*, 2011). Cargo plasmids were introduced into WT by genetic transformation and antibiotics were used for the selection of the desired mutation. Complete segregation of the mutant alleles was confirmed by PCR, and in some cases the PCR products were subjected to restriction analysis and sequencing to ascertain the identity of the amplified fragments.

### *Recombinant NtcA expression, purification and gel retardation assays.*

An *Nde*I/*Xho*I fragment encompassing the entire *Synechocystis ntcA* gene was synthesized by PCR from genomic DNA using the oligonucleotides 5′-CTAGCATATGGATCAGTCCCTAACCC-3′/5′-GCTACTCGAGGGTAAACTGTTGACTGAG-3′. This fragment was cloned into the pET24a(+) plasmid (Novagen) to generate pSNtcA. Exponentially growing *Escherichia coli* BL21 cells transformed with pSNtcA were treated with 0.5 mM IPTG for 4 h. The C-terminal His-tagged version of *Synechocystis* NtcA was purified by nickel-affinity chromatography using His-Bind matrix (Novagen) following the manufacturer's instructions. For further purification, the sample was subjected to gel filtration chromatography using a HiLoad 16/60 Superdex 75 column (GE Healthcare) running on an Akta FPLC system.

*Synechocystis* NtcA-$His_6$, expressed and purified as described above, was used in gel retardation assays. The $P_{petH}$ promoter probes were obtained by *Spe*I/*Sna*BI digestion of plasmids carrying WT or modified versions of the NtcA-binding site. DNA fragments were end-labelled with [α-$^{32}$P]dCTP using Sequenase version 2.0 enzyme. The binding reactions and electrophoresis were carried out as described previously (Muro-Pastor *et al.*, 1996).

### *Electrophoresis and immunoblotting of cell extracts.*

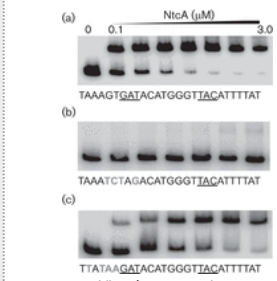
The experiments were performed as in our previous work (Omairi-Nasser *et al.*, 2011). For *Synechococcus* PCC7002 and *Anabaena* PCC7120 FNR

immunodetection, the amount of total-cell extracts loaded per well was doubled compared with *Synechocystis* (evaluated by chlorophyll concentration to 1 μg) and the antibody (raised against the *Synechocystis* FNR) was two times more concentrated (1/5000) than it was for *Synechocystis* (1/10000). *Anabaena* heterocysts were separated from vegetative cells according to Golden *et al.* (1985).

## Results

### NtcA binding to WT and modified versions of the *Synechocystis petH* promoter

To test whether NtcA binds to the *Synechocystis petH* promoter, electrophoretic mobility shift assays (EMSAs) using purified *Synechocystis* NtcA protein were performed. The protein was expressed in *E. coli* and purified as a His-tagged version. Binding assays were performed using an *Spe*I/*Sna*BI DNA fragment, which spans positions −85 to +144 with respect to the distal *petH* transcription start point. It has been claimed that >8 nt might separate the NtcA-binding triplets (Jiang *et al.*, 2000), so two putative binding sites ($GATN_8TAC$ and $GTGN_{10}TAC$), centred at −41.5 and −42.5 upstream from the distal *petH* transcription start point, were considered. We tested fragments containing modified versions of both putative sites; X, creating an *Xba*I restriction site, and P, creating a *Psi*I site where only the putative site $N_{10}$ was modified (Fig. 1).



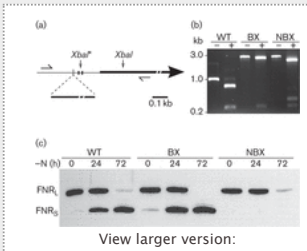

View larger version:
»In this page »In a new window
»Download as PowerPoint Slide

**Fig. 1.** NtcA binds to the *Synechocystis petH* promoter *in vitro*. EMSAs were performed with a 232 bp DNA fragment, encompassing the distal and the proximal *petH* promoters. The (a) WT fragment or mutant fragments, (b) X and (c) P, carrying changes to the grey bases, were used for binding reactions. Sequences of the putative NtcA-binding site, with the consensus triplets underlined when present, are shown below each EMSA. DNA probes (0.86 nM) were incubated in the presence of purified NtcA protein (0.1–3.0 μM).

