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Transcription and Regulation of the Bidirectional Hydrogenase in the Cyanobacterium *Nostoc* sp. Strain PCC 7120[▽]

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The filamentous, heterocystous cyanobacterium *Nostoc* sp. strain PCC 7120 (*Anabaena* sp. strain PCC 7120) possesses an uptake hydrogenase and a bidirectional enzyme, the latter being capable of catalyzing both H_2 production and evolution. The completely sequenced genome of *Nostoc* sp. strain PCC 7120 reveals that the five structural genes encoding the bidirectional hydrogenase (*hoxEFUYH*) are separated in two clusters at a distance of approximately 8.8 kb. The transcription of the *hox* genes was examined under nitrogen-fixing conditions, and the results demonstrate that the cluster containing *hoxE* and *hoxF* can be transcribed as one polycistronic unit together with the open reading frame alr0750. The second cluster, containing *hoxU*, *hoxY*, and *hoxH*, is transcribed together with alr0763 and alr0765, located between the *hox* genes. Moreover, alr0760 and alr0761 form an additional larger operon. Nevertheless, Northern blot hybridizations revealed a rather complex transcription pattern in which the different *hox* genes are expressed differently. Transcriptional start points (TSPs) were identified 66 and 57 bp upstream from the start codon of alr0750 and *hoxU*, respectively. The transcriptions of the two clusters containing the *hox* genes are both induced under anaerobic conditions concomitantly with the induction of a higher level of hydrogenase activity. An additional TSP, within the annotated alr0760, 244 bp downstream from the suggested translation start codon, was identified. Electrophoretic mobility shift assays with purified LexA from *Nostoc* sp. strain PCC 7120 demonstrated specific interactions between the transcriptional regulator and both *hox* promoter regions. However, when LexA from *Synechocystis* sp. strain PCC 6803 was used, the purified protein interacted only with the promoter region of the alr0750-*hoxE*-*hoxF* operon. A search of the whole *Nostoc* sp. strain PCC 7120 genome demonstrated the presence of 216 putative LexA binding sites in total, including *recA* and *recF*. This indicates that, in addition to the bidirectional hydrogenase gene, a number of other genes, including open reading frames connected to DNA replication, recombination, and repair, may be part of the LexA regulatory network in *Nostoc* sp. strain PCC 7120.

Cyanobacteria are capable of synthesizing two functionally different hydrogenases: an uptake hydrogenase and a bidirectional enzyme (18, 49). So far, the cyanobacterial uptake hydrogenase has been, in all known cases, found only in nitrogen-fixing strains and has the evident function of recycling the H_2 produced by the nitrogen-fixing enzyme, nitrogenase. However, the soluble or loosely membrane-associated bidirectional hydrogenase is an enzyme present in both nitrogen-fixing and non-nitrogen-fixing cyanobacteria (49), and it can catalyze both H_2 uptake and evolution.

The physiological role of the cyanobacterial bidirectional hydrogenase is not fully understood, and several functions have been proposed. It was thought to function predominantly in anaerobic or microaerobic environments catalyzing an uptake of hydrogen produced by other microorganisms (19, 20). It was also proposed as a mediator in the release of excess of reducing equivalents during fermentative growth (47, 51). However, the low K_m for H_2 (2.3 μM) suggests that the enzyme normally operates in the uptake direction (19, 20), and as the proton gradient is directed outward in cyanobacteria, the enzyme might reside at the periplasmic face of the cytoplasmic membrane and allocate electrons to the respiratory chain (22, 42). The coupling of the bidirectional hydrogenase activity to the respiratory chain has also been emphasized by the homology to subunits of the NADH-ubiquinone oxidoreductase (complex I) (2, 10, 39). In cyanobacteria, only an incomplete version of complex I, containing 11 out of the 14 subunits that are strictly conserved in other prokaryotes like *Escherichia coli*, can be found. However, studies of respiration in *Nostoc punctiforme*, a species naturally lacking the bidirectional hydrogenase, demonstrated comparable rates to cyanobacteria having the enzyme (8). In addition, *hoxU* mutants of *Synechococcus* sp. strain PCC 6301 (10) and *hoxEF* mutants of *Synechocystis* sp. strain PCC 6803 showed nonimpaired respiratory O_2 uptake while being affected in H_2 evolution (21). Several strains naturally lack the bidirectional hydrogenase completely, and it seems that it does not play an essential role in strains in which it is present, since inactivation of *hoxH* in both *Synechocystis* sp. strain PCC 6803 and *Nostoc* sp. strain PCC 7120 resulted in only a small decrease in the growth rate compared to that of the wild type (3, 26). In the unicellular, non-nitrogen-fixing *Synechocystis* sp. strain PCC 6803, this enzyme has been suggested to act as an electron valve for low-potential electrons generated during the light reaction of photosynthesis, thus preventing a slowing down of electron transport (3).

