Constitutive and nitrogen-regulated promoters of the *petH* gene encoding ferredoxin:NADP$^+$ reductase in the heterocyst-forming cyanobacterium *Anabaena* sp.

Ana Valladares[a], Alicia M. Muro-Pastor[a], María F. Fillat[b], Antonia Herrero[a], Enrique Flores[a,*]

[a] *Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla, Centro de Investigaciones Científicas Isla de la Cartuja, Avda. Américo Vespucio s/n, E-41092 Sevilla, Spain*
[b] *Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Ciencias, Universidad de Zaragoza, E-50009 Zaragoza, Spain*

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Abstract Determination of the putative transcription start points of the *petH* gene encoding ferredoxin:NADP$^+$ reductase in the heterocyst-forming cyanobacteria *Anabaena* sp. PCC 7119 and PCC 7120 showed that this gene is transcribed from two promoters, one constitutively used under different conditions of nitrogen nutrition and the other one used in cells subjected to nitrogen stepdown and in nitrogen-fixing filaments. The latter promoter, whose use was NtcA-dependent but HetR-independent, was functional in heterocysts. The N-control transcriptional regulator NtcA was observed to bind in vitro to this promoter. For the sake of comparison, the transcription start points of the *nifHDK* operon in strain PCC 7120 and binding of NtcA to the *nifHDK* promoter were also examined.

Key words: Ferredoxin:NADP$^+$ reductase; *nifH*; NtcA; *petH*; Nitrogen fixation; *Anabaena* sp.

1. Introduction

Ferredoxin:NADP$^+$ reductase, the *petH* gene product, is a key enzyme in the phototrophic metabolism. It catalyzes the reduction of NADP$^+$ using reduced ferredoxin (or flavodoxin) which is produced in the oxygenic photosynthesis as an electron donor. Cyanobacteria are characterized by their ability to perform oxygenic photosynthesis. They generally use ammonium or nitrate as a source of nitrogen and many strains can fix molecular nitrogen [1]. Some filamentous cyanobacteria, like *Anabaena* sp., can develop differentiated cells called heterocysts which are specialized in nitrogen fixation [2]. In nitrogen-fixing filaments of *Anabaena* sp., about 5–10% of the cells are heterocysts and these have been shown to contain an increased concentration of ferredoxin:NADP$^+$ reductase, about 14-fold of that found in vegetative cells [3].

Cyanobacteria generally assimilate ammonium with preference over other nitrogen sources like nitrate or dinitrogen and the heterocyst development is repressed by growth of the cyanobacterial filaments in the presence of a source of combined nitrogen such as ammonium or nitrate [1,2]. The *ntcA* gene of cyanobacteria encodes a protein, NtcA, which belongs to the CRP family of bacterial transcriptional regulators and is required for the expression of genes encoding proteins involved in the assimilation of nitrogen sources alternative to ammonium [4–7]. NtcA is also required for the heterocyst development in *Anabaena* sp. PCC 7120 [8,9]. The NtcA-binding site on DNA is characterized by the sequence signature GTAN$_8$TAC which is found circa 22 bp upstream from a −10 box, TAN$_3$T, in the promoter region of the NtcA-activated genes [10,11]. Another gene required for heterocyst development and characterized in *Anabaena* sp. PCC 7120 is *hetR*, whose expression is increased after a nitrogen stepdown in those cells that will become heterocysts [12,13]. Induction of *hetR* is dependent on NtcA [8]. While NtcA appears to link the heterocyst development to the nitrogen status of the cells, HetR, which has recently been proposed to be a protease [14], would represent an early regulatory element for differentiation to proceed. The mature heterocyst has been shown to be the site of expression of the *nifHDK* operon (comprising the structural genes for nitrogenase [2]) in filaments of *Anabaena* sp. PCC 7120 grown under aerobic conditions [15].