When purified NtcA was incubated with the WT-labelled probe, an NtcA-DNA complex was clearly detected (Fig. 1a); however, when the X probe was used, no NtcA-DNA complex was detected (Fig. 1b). The mutations present in the P probe did not prevent NtcA binding *in vitro* (Fig. 1c). These results indicate that NtcA binds *in vitro* to the $GATN_8TAC$ site, which is centred at −41.5 upstream from the distal *petH* transcription start point.

### NtcA binding is required for FNRS accumulation in *Synechocystis*

To determine whether the identified NtcA-binding site operated *in vivo*, the X mutation linked to the Ω cassette was introduced in *Synechocystis*, yielding mutant NBX (Fig. 2a). Total segregation of the chromosomes was confirmed by PCR and restriction analysis, in which the presence of the *Xba*I site confirmed the integration of the modified NtcA-binding site into the segregated chromosome (Fig. 2b).




View larger version:
»In this page »In a new window
»Download as PowerPoint Slide

**Fig. 2.** Construction and characterization of a mutant lacking the NtcA-binding site. (a) Structure of the mutagenized *petH* gene; a solid line represents the 5′-non-coding region where the X mutation, which abolished NtcA binding (two black boxes) and created an *Xba*I site (*Xba*I*), is linked to the Ω cassette. A black arrow represents the *petH* ORF and half arrows indicate the location of PCR primers. (b) PCR and restriction analysis of the mutant: –, PCR produced a 1 kb fragment from the WT and 3 kb fragments from BX and NBX, due to the Ω cassette; +, *Xba*I digestion of the PCR products yielded an additional 0.4 kb fragment in NBX. (c) Immunodetection of the FNR in cell extracts from the WT, BX and

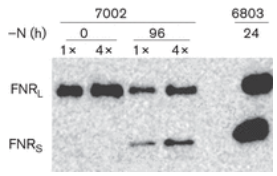
NBX under standard conditions (0), and upon nitrogen starvation for 24 and 72 h. Unlike WT and BX, NBX failed to induce FNRS synthesis.

FNR isoform accumulation upon nitrogen starvation was examined in total-protein extracts from NBX, which carried the X mutation plus an Ω cassette insertion 115 bases upstream from the distal transcription start point, the WT and BX, a strain carrying a WT *petH* allele plus the omega cassette at the same site as NBX. Fig. 2(c) shows that, unlike the WT and BX, NBX did not accumulate FNRS even after 3 days of nitrogen starvation. A similar experiment performed with the P mutation showed a WT behaviour for FNR isoform accumulation, which excludes NtcA binding to the putative $N_{10}$ site *in vivo* (data not shown).

We have shown previously that FNRS accumulation depends on the *petH* distal promoter, producing a long transcript whose translation yields FNRS (Omairi-Nasser *et al.*, 2011). The absence of FNRS in a strain lacking the NtcA-binding site implies that NtcA is required for *petH* transcription from the distal promoter. These results confirmed the requirement of the longer transcript for the synthesis of FNRS and established its transcription activation by NtcA.

### FNRS accumulation in *Synechococcus* sp. PCC7002

Recent transcriptome studies in *Synechococcus* sp. PCC7002 showed that *petH* transcription proceeds from a proximal transcription start point, located 87 bases upstream from the ORF, under standard conditions, and from a distal transcription start point, located 283 bases upstream from the ORF, operating under nitrogen starvation (Ludwig & Bryant, 2012). We performed Western blots on total extracts from *Synechococcus* sp. PCC7002 grown under standard and nitrogen starvation conditions. Fig. 3 shows that FNRS was expressed upon nitrogen starvation, consistent with the transcript results.



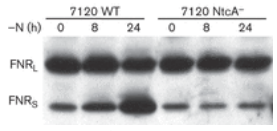

View larger version:
»In this page »In a new window
»Download as PowerPoint Slide

Fig. 3. FNR immunodetection in cell extracts from WT *Synechococcus* sp. PCC7002 grown with nitrate (0) or nitrogen starved for 96 h (two amounts were loaded from each sample); the last well contains extracts from WT *Synechocystis* starved for 24 h for comparison.

A putative NtcA-binding site was found upstream from the distal *petH* promoter in *Synechococcus* sp. PCC7002 (Fig. 6a, *b*). Thus, the situation in this cyanobacterium turns out to be similar to the situation described for *Synechocystis*, where FNRS accumulation follows an NtcA-induced and longer *petH* transcript.