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Five subunits have been demonstrated to form the bidirectional hydrogenase in cyanobacteria (41, 42). The enzyme consists of a diaphorase part, encoded by *hoxEFU*, and a hydrogenase part, encoded by *hoxYH*. The physical organizations of the structural genes encoding the bidirectional hydrogenase have strong similarities among different strains. In *Anabaena variabilis* ATCC 29413, *Nostoc* sp. strain PCC 7120, *Synechococcus* sp. strain PCC 6301, and *Synechocystis* sp. strain PCC 6803, one or several additional open reading frames (ORFs) between some of the structural genes have been identified. The intergenic region between *hoxF* and *hoxU* in *Synechococcus* sp. strain PCC 6301 and that in *Nostoc* sp. strain PCC 7120 are longer (333 kb and 8.8 kb, respectively) than those in other strains (49). Transcriptional studies using reverse transcription-PCR (RT-PCR) indicate that the structural *hox* genes form a single transcript together with two ORFs of unknown function in *Anabaena variabilis* ATCC 29413 (9) and forms a single transcript together with three additional ORFs in *Synechocystis* sp. strain PCC 6803 (30). In contrast, the *hox* genes in the unicellular *Synechococcus* sp. strain PCC 6301 are located on two different transcripts. *hoxEF* forms one transcript, and *hoxUYH* is part of a second transcript together with *hoxW*, *hypA*, and *hypB* (9). In *Synechococcus* sp. strain PCC 7942, *hoxEF* and *hoxUYHW* are located on two different transcripts (40). *hoxUYHW* may be polycistronic, with a second promoter located between *hoxH* and *hoxW* (40). The differences in structural gene organization and transcription units may imply a difference in transcriptional regulation between different strains.

The activity of the bidirectional hydrogenase has been investigated previously for both unicellular and filamentous cyanobacteria. In several studies, this activity was demonstrated to be induced under anaerobic conditions (19, 20, 39, 44). The bidirectional hydrogenase in *Nostoc* sp. strain PCC 7120 is present and active in both vegetative cells and heterocysts in aerobically grown filaments. When the cells were transferred to anaerobic conditions, the level of activity of the bidirectional hydrogenase increased by about two orders of magnitude, with roughly equal specific activities in both cell types (19, 20). Similar results were observed for *Anabaena variabilis* ATCC 29413 (44). However, the activity of the bidirectional hydrogenase in the unicellular *Gloeocapsa alpicola* and *Chroococcidiopsis thermalis* is not directly dependent on oxygen (45, 51). Relatively little is known about the transcriptional regulation of the bidirectional hydrogenase in cyanobacteria. The relative abundances of *hoxY* and *hoxH* transcript levels in *Gleocapsa alpicola* did not change significantly under nitrogen-limiting conditions (46), while the activity of the enzyme increased considerably (46, 51). The *hoxH* transcript level was not affected by a shift from ammonium-grown cells to N_2-fixing conditions or the addition of hydrogen for *Nostoc muscorum* (6, 7).

Recently, it was demonstrated that the transcription factor LexA interacts with the regulatory promoter region of the *hox* operon in *Synechocystis* sp. strain PCC 6803 (30), suggested to function as a transcription activator (16). Large-scale analyses demonstrated that the transcriptions of many genes are affected in a *Synechocystis* sp. strain PCC 6803 *lexA*-depleted mutant, i.e., it was possible to identify numerous genes whose expression was either activated or repressed in response to LexA depletion (14). In addition, *Synechocystis* sp. strain PCC 6803 LexA was recently suggested to function as a general regulator of redox-responsive gene expression (1, 33), a concept that discards the classical model of LexA as a DNA binding protein directly involved in the SOS response.