The *petH* gene from several cyanobacteria has been cloned and sequenced [16–18]. It encodes a protein of circa 49 kDa with an N-terminal domain that is missing from higher plant ferredoxin:NADP$^+$ reductase and whose first 80 amino acids present a sequence homologous to that of the CpcD phycobilisome linker polypeptide. Consistent with data which showed the presence of a high level of ferredoxin:NADP$^+$ reductase in heterocysts, *petH* mRNA levels have been shown to be higher in nitrogen-fixing cultures than in those grown with nitrate [3]. However, no investigation of the structure of the *petH* promoters in any heterocyst-forming cyanobacterium has been reported to date. In this work, we have characterized the putative promoter regions of *petH* in two strains of *Anabaena* sp., PCC 7119 and PCC 7120, and in *ntcA* and *hetR* mutants of strain PCC 7120. For the sake of comparison, the *Anabaena* sp. PCC 7120 *nifH* promoter has been reinvestigated.

2. Materials and methods

2.1. Bacterial strains and plasmids

This study was carried out with the heterocyst-forming cyanobacteria *Anabaena* sp. strains PCC 7119 and PCC 7120 and two mutants of the latter, strain CSE2 (an insertional mutant of the *ntcA* gene [8]) and strain DR884a (an insertional mutant of the *hetR* gene [13]). They were grown photoautotrophically at 30°C in BG11$_0$C medium (BG11$_0$ medium supplemented with 0.84 g/l NaHCO$_3$ [19]), bubbled with a mixture of CO$_2$ (1% v/v) and air and supplemented with 2 μg/ml streptomycin and 2 μg/ml spectinomycin in the case of strain CSE2 and 5 μg/ml neomycin in the case of strain DR884a. When indicated,

*Corresponding author. Fax: (34) (954) 46 00 65.
E-mail: flores@cica.es

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8 mM NH_4Cl (plus 16 mM TES-NaOH buffer (pH 7.5)) or 17.6 mM $NaNO_3$ was added as a nitrogen source.

For RNA isolation, cells growing exponentially in $BG11_0C$ medium (nitrogen-free) or $BG11_0C$ supplemented with NH_4Cl were used. For induction experiments, cells growing exponentially in $BG11_0C$ medium supplemented with NH_4Cl were harvested at room temperature, washed with $BG11_0C$ medium, resuspended in $BG11_0C$ medium supplemented or not with NH_4Cl or $NaNO_3$ and further incubated under culture conditions for the indicated number of h. Cultures for heterocyst isolation were grown in $BG11_0C$ medium supplemented with NH_4Cl until they reached the exponential phase. Cells were then washed with and resuspended in $BG11_0C$ medium and further incubated until mature heterocysts were observed (19 h). Heterocysts were isolated as described [20].

We have previously determined that a strain PCC 7120 *Ase*I DNA fragment of about 2.3 kb hybridized to a probe containing the 5′ end of *petH*. To clone the promoter region of the *petH* gene from *Anabaena* sp. PCC 7119 and PCC 7120, chromosomal DNA was digested with *Ase*I, fragments in the size range of 2–2.7 kb were isolated and re-ligated and DNA was amplified by PCR using oligonucleotides PH1 (5′-GTTTCTTCGTTCTGACGCATACCCA-3′, complementary to positions +98–+75 relative to the translation start of *petH* from strain PCC 7119) and PH2 (5′-CCGCCTAACTTACGCTATCAGCCG-3′, corresponds to positions +1058–+1081 relative to the translation start of *petH* from strain PCC 7119). The PCR products, of about 1.1 kb, were cloned into plasmid pGEM-T (Promega). The regions corresponding to the *petH* promoters from both strains were sequenced using the oligonucleotides PH1 and PH5 (5′-GCACTCTGAGAAAGTAGC-3′, complementary to positions −98–−115 relative to the translation start of *petH*). Several products from different PCR amplifications were analyzed rendering identical sequences. The plasmids containing the *petH* promoter of strain PCC 7119 and PCC 7120 were named pCSMFF1 and pCSAV13, respectively.