### FNRS accumulation in *Anabaena* PCC7120

In *Anabaena* PCC7120, *petH* is transcribed from a constitutive transcription start point, located 63 bases upstream from the ORF, and from an NtcA-regulated transcription start point, located 188 bases upstream from the ORF (Valladares *et al.*, 1999). To test whether the NtcA-regulated long transcript is translated into a small FNR isoform, we performed Western blots on total extracts from the WT as well as from an *ntcA* insertional mutant (CSE2; Frías *et al.*, 1994) grown with ammonia or in the absence of combined nitrogen. Fig. 4 shows that FNRS accumulates upon nitrogen step-down in WT cells, whilst it failed to accumulate in the *ntcA* mutant. A small amount of FNRS was detected in the presence of nitrogen in the WT (7120WT, 0), but also when NtcA was absent ($NtcA^-$, all wells).

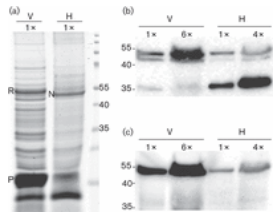



View larger version:
»In this page »In a new window
»Download as PowerPoint Slide

Fig. 4. FNRS accumulation in *Anabaena* sp. PCC7120. FNR immunodetection in WT and NtcA-deficient mutant, CSE2, grown with ammonium (0) or in the absence of combined nitrogen for 8 and 24 h.

As the distal promoter was shown to be the main promoter operative in the heterocysts (Valladares *et al.*, 1999), purified heterocysts were tested for the presence of FNRS. Fig. 5(b) shows clearly that FNRS is the major isoform in purified heterocysts. Heterocyst extract purity was estimated by evaluating

their relative content of ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (RbcL, which is expressed only in vegetative cells). As shown in Fig. 5, isolated heterocysts had similar amounts of RbcL and $FNR_L$, which suggested that mature heterocysts contain almost exclusively $FNR_S$.




View larger version:
»In this page »In a new window
»Download as PowerPoint Slide

**Fig. 5.**
*Anabaena* sp. PCC7120 expresses $FNR_L$ in vegetative cells and $FNR_S$ in heterocysts. Different amounts of total extracts were migrated using SDS-PAGE. The first two lanes were Coomassie stained (a), and the rest were blotted on a membrane and hybridized with an FNR antibody (b); the membrane was striped and rehybridized with an RbcL antibody to evaluate heterocyst purity (c). Numbers to the right of the Coomassie-stained gel and to the left of the blots indicate the sizes of the molecular mass markers; R, P and N indicate possible positions of specific and abundant proteins (RbcL, phycocyanin and nitrogenase, respectively).

## Discussion

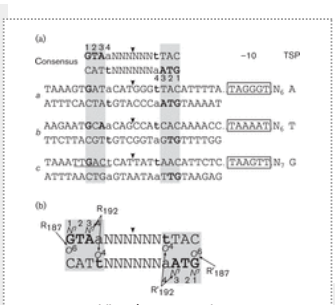
This work shows that *petH* regulation is similar in cyanobacteria capable of $FNR_S$ synthesis. An ORF-proximal transcription start point produces a transcript that is translated into $FNR_L$, whilst a distal transcription start point controlled by NtcA, produces a longer transcript that is translated into $FNR_S$.

When *Synechocystis* is grown under standard conditions, $FNR_L$ is the major isoform but traces of $FNR_S$ are present. The distal *petH* transcription start point was detected in addition to the proximal transcription start point under standard conditions in the genome map of *Synechocystis* transcription start points (Mitschke *et al.*, 2011a). Here, we show that NtcA binding to the distal promoter is required for $FNR_S$ synthesis that depends on the distal *petH* promoter activity (Omairi-Nasser *et al.*, 2011). NtcA was shown to be essential for *Synechocystis* under all known conditions (García-Domínguez *et al.*, 2000), which implies that NtcA is present even when nitrogen is available. Therefore, the slight accumulation of $FNR_S$ and the presence of the longer transcript in the WT *Synechocystis* are due to the presence of NtcA under standard conditions.

A slight difference was found in *Synechococcus* sp. PCC7002. Whilst $FNR_S$ accumulates under nitrogen starvation, it was not detected under standard conditions. In a recent transcriptome work, the long *petH* transcript, in addition to the short transcript, was detected under standard conditions (Ludwig & Bryant, 2012; M. Ludwig & D. A. Bryant, personal communication). The absence of $FNR_S$, under the standard conditions used in our work, might either result from a poor recognition of the *Synechococcus* sp. PCC7002 $FNR_S$, by the *Synechocystis* antibody, or from the different growth conditions used in each laboratory.