In the present study, the transcription of the structural genes encoding the bidirectional hydrogenase, the *hox* genes, in the filamentous, heterocystous cyanobacterium *Nostoc* sp. strain PCC 7120 was investigated. The regulation of the *hox* operons was examined during a transfer from aerobic conditions to anaerobic conditions. In addition, transcription start sites have been determined, and specific interactions between the transcriptional regulator LexA and the *hox* promoter regions have been observed.

MATERIALS AND METHODS

Organisms and growth conditions. The filamentous heterocystous cyanobacterium *Nostoc* sp. strain PCC 7120 (= *Anabaena* sp. strain PCC 7120) was routinely grown in $BG11_0$ liquid medium (37) at 23°C under a continuous illumination of 40 μmol of photons m^{-2} s^{-1} and by sparging with air. Dark, anaerobic conditions were achieved by replacing the air with 100% argon and covering the culture with aluminum foil. All cultures were mixed with a magnetic stirrer to obtain a homogeneous suspension. *Escherichia coli* strains were grown in LB medium or on agar plates at 37°C.

Nucleic acid extraction. Genomic DNA was isolated from *Nostoc* sp. strain PCC 7120 cultures grown with air. Cells were harvested (4,500 × *g* for 10 min) at room temperature, and DNA was extracted as described previously (48) and resuspended in water. Plasmid DNA was isolated from *E. coli* by using the GenElute plasmid miniprep kit (Sigma-Aldrich). For the isolation of total RNA, *Nostoc* sp. strain PCC 7120 cells were harvested at room temperature and washed once with 2 ml RNAlater (Ambion) followed by centrifugation at 20,000 × *g* for 60 seconds. The cells were disrupted in a Fast-Prep FP 120 BIO 101 homogenizer (Savant) at full speed for 1 min together with 0.6 g acid-washed glass beads (0.6-mm diameter) and 1 ml TRI Reagent (Molecular Research Center, Inc.), followed by a quick cooling on ice. After incubation at room temperature for 8 min, the suspension was transferred to a 2-ml Phase Lock Gel tube (Eppendorf) to which 100 μl BCP (1-bromo-3-chloropropane) and 100 μl water were added. Phases were separated by centrifugation at 12,000 × *g* for 5 min at 4°C. The upper aqueous layer was taken out, and the RNA was precipitated with 250 μl isopropanol and 250 μl RNA precipitation buffer (0.8 M sodium citrate and 1.2 M NaCl) for several hours at −20°C. The RNA was collected by centrifugation at 12,000 × *g* for 15 min, washed twice with 75% ethanol, and resuspended in RNA resuspension solution (1 mM sodium citrate, pH 6.4). RNA samples were further treated with DNase I according to the instructions of the manufacturer (Fermentas).

Agarose gel electrophoresis, PCR, DNA recovery, and sequencing. Agarose gel electrophoresis was used to separate and analyze DNA and RNA according to standard procedures (38). PCRs were carried out in a thermal cycler (Gene Amp PCR system 2400; Applied Biosystems) with either *Taq* DNA polymerase (Fermentas) as described previously (48) or Phusion DNA polymerase (Finnzymes) according to guidelines provided from the supplier. All the oligonucleotides that were used are listed in Table 1. Obtained DNA fragments were isolated from agarose gels with the GFX PCR DNA and Gel Band Purification kit (Amersham Biosciences) according to the manufacturer's instructions. Probes used for electrophoretic mobility shift assay (EMSA) and Northern blotting were analyzed by sequencing before use. Sequencing reactions were performed at Macrogen Inc.

Transcription analysis. RT of 2 μg total RNA extracted from cells grown under dark, anaerobic conditions for 24 h were carried out with the Revert Aid first-strand cDNA synthesis kit (Fermentas), according to the instructions of the manufacturer. The antisense primers used for the cDNA synthesis were AhoxFR and AhoxHR (Table 1). PCR amplification of the *hox* cluster genes (Fig. 1) were performed using corresponding primers (Table 1). Genomic DNA from *Nostoc* sp. strain PCC 7120 was used as a positive control. Negative controls included the omission of reverse transcriptase in the RT reaction and a PCR to which no template was added. For Northern blotting, probes were obtained by PCR using *Nostoc* sp. strain PCC 7120 genomic DNA as the template and primer pairs listed in Table 1. RNA separation, transfer and hybridization were done according to the Hybond-N+ protocol (Amersham Biosciences), making use of probes labeled with [α-^{32}P]dCTP. The even loading of the total RNA aliquots was controlled by verification of equal abundance of the rRNA bands and of the con-