The promoter region of *nifH* from *Anabaena* sp. PCC 7120 was cloned as a 381 bp fragment which contained the final part of *nifU*, the initial part of *nifH* and the intergenic region between both genes. This fragment was amplified by PCR, using the oligonucleotides NH1 (5′-GTACTGCAAGGGGCGTGTGGC-3′, corresponds to positions −334–−314 relative to the start codon of *nifH*) and NH3 (5′-CCGCCTTTACCGTAGAAAGC-3′, complementary to positions +47–+28 relative to the translation start of *nifH*) and chromosomal DNA from strain PCC 7120 as template and cloned into plasmid pGEM-T rendering pCSAV57.

2.2. DNA and RNA isolation and manipulation

Total DNA [21] and RNA [22] from cyanobacteria were isolated as previously described. Sequencing was carried out by the dideoxy chain termination method, using a T^7Sequencing kit (Pharmacia Biotech) and [α-^{35}S]-thio dATP. DNA fragments were purified from agarose gels with the Geneclean II kit (Bio101). Plasmid isolation from *Escherichia coli*, transformation of *E. coli* DH5α, used for all plasmid constructions, digestion of DNA with restriction endonucleases, ligation with T4 DNA ligase and PCR amplification were performed by standard procedures [23,24].

2.3. Primer extension analysis

The oligonucleotide used for the primer extension analysis of the *petH* transcript was PH1 (see above). Plasmids pCSMFF1 and pCSAV13 were used to generate dideoxy sequencing ladders using the same primer. The oligonucleotide used for primer extension analysis of the *nifH* transcript was NH3. Plasmid pCSAV57, that contains the promoter region of the *nifH* gene, was used to generate dideoxy sequencing ladders using the same primer. Oligonucleotide NH2 (5′-CACGCCTGTTTGAGCTATGGC-3′, complementary to positions −51–−71 relative to the translation start of *nifH*) was used to confirm the *nifH* transcription start point (*tsp*).

Oligonucleotides were end-labelled with T4 polynucleotide kinase (Boehringer) and [γ-^{32}P]dATP as described [24] and mixed with 25–30 μg of total RNA in the presence of 10 mM Tris-HCl (pH 8.0), 150 mM KCl and 1 mM EDTA. The mixtures were incubated first at 85°C for 10 min for denaturation of RNA and then at 50°C for 1 h for annealing. The extension reactions were carried out at 47°C for 1 h in a final volume of 45 μl containing the whole annealing reaction, 0.25 mM of each deoxynucleoside triphosphate, 200 U of reverse transcriptase (Superscript II, Gibco-BRL) and the buffer recommended by the transcriptase provider. (In the case of the experiment shown in Fig. 2A, 50 μg of RNA and 25 U of AMV reverse transcriptase were used and annealing was at 50°C for 3 h). Reaction mixtures were then treated with RNase A (DNase-free, Boehringer) and extracted with phenol and chloroform. The extended fragments were precipitated with sodium acetate and ethanol, resuspended in formamide loading dye and loaded onto 6% polyacrylamide-urea sequencing gels next to the corresponding sequencing ladder. Images of radioactive gels were obtained using a Cyclone storage phosphor system (Packard).

2.4. Band shift assays

DNA fragments to be used in electrophoretic mobility band shift assays were obtained by PCR. The oligonucleotides PH1 (see above) and PH3 (5′-CAAGACTCAGCACTCAGGAC-3′, corresponding to positions −316–−297 relative to the translation start of *petH*) and plasmid pCSAV13 were used in the case of the *petH* upstream region. Oligonucleotides NH1 and NH3 (see above) and plasmid pCSAV57 were used in the case of the *nifH* promoter region. For the *glnA* promoter region, oligonucleotides GA3 (5′-GGATTTTATGTCAAAGTTGACCCC-3′, corresponding to positions −238–−215 relative to the translation start of *glnA*) and GA6 (5′-CGAAACAAAGTTGATGAC-3′, complementary to positions −70–−87 relative to the translation start of *glnA*) and plasmid pAN503 [25] were used. Binding assays were carried out as described [26] using as a source of NtcA, a cell-free extract (1.4 μg of protein) of *E. coli* BL21 (DE3) (pREP-4) in which NtcA from *Anabaena* sp. PCC 7120 was over-produced as an histidine-tagged protein from the isopropyl-β-D-thiogalactopyranoside inducible promoter of vector pQE9 (Quiagen) (A. M. Muro-Pastor, unpublished construction).