The low level of $FNR_S$ detected in *Anabaena* grown in the presence of ammonia, as well as in the NtcA-deficient mutant (Fig. 4), suggests that NtcA does not tightly control transcription from the distal promoter in this cyanobacterium. It is noteworthy that a putative −35 element overlaps the NtcA-binding site in *Anabaena* (Fig. 6a, *c*); this might be responsible for the low level of $FNR_S$ detected in the absence of NtcA. It was shown that NtcA was responsible for the upregulation of the distal *petH* promoter in *Anabaena* (Valladares *et al.*, 1999); here, we showed that NtcA is required for the accumulation of a higher level of $FNR_S$.

**Fig. 6.**
Structure of the NtcA-binding sites associated with the distal *petH* promoters. (a) The consensus NtcA-binding site is shown in bold upper-case letters. NtcA-binding sites found at the distal *petH* promoter in *a Synechocystis* sp. PCC6803, *b Synechococcus* sp. PCC7002 and *c Anabaena* sp. PCC7120. Bold upper-case letters indicate nucleotides that are identical to consensus sequence; lower-case letters indicate nucleotides that might interact with the conserved Arg192 of NtcA. An arrowhead indicates the symmetry axis of the NtcA-binding site, which is located −41.5, −40.5 and −42.5 upstream from the distal *petH* transcription start point (TSP) in *a*, *b* and c, respectively. The underlined sequence in *c* could constitute a −35 promoter element in *Anabaena* sp. PCC7120;



boxed sequences represent putative −10 promoter elements. (b) Summary of the proposed NtcA F-helix residue interaction with the site. NtcA arginine residues (the numbers are those of *Synechocystis*) are labelled R and R′ to indicate that they belong to different subunits of the dimer.

View larger version:
»In this page »In a new window
»Download as PowerPoint Slide

In addition to its photoautotrophic growth ability, *Anabaena* can fix molecular nitrogen when combined nitrogen is absent. Differentiated cells called heterocysts achieve this function for the adjacent vegetative (photosynthetic) cells, which in turn supply carbohydrates to the non-photosynthetic heterocysts. Heterocysts have been shown to contain 14 times more FNR than vegetative cells (Razquin *et al.*, 1996). Since ~10% of the cells in a nitrogen-fixing culture are heterocysts, the equal amounts of $FNR_S$ and $FNR_L$ in a nitrogen-fixing culture (Fig. 4, 7120WT, 24) indicate that $FNR_S$ is associated with heterocysts. In another experiment, we showed that purified heterocysts contained almost exclusively $FNR_S$, whilst $FNR_L$ is the major isoform in ammonia-grown vegetative cells. This suggests that $FNR_L$ function is related to photosynthesis and sugar anabolism, whilst $FNR_S$ functions in an environment where sugar catabolism is activated to sustain nitrogen fixation.

Obligate photoautotrophs like *Synechococcus elongatus* and *Thermosynechococcus elongatus* do not contain a second initiating codon and do not accumulate $FNR_S$ (Thomas *et al.*, 2006). In a recent transcriptome study of *S. elongatus*, the *petH* transcription start point was located 135 bases upstream from the ORF under standard conditions (Vijayan *et al.*, 2011). Unfortunately, no transcription data are available for this cyanobacterium under nitrogen-limited conditions. However, the fact that strains capable of $FNR_S$ synthesis such as *Synechocystis* sp. PCC6803, *Synechococcus* sp. PCC7002 and *Anabaena* sp. PCC7120 are facultative heterotrophs strongly suggests that $FNR_S$ function is associated with catabolism.