TABLE 1. Oligonucleotides used in this study[a]

Gene, ORF, or vector in indicated expt	Sense primer		Antisense primer		Size (bp)	Reference/ source
	Name	Sequence (5′→3′)	Name	Sequence (5′→3′)		
RT-PCR and Northern blot analysis						
alr0750	Aalr0750F	AGTTGCATTGGATCGCTC CGATAT	Aalr0750R	CTCTGCCCAAATCATGCAC TATATG	467	5
hoxE	AhoxEF	GTGGTGTGTACAGGTA CGGC	AhoxER	TTACCTAAGACTTTGC CATC	188	5
hoxF	AhoxFF	ACATTGGCAGACAAGA ACGC	AhoxFR	TTGCTGACGCATCTTC AGGC	270	5
alr0760	Aalr0760F	TACCCTTCCAGTTCTCCA ATCTTC	Aalr0760R	ATCTATCAACTTCATCTAA GCCG	482	5
alr0761	Aalr0761F	ACAGACAATTTCATGTTA CAAGC	Aalr0761R	TATCAACTTGGATTTGATA TCTAC	424	This work
hoxU	AhoxUF	ACGACCAACTCATTA GCGC	AhoxUR	AGCCACGCAAACAGAAC	290	5
alr0763	Aalr0763F	GACAAGCAACAACCAGA	Aalr0763R	CTGGTGCTTCATCTAGGAA	417	This work
hoxY	AhoxYF	TGAAGTTAGCAACAGT ATGG	AhoxYR	AAGACTGATTCAGCAC TACC	307	This work
alr0765	Aalr0765F	GCCACGAACAAGATGCT	Aalr0765R	TTTCGGCTCGTTGGTGT	417	This work
hoxH	AhoxHF	GACTATTGCCTTGGA TGCT	AhoxHR	GACAGAATATCTGGGT CGTT	513	This work
rnpB	rnpBF	GACCAAACTTGCTGGAT AACG	rnpBR	TTGCGAGGGCAGTTATC TATC	331	This work
lexA	LexAF	TCGTCAGATGATGCAGGC GATGAA	LexAR	AGTGATGCGATCGCCACTT CGATA	424	This work
Cloning—*lexA*	AlexAF2	GGATCCGAACGCCTAACA GAAGCGCA	AlexAR2	AAGCTTTCACATATAACCG CGCCACA	618	This work
	AlexAF3	GAATTCATGGAACGCCTA ACAGAAGCG	AlexAR3	GGATCCTCACATATAACCG CGCCACAC	618	This work
Identification of TSPs						
alr0750			Aalr0750R3	CTCTGCCCAAATCATGCAC TATA		This work
			Aalr0750R4	GTATTACCTGCGGTTGTT		This work
hoxU			AhoxUR	AGCCACGCAAACAGAAC		This work
EMSA						
alr0750 fragment	Aalr0750F2	AAGTAAGCTAGAAGGCG CTTGC	Aalr0750R2	ATATCGGAGCGATCCAAT GCAACT	499	This work
hoxU fragment	Aalr0761F3	GCATCTGGTTTAAATGG TTAC	AhoxUR2	GCGCTAATGAGTTGG TCGT	535	This work
pQE-30	pQEF	CCCGAAAAGTGCCACCTG	pQER	GTTCTGAGGTCATTACTGG	334	This work

[a] The underlined base pairs correspond to restriction sites.

stitutive RNA component of the ribozyme RNase P (52). The relative positions of the isotope were visualized using a BAS-2000II bioimaging analyzer (Fuji Film) or a Pharos FX Plus molecular imager (Bio-Rad).

Identification of TSPs. Transcription start points (TSPs) were localized with the a system for rapid amplification of cDNA ends (5′ RACE; Invitrogen). First-strand cDNA synthesis was performed using 3 μg of total RNA under aerobic and dark, anaerobic conditions together with random hexamer primers (Fermentas). PCR products obtained with the gene-specific primers listed in Table 1 were cloned into the pCR2.1-TOPO vector (Invitrogen) according to the manufacturer's instructions before being sequenced.