2.5. Nucleotide sequences

The sequences of the DNA fragments analyzed in this work are presented in Fig. 1, (the sequence of the *petH* promoter region analyzed is identical in strains PCC 7119 and PCC 7120). Nucleotide sequences for the *petH* promoter region have been deposited under accession numbers X72394 (strain PCC 7119) and AJ132476 (strain PCC 7120). Other sequences can be found under accession numbers J05111 (*nifH* from strain PCC 7120) and X00147 (*glnA* from strain PCC 7120).

3. Results

3.1. tsps of petH

Putative *tsp*s of the *Anabaena* sp. PCC 7119 and PCC 7120 *petH* genes were identified by primer extension analysis using as template, RNA isolated from cells grown using ammonium or dinitrogen as the nitrogen source. Identical results were obtained in both strains (see data for strain PCC 7120 in Fig. 2A). An extension product, present independently of the nitrogen source used for growth, was localized at position −63 with respect to the translation start of the gene. A second extension product was observed with RNA isolated from dinitrogen grown cells which was localized at position −188. We named the gene proximal putative *tsp* as *tsp*1, the distal one *tsp*2 and the corresponding RNAs, RNA-1 and RNA-2, respectively. The use of these *tsp*s was further investigated in strain PCC 7120, for which *ntcA* and *hetR* mutants are available.

To investigate the *petH tsp*s in the *Anabaena* sp. PCC 7120 *ntcA* mutant, strain CSE2, which is unable to grow on dinitrogen or nitrate or to develop heterocysts [8], the RNA was isolated from ammonium grown cells further incubated for 6 h in medium containing nitrate or ammonium or lacking any source of combined nitrogen. RNA was also isolated from cells of strain PCC 7120 incubated under the same conditions. Confirming and extending the results described above, RNA-2 was observed in strain PCC 7120 only with RNA isolated from cells subjected to a nitrogen stepdown, while RNA-1