**Potential interaction of NtcA with its binding site**

Comparison of the *petH* NtcA-binding sites reveals a possible role for the first nucleotide position after the consensus triplet. As in the Crp structure, the F-helix of NtcA ensures DNA recognition. The F-helix of Crp was shown to exhibit sequence preferences at specific positions (GTGA) within each DNA half-site, which are mediated by Arg180, Glu181 and Arg185 (Lawson *et al.*, 2004). In the Crp-DNA complex, Arg180 interacts with guanine ($O^6$ and $N^7$ atoms) of the GC pair at position 1 of its consensus; a similar interaction may occur in the NtcA-DNA complex through Arg187 (or 186, depending on amino acid numbering) (position 1, Fig. 6b). In place of Glu181 of Crp, a Val is found in NtcA, which is believed to accommodate an AT (instead of a GC) pair at position 3 and explains the GTA triplet in the NtcA-binding consensus (Llácer *et al.*, 2010). However, Arg185 in the Crp-DNA complex interacts with guanine ($O^6$ and/or $N^7$) of GC at position 3, but also with thymine ($O^4$) of the AT pair at position 4 (Lawson *et al.*, 2004). In the NtcA complex, a similar interaction could occur between Arg192 (or 191) and the adenine ($N^7$) of the AT pair at position 3 (Fig. 6b). In this context, NtcA binding would be strengthened by the presence of an AT pair at position 4, as Arg192 would also interact with the thymidine ($O^4$).

In *Synechocystis*, the position 3 AT pair is not found in the 5′ half of the *petH* NtcA-binding site, but an AT pair is present in both half-sites at position 4 (Fig. 6a, *a*). In *Synechococcus* PCC7002, the position 3 AT pair is not conserved in the 3′ half, but again both half-sites retained an AT pair at position 4 (Fig. 6a, *b*). In *Anabaena*, none of the position-3 AT pairs are conserved and only the one at position 4 of the 3′ half is conserved (Fig. 6a, *c*).

Therefore, the first nucleotide after the GTA consensus triplet could play a role in NtcA binding. Further experiments are required to test this proposal.

## Acknowledgements

We are indebted to Alicia M. Muro-Pastor for providing the Western blot used in Fig. 4. The work in France was financially supported by the Centre National de la Recherche Scientifique (UMR8221) and the Commisariat à l'Energie Atomique (Bioénergies DSV, IBITEC-S). The work in Spain was supported by Ministerio de Economía y Competitividad co-financed by FEDER (grant BFU2010-15708) and

by Junta de Andalucía (grant P07-CVI-02792 and group BIO-284). The anonymous reviewers are acknowledged for their constructive comments.

BFU2010-15708FEDER
Centre National de la Recherche Scientifique
Commisariat à l'Energie Atomique
Ministerio de Economía y Competitividad
Junta de Andalucía

## References

Frías J. E., Flores E., Herrero A. (1994) Requirement of the regulatory protein NtcA for the expression of nitrogen assimilation and heterocyst development genes in the cyanobacterium *Anabaena* sp. PCC 7120. *Mol Microbiol* **14**, 823–832. » CrossRef » Medline » Search Google Scholar

García-Domínguez M., Reyes J. C., Florencio F. J. (2000) NtcA represses transcription of *gifA* and *gifB* genes that encode inhibitors of glutamine synthetase type I from *Synechocystis* sp. PCC 6803. *Mol Microbiol* **35**, 1192–1201. » CrossRef » Medline » Search Google Scholar

Golden J. W., Robinson S. J., Haselkorn R. (1985) Rearrangement of nitrogen fixation genes during heterocyst differentiation in the cyanobacterium *Anabaena*. *Nature* **314**, 419–423. » CrossRef » Medline » Search Google Scholar

Herrero A., Muro-Pastor A. M., Flores E. (2001) Nitrogen control in cyanobacteria. *J Bacteriol* **183**, 411–425. » FREE Full Text

Jiang F., Wisén S., Widersten M., Bergman B., Mannervik B. (2006) Examination of the transcription factor NtcA binding motif by *in vitro* selection of DNA sequences from a random library. *J Mol Biol* **361**, 783–793. » CrossRef » Medline » Search Google Scholar

Körner H., Sofia H. J., Zumft W. G. (2003) Phylogeny of the bacterial superfamily of Crp-Fnr transcription regulators: exploiting the metabolic spectrum by controlling alternative gene programs. *FEMS Microbiol Rev* **27**, 559–592. » CrossRef » Medline » Search Google Scholar

Lawson C. L., Swigon D., Murakami K. S., Darst S. A., Berman H. M., Ebright R. H. (2004) Catabolite activator protein: DNA binding and transcription activation. *Curr Opin Struct Biol* **14**, 10–20. » CrossRef » Medline » Search Google Scholar

López-Landavery E. A., Espinosa-Cortés L., Castells M. A., Contreras A., Forchhammer K., Rubio V. (2011) Structural basis for the regulation of NtcA-dependent transcription by proteins PipX and PII. *Proc Natl Acad Sci U S A* **107**, 15397–15402. » Abstract // FREE Full Text