Cloning of *lexA* and purification of the gene product. *Nostoc* sp. strain PCC 7120 *lexA* was amplified from genomic DNA by using the primers AlexAF2 and AlexAR2 (Table 1). The obtained PCR product was cloned into the pCR 2.1-TOPO (Invitrogen) vector and confirmed by sequencing. The *Nostoc* sp. strain PCC 7120 *lexA* was further subcloned into the pQE-30 (QIAGEN) vector by using the restriction enzymes BamHI and HindIII and introduced into M15 (pREP4) cells (QIAGEN). After DNA sequencing confirmed that no mutations had been introduced, *Nostoc* sp. strain PCC 7120 LexA was overexpressed by induction with IPTG (isopropyl-β-D-thiogalactopyranoside) and purified using Ni-nitrilotriacetic acid Superflow resin (QIAGEN) according to the instructions of the manufacturer. The obtained *Nostoc* sp. strain PCC 7120 LexA His-tagged protein was analyzed by Coomassie blue staining of a sodium dodecyl sulfate-polyacrylamide gel (see Fig. 4). *Synechocystis* sp. strain PCC 6803 His-tagged LexA was purified as described previously (30).

EMSA. The different DNA fragments were obtained by PCR using *Nostoc* sp. strain PCC 7120 genomic DNA or pQE-30 vector (QIAGEN) as the template (for details, see Table 1 and Fig. 3). All fragments were end labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase (Fermentas) as described (31). Various amounts of purified LexA from *Nostoc* sp. strain PCC 7120 or *Synechocystis* sp. strain PCC 6803 were incubated with 20 fmol of each fragment alone or together with 20 fmol of labeled unrelated DNA. For binding competition experiments, an excess of either specific or nonspecific unlabeled fragments was included. LexA from *Nostoc* sp. strain PCC 7120 was used in assays carried out as described previously (28), except that 1 μg of salmon sperm DNA was included in the incubation buffer. Assay mixtures were incubated at 30°C for 30 min before separation on a 6% (wt/vol) nondenaturing polyacrylamide gel at 200 V. The assays performed with *Synechocystis* sp. strain PCC 6803 LexA were carried out as described previously (30), and the reaction mixtures were separated by electrophoresis on a 10 to 15% (wt/vol) gradient native polyacrylamide gel by using the PhastSystem (Amersham Biosciences). All gels were visualized using a BAS-2000II bioimaging analyzer (Fuji Film).

In vivo bidirectional hydrogenase activity measurement. The activity of the bidirectional hydrogenase was assayed by determining the evolution of H_2 from methyl viologen reduced by sodium dithionite as described previously (48), using