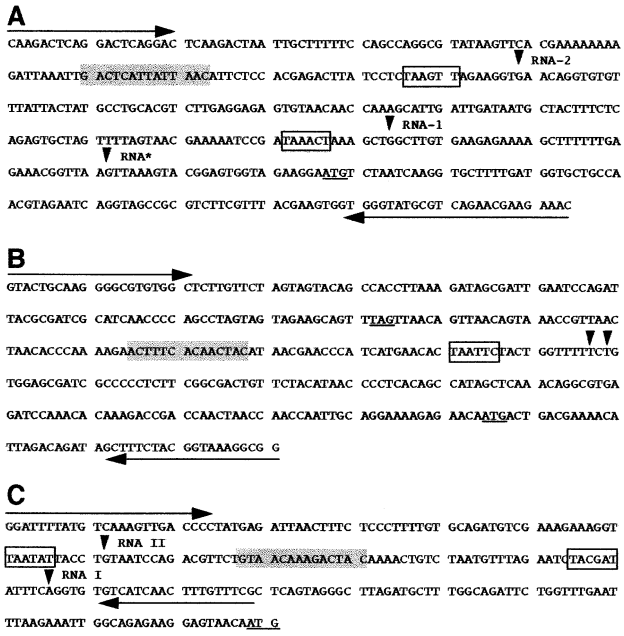

Fig. 1. Nucleotide sequences of the DNA regions upstream from the *petH* (A), *nifH* (B) and *glnA* (C) genes of *Anabaena* sp. PCC 7120. The corresponding sequence of the *petH* upstream region in strain PCC 7119 is identical to that of strain PCC 7120. The location of the oligonucleotides used for PCR amplification of fragments for DNA-binding is indicated by horizontal arrows. The ATG translation start of the three genes, as well as the TAG termination codon of the *nifU* gene which is located upstream from *nifH*, are underlined. Putative *tsp*s are indicated by solid triangles. Putative promoter −10 boxes are indicated by open boxes. The NtcA-binding site upstream from *glnA* and the putative NtcA-binding sites found upstream from the *nifH tsp* and the *petH tsp*2 are indicated by gray boxes.

was detected independently of the nitrogen status of the cells (Fig. 2B). With regard to mutant strain CSE2, only RNA-1 was observed even with RNA isolated from cells incubated with no combined nitrogen (Fig. 2B). While *tsp*1 appears therefore to be constitutively used, the use of *tsp*2 requires an intact *ntcA* gene and incubation of the cells in the absence of nitrate or ammonium, i.e. incubation under conditions that lead to heterocyst development.

The *petH tsp*s were then studied using as template RNA obtained from isolated heterocysts. This RNA preparation did not show hybridization to a probe of the *rbcL* gene, encoding the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase, which is expressed only in vegetative cells [15] (not shown). While RNA-1 was barely detectable with this RNA preparation, RNA-2 was abundant (Fig. 2B). Whether other extension products obtained with heterocyst RNA (note, e.g., RNA* which was also observed with RNA from whole filaments incubated with no combined nitrogen) correspond to real *tsp*s or to degraded mRNA molecules is unknown.

The *petH tsp*s were also investigated in cells of an *Anabaena* sp. PCC 7120 *hetR* mutant, strain DR884a, which is also unable to grow on dinitrogen and to develop heterocysts [13]. RNA isolated from ammonium grown cells or from ammonium grown cells incubated for 6 h in the absence of any source of combined nitrogen was used in this case. As in the wild-type (see above), RNA-1 was observed with both RNA preparations and RNA-2 was more abundant in the RNA isolated from cells subjected to a nitrogen stepdown (Fig. 2C). Note that RNA* was detectable at a low level in the latter RNA preparation.

3.2. *tsp of nifH*

The *nifHDK* operon can be considered as the paradigm of genes expressed only in the heterocyst. The *tsp* of *nifH* in strain PCC 7120, studied by S1 nuclease protection, has been localized to nucleotide −126 or −132 with respect to the translation start of the gene [27]. We were interested in checking the *nifH tsp* with our heterocyst RNA preparation. As a control, we studied the *nifH tsp* using RNA isolated from ammonium grown cells incubated for 20 h in medium containing nitrate or ammonium or no source of combined nitrogen. Either with heterocyst RNA (Fig. 3B) or with the RNA preparation from cells incubated with no combined nitrogen (Fig. 3A), a doublet of extension products was localized at nucleotides −126/−128 with respect to the *nifH* initiation codon. A very low level of this putative *tsp* was also detected with RNA from the culture incubated in the presence of nitrate (Fig. 3A), probably originating from a small percentage of heterocysts present in this culture. This *tsp* was confirmed with oligonucleotide NH2.