Ludwig M., Bryant D. A. (2011) Acclimation of the global transcriptome of the cyanobacterium *Synechococcus* sp. strain PCC 7002 to nutrient limitations and different nitrogen sources. *Front Microbiol* **2**, 145. » CrossRef » Medline » Search Google Scholar

Luque I., Forchhammer K. (2008) Nitrogen assimilation and C/N balance sensing. in *The Cyanobacteria: Molecular Biology, Genomics and Evolution*, pp. 335–382. Edited by Herrero A., Flore E. . Wymondham: Caister Academic Press. » Search Google Scholar

Mitschke J., Georg J., Scholz I., Sharma C. M., Dienst D., Bantscheff J., Voss B., Steglich C., Wilde A., Vogel J., Hess W. R. (2011) An experimentally anchored map of transcriptional start sites in the model cyanobacterium *Synechocystis* sp. PCC6803. *Proc Natl Acad Sci U S A* **108**, 2124–2129. » Abstract // FREE Full Text

Mitschke J., Vioque A., Haas F., Hess W. R., Muro-Pastor A. M. (2011) Dynamics of transcriptional start site selection during nitrogen stress-induced cell differentiation in *Anabaena* sp. PCC7120. *Proc Natl Acad Sci U S A* **108**, 20130–20135. » Abstract // FREE Full Text

Muro-Pastor M. I., Reyes J. C., Florencio F. J. (1996) The NADP$^+$ isocitrate dehydrogenase gene (*icd*) is nitrogen regulated in cyanobacteria. *J Bacteriol* **178**, 4070–4076. » Abstract // FREE Full Text

Muro-Pastor M. I., Reyes J. C., Florencio F. J. (2001) Cyanobacteria perceive nitrogen status by sensing intracellular 2-oxoglutarate levels. *J Biol Chem* **276**, 38320–38328. » Abstract // FREE Full Text

Omairi-Nasser A., de Gracia A. G., Ailani G. (2011) A larger transcript is required for the synthesis of the smaller isoform of ferredoxin:NADP oxidoreductase. *Mol Microbiol* **81**, 1178–1189. » CrossRef » Medline » Search Google Scholar

Osanai T., Imamura S., Asayama M., Shirai M., Suzuki I., Murata N., Tanaka K. (2006) Nitrogen induction of sugar catabolic gene expression in *Synechocystis* sp. PCC 6803. *DNA Res* **13**, 185–195. » Abstract // FREE Full Text

Razquin P., Fillat M. F., Schmitz S., Stricker O., Böhme H., Gómez-Moreno C., Peleato M. L. (1996) Expression of ferredoxin-NADP$^+$ reductase in heterocysts from *Anabaena* sp. *Biochem J* **316**, 157–160. » Medline » Search Google Scholar

Stevens S. E., Porter R. D. (1980) Transformation in *Agmenellum quadruplicatum*. *Proc Natl Acad Sci U S A* **77**, 6052–6056. » Abstract // FREE Full Text

Thomas J.-C., Ughy B., Lagoutte B., Ajlani G. (2006) A second isoform of the ferredoxin:NADP oxidoreductase generated by an in-frame initiation of translation. *Proc Natl Acad Sci U S A* **103**, 18368–18373. » Abstract // FREE Full Text

Ughy, B., Ajlani, G. (2004) Phycobilisome rod mutants in *Synechocystis* sp. strain PCC6803. *Microbiology* **150**, 4147–4156. » Abstract // FREE Full Text

Valladares, A., Muro-Pastor, A. M., Fillat, M. F., Herrero, A., Flores, E. (1999) Constitutive and nitrogen-regulated promoters of the *patH* gene encoding ferredoxin:NADP$^+$ reductase in the heterocyst-forming cyanobacterium *Anabaena* sp. *FEBS Lett.* **449**, 159–164. » CrossRef » Medline » Search Google Scholar

Vijayan, V., Jain, I. H., O'Shea, E. K. (2011) A high resolution map of a cyanobacterial transcriptome. *Genome Biol.* **12**, R47. » CrossRef » Medline » Search Google Scholar

Zhao, M. X., Jiang, Y. L., He, Y. X., Chen, Y. F., Teng, Y. B., Chen, Y. Zhang, C. C., Zhou, C. Z. (2010) Structural basis for the allosteric control of the global transcription factor NtcA by the nitrogen starvation signal 2-oxoglutarate. *Proc Natl Acad Sci U S A* **107**, 12487–12492. » Abstract // FREE Full Text