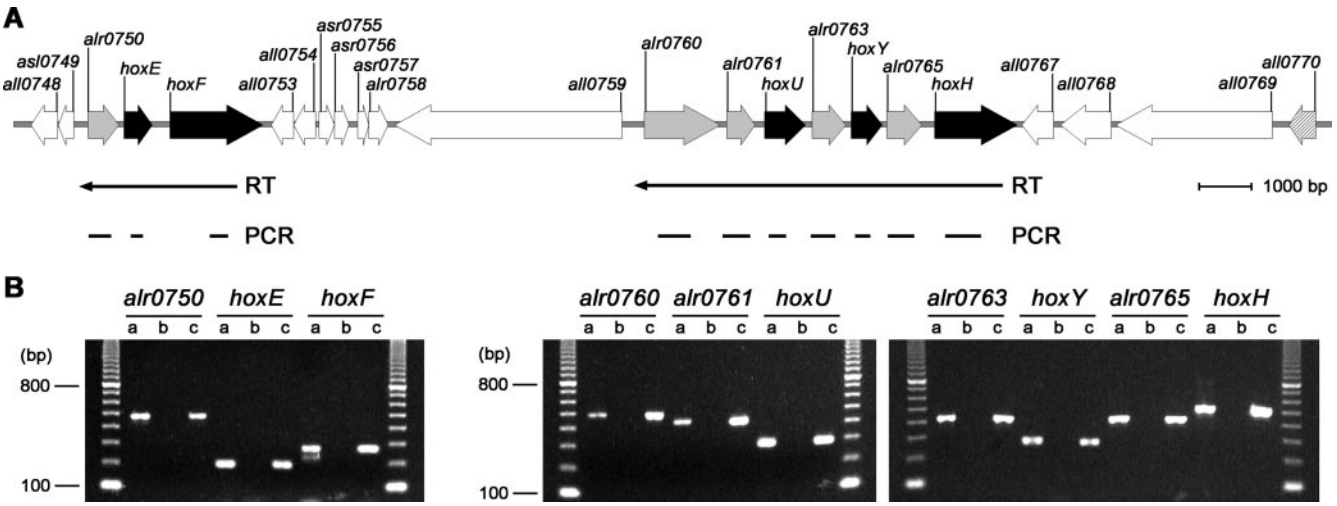

FIG. 1. (A) Physical map of the *hox* gene clusters (depicted in black) in *Nostoc* sp. strain PCC 7120. ORFs present on the same transcript as the *hox* genes are depicted in gray, and the ORF corresponding to the putative bidirectional-hydrogenase-specific C-terminal endopeptidase, *hoxW* (all0770), is striped. (B) RT-PCR analysis of the two *hox* gene clusters. Total RNA was isolated from cells grown under dark, anaerobic conditions for 24 h. The RT reactions were performed with primers against *hoxF*, followed by PCRs for alr0750, *hoxE*, *hoxF*, and *hoxH*, followed by PCRs for alr0760, alr0761, *hoxU*, alr0763, *hoxY*, alr0765, and *hoxH* (primers are listed in Table 1). Lanes: a, RT-PCR product; b, negative control without reverse transcriptase in the RT reaction prior to the PCR; c, PCR-positive control; marker, 100-base-pair ladder (Amersham Biosciences) where the lowest visible band corresponds to 100 base pairs.

a Clarus 500 gas chromatograph with a Molecular Sieve 5A 60/80 mesh column (Perkin Elmer) and Ar as the carrier gas.

RESULTS

Characterization of the *hox* transcription units. The *hox* genes encoding the bidirectional hydrogenase in the filamentous, nitrogen-fixing cyanobacterium *Nostoc* sp. strain PCC 7120 (= *Anabaena* sp. strain PCC 7120) are located in two different gene clusters separated by approximately 8.8 kb (Fig. 1A). To investigate the transcription units of the *hox* genes, RT reactions were carried out using total RNA extracts from *Nostoc* sp. strain PCC 7120. The two primers AhoxFR and AhoxHR (Table 1) were used as *hoxF*- and *hoxH*-specific antisense primers, respectively, and the resulting cDNA was used as the template in PCR amplifications. From the RT reaction originating from *hoxF*, it was possible to obtain PCR products for *hoxF*, *hoxE*, and the ORF alr0750, suggesting that all three genes are located on at least one single transcript (Fig. 1B). From the RT reaction originating from *hoxH*, PCR products were obtained from all six upstream genes, including the four ORFs located before and between the *hox* genes, suggesting a larger transcript harboring alr0760, alr0761, *hoxU*, alr0763, *hoxY*, alr0765, and *hoxH*. However, when using probes against genes in the two putative operons and Northern blot hybridizations, the transcript levels of the two ORFs located upstream from *hoxU* (alr0760 and alr0761) were significantly lower than those for any other genes (see Fig. 3A, for cells with induced hydrogenase activity, and see below).