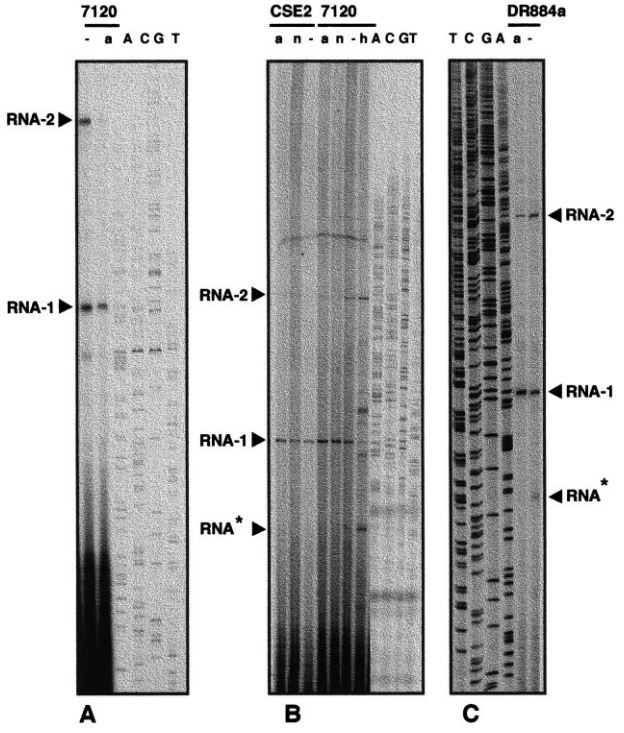

Fig. 2. Primer extension analysis of the expression of *petH* in *Anabaena* sp. PCC 7120 and mutant strains CSE2 (*ntcA*$^-$) and DR884a (*hetR*$^-$). Primer extension assays were carried out with oligonucleotide PH1 and the indicated RNA preparations. (A) RNA isolated from strain PCC 7120 cells grown on dinitrogen (−) or ammonium (a). (B) RNA isolated from PCC 7120 or CSE2 cells grown with ammonium and incubated for 6 h in fresh ammonium medium (a), nitrate medium (n) or medium lacking combined nitrogen (−) or RNA prepared from isolated heterocysts (h). (C) RNA isolated from DR884a cells grown on ammonium (a) or incubated for 6 h in medium lacking combined nitrogen (−). The sequencing ladders shown were generated with oligonucleotide PH1 and plasmid pCSAV13.

3.3. Binding of NtcA

In view of the NtcA-dependence of the use of the *petH tsp2*, we tested the binding of NtcA to a DNA fragment carrying the corresponding promoter. A DNA fragment from the upstream region of *nifH* has previously been reported to bind NtcA, although very weakly [28]. We have included in our analysis a DNA fragment carrying the *nifH* promoter. Finally, as a positive control, a DNA fragment carrying an NtcA-activated promoter of the *Anabaena* sp. PCC 7120 *glnA* gene (the promoter that corresponds to the *glnA* RNA_I [8,25]) was also included. As a source of NtcA, an extract of an *E. coli* strain over-expressing the strain PCC 7120 *ntcA* gene was used. NtcA-dependent band shifts were observed with both the *petH* and *nifH* promoters, but the fraction of retarded fragment was smaller with the *nifH* fragment than with the *petH* one (Fig. 4). In each case, however, retardation was poorer than that observed with the *glnA* RNA_I promoter. On the other hand, no band shift was observed with any of the tested DNA fragments when an extract from *E. coli* (pQE9) was used instead of the NtcA-carrying extract or with a 233 bp fragment from pBluescript SK(+) and the NtcA-containing extract (not shown).