Attempts to identify TSPs upstream from alr0750, alr0760, and *hoxU* were performed by using the 5′ RACE system. Although no TSP could be found upstream from alr0760, a TSP was identified within the annotated ORF, 244 bp downstream from the translation start codon. Interestingly, it was possible to find additional ORFs within the annotated alr0760 corresponding to shorter versions, since the putative translation start points are found in the same frame as in the original alr0760. One of those additional ORFs starts 321 nucleotides downstream from the annotated ATG start codon and 78 bp downstream from the putative TSP identified in this work by use of 5′ RACE. The N-terminal region of Alr0760, in particular, the first 80 amino acids, shows significantly lower similarity to this TSP than the remaining part of the protein in BLAST searches. This may indicate that the identified TSP could be real and located within a falsely annotated gene. The presence of multiple TSPs also cannot be excluded, and this occurrence has been reported previously, e.g., *ntcA* of *Nostoc* sp. strain PCC 7120 shows multiple transcripts with different 5′ ends depending on nitrogen availability (35). However, no significant sequence motifs could be found either upstream from the identified TSP within alr0760 or upstream from the translation start codon of the annotated ORF. TSPs were also identified upstream from alr0750 and *hoxU*, located 66 bp and 57 bp upstream from the respective start codons. The 5′ RACE experiments were performed using RNA extracted from cells of *Nostoc* sp. strain PCC 7120 grown aerobically and from cells induced in dark, anaerobic conditions for 24 h with identical results. Furthermore, when analyzing the different promoter regions, it was possible to identify putative sigma^{70} promoter sequences and ribosomal binding sites upstream from alr0750 and *hoxU* (Fig. 2). The positions and characters of the −10 and −35 elements show similarity to known promoters from other genes in cyanobacteria (13). Interestingly, the alr0750 and *hoxU* promoter regions have segments of 40 nucleotides that share a high degree of homology, suggesting a similar regulation of the two different promoter regions. No significant sequence motifs could be found upstream from the identified TSP within the annotated ORF alr0760. By use of Northern hybridizations, accumulations of transcripts of approximately