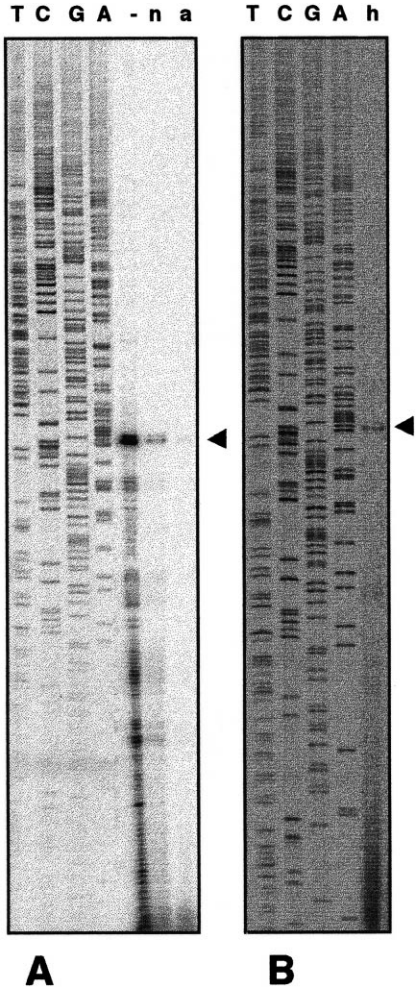

Fig. 3. Primer extension analysis of the expression of *nifH* in *Anabaena* sp. PCC 7120. Primer extension assays were carried out with oligonucleotide NH3 and the indicated RNA preparations. (A) RNA isolated from ammonium grown cells incubated for 20 h in medium lacking combined nitrogen (−) or supplemented with nitrate (n) or ammonium (a). (B) RNA isolated from heterocysts (h). The sequencing ladders shown were generated with oligonucleotide NH3 and plasmid pCSAV57. Arrowheads point to the extension products located at nucleotides −126/−128 with respect to the *nifH* translational start.

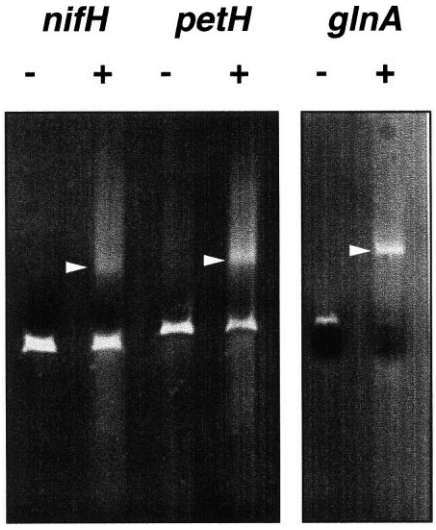

Fig. 4. Band shift assays with NtcA protein. Assays were carried out as described [26] using fragments from the promoter regions of *nifH, petH* or *glnA* from *Anabaena* sp. PCC 7120 (see Fig. 1). Assays were carried out with either no extract (−) or extracts from *E. coli* BL21 containing NtcA (+). Arrowheads point to retarded DNA fragments.

4. Discussion

The results presented in this work indicate that the *petH* gene is expressed in two strains of *Anabaena* sp., PCC 7119 and PCC 7120, from at least two promoters. The gene proximal promoter generates RNA-1 and appears to represent a constitutive promoter that is functional independently of the nitrogen regime of the cells. Seven nucleotides upstream from *tsp*1, a sequence, TAAACT, is found (Fig. 1A) that conforms to a typical cyanobacterial −10 hexamer, TAN_3T, that is similar to the −10 box of the *E. coli* σ^{70} promoters [10,29,30]. No obvious −35 box is found, however, in this putative *petH* promoter. While the presence of the TAN_3T hexamer would reflect recognition by the RNA polymerase bearing the SigA σ factor, which is homologous to σ^{70} [31], the absence of a −35 box suggests that an additional transcription factor is required to assist RNA polymerase-SigA in the use of this promoter.

The *petH* distal promoter generates RNA-2 and is used preferentially in the absence of combined nitrogen. It appears to be the main *petH* promoter operative in the heterocysts but is also used in the *hetR* mutant and in wild-type filaments, after a nitrogen stepdown, before mature heterocysts have developed. Thus, the *tsp*2 promoter is functional in the heterocyst but does not require a developed heterocyst to be used. Expression of heterocyst-specific genes *fdxH* (encoding a ferredoxin) and *nifH* before complete maturation of the heterocysts has also been observed in response to iron stress [32]. Like many other cyanobacterial promoters activated after a nitrogen stepdown [11], the *tsp*2 promoter requires NtcA to be used. A sequence that conforms to the TAN_3T −10 box is found eight nucleotides upstream from *tsp*2 and centered

around 29/30 nucleotides upstream from this hexamer, a sequence is found that exhibits some of the features of the NtcA-binding sites (Fig. 1A). Common features of the NtcA-binding sites are, in addition to the $GTAN_8TAC$ sequence signature, the presence of a string of A or T, 5′ from the GTA triplet and 3′ from the TAC triplet, and the presence of a C and an A in the second and third positions, respectively, 3′ from the GTA triplet [10,11]. The *petH tsp*2 promoter bears these features of an NtcA-binding site but, instead of the $GTAN_8TAC$ sequence signature, a $GACN_8AAC$ sequence is found.

As mentioned above, the *nifHDK* operon is expressed in *Anabaena* sp. PCC 7120 exclusively in the heterocysts. Consistently, it is not expressed in the *ntcA* [8] and *hetR* (our unpublished results) mutants. The *nifH tsp* that we have detected with RNA isolated from either whole filaments or heterocysts is localized essentially at the position previously reported [27] and is close to the *tsp* described for *Anabaena azollae nifH* [33]. A putative −10 box might be recognized in this promoter (Fig. 1B) and 23 nucleotides upstream from this box, a TAC triplet is found that might represent the 3′ half of an NtcA-binding site. However, while the 5′ and 3′ strings of A or T are present, the GTA triplet is not.

Compared to the strong binding of the NtcA protein to the *glnA* RNA_I promoter, we have observed a weak binding of NtcA to the *petH tsp*2 promoter and, consistently with previously reported results [28], an even weaker binding to the *nifH* promoter (Fig. 4). It is possible that these promoters are recognized more efficiently in vivo by a modified NtcA protein or by NtcA assisted by another regulatory element. Poor NtcA recognition sequences and a consequent poor binding of NtcA might represent a mechanism for regulation of some promoters that are activated in the absence of combined nitrogen. Thus, those promoters for which NtcA would exhibit a low affinity would be activated only under conditions of nitrogen deficiency that would lead to maximum levels of active NtcA protein.

Ferredoxin:$NADP^+$ reductase is required to generate NADPH for the general metabolism and has to be present in the cells independently of the nitrogen source used for growth. Consistently, a constitutive promoter, the one corresponding to *tsp*1, has been found for *petH* in this work. On the other hand, high levels of ferredoxin:$NADP^+$ reductase are present in heterocysts [3], cells whose metabolism is heavily oriented to electron transfer reactions required to provide reducing equivalents to nitrogenase as well as to respiratory chains in order to keep a low cellular O_2 tension [2]. It is interesting that transcription of *petH* in these differentiated cells takes place mainly from another promoter, the one originating RNA-2. The organization of *petH* promoters in *Anabaena* sp. PCC 7119 and PCC 7120 is markedly different from the situation reported for *petH* in *Synechocystis* sp. PCC 6803 [18]. In this non-nitrogen-fixing cyanobacterium, the only *tsp* detected for *petH* was mapped 523 bp upstream from the predicted translation start of the gene. This produces a large 5′ untranslated region which overlaps with the promoter of the *prk* gene encoding phosphoribulokinase.

The use of more than one promoter for a cyanobacterial gene was first reported for *glnA* (glutamine synthetase) in *Anabaena* sp. PCC 7120 [25]. Glutamine synthetase has a role in the ammonium incorporation into carbon skeletons independently of the nitrogen source used for growth, but higher levels of glutamine synthetase are found in cells grown using nitrate or dinitrogen than in ammonium grown cells [1]. At least three promoters are involved in the expression of *glnA* and one of them, the one that corresponds to RNA_I, strictly requires NtcA to be used [8]. Other genes from heterocyst-forming cyanobacteria that appear to be transcribed from more than one promoter include *argD* (*N*-acetylornithine aminotransferase) from *Anabaena* sp. PCC 7120 [34] and *zwf* (glucose-6-phosphate dehydrogenase) from *Nostoc punctiforme* [35]. Transcription from different promoters may represent a mechanism widely used in heterocyst-forming cyanobacteria to adjust the expression of genes required in both types of cells to the different programs of gene expression operative in heterocysts and vegetative cells.

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