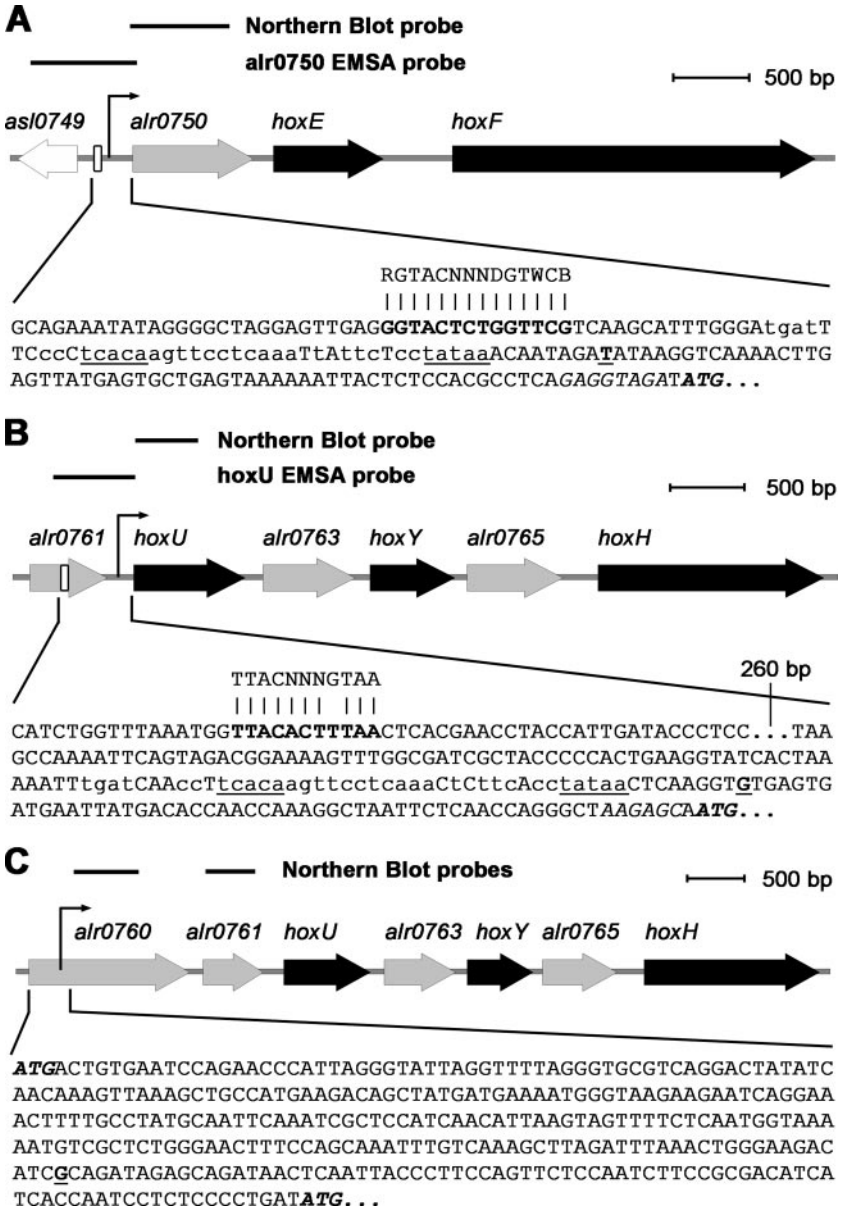

FIG. 2. Schematic representation of the *Nostoc* sp. strain PCC 7120 *hox* gene clusters and the regulatory promoter regions of the two operons. Positions of the DNA probes used for Northern blot hybridizations (Fig. 3) and DNA fragments used in EMSAs (Fig. 5 and 6) are depicted as lines above the respective maps. The identified TSPs are indicated by arrows and depicted in boldface and underlined in the sequence. Putative −10 and −35 regulating sequences are underlined, and the segments of 40 nucleotides that show a high degree of homology in the two promoter regions of alr0750 and *hoxU* are indicated with the matching nucleotides shown in lowercase. The ribosomal binding site is shown in italics, and the start codons of *hoxE* (A), *hoxU* (B), and alr0760 (C) are shown in boldface and italics. The putative LexA binding sites are indicated with a white box in the map and depicted in boldface in the sequences. In addition, the suggested consensus LexA binding sites (11, 27) are shown above the *Nostoc* sp. strain PCC 7120 sequences.

1,200 and 1,900 nucleotides were observed when using alr0750 and *hoxU* as probes, respectively (Fig. 3B). The 1,200-nucleotide transcript, starting from the identified TSP, would cover alr0750 and *hoxE*, ending in the intergenic region between *hoxE* and *hoxF*. However, no TSP upstream from *hoxF* could be identified (5). The 1,900-nucleotide transcript may cover *hoxU*, alr0763, and part of *hoxY*.

Anaerobic induction of the two *hox* gene clusters. It has been demonstrated that anaerobic conditions induce higher transcription levels of *hoxH* transcript in the filamentous, heterocystous *Nostoc muscorum* and *Anabaena variabilis* ATCC 29413 (6, 46). In the present study, when cells of *Nostoc* sp. strain PCC 7120 were transferred to anaerobic conditions and the relative amounts of alr0750 and *hoxU* transcript were monitored by Northern blot hybridization, significantly higher transcript levels were observed (Fig. 3B) with a simultaneous increase in the enzyme activity (from 3.29 ± 1.75 nmol H_2 evolved · h^{-1} · μg chlorophyll a^{-1} under aerobic conditions to

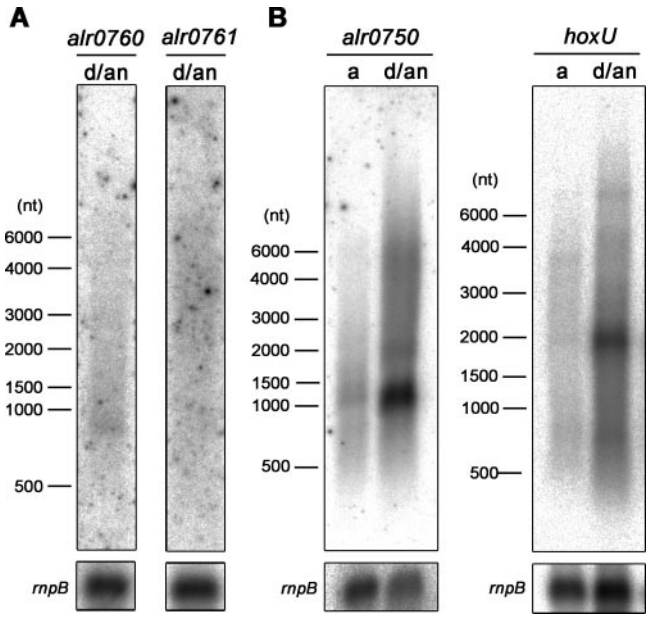

FIG. 3. Northern blot analysis of alr0760 and alr0761 (A) and alr0750 and *hoxU* (B) in *Nostoc* sp. strain PCC 7120 under aerobic (a) and dark, anaerobic (d/an) conditions. Probes for the Northern blot hybridization were obtained by PCR using *Nostoc* sp. strain PCC 7120 genomic DNA as the template and primer pairs Aalr0760F-Aalr0760R and Aalr0761F-Aalr0761R (A) and Aalr0750F-Aalr0750R and AhoxUF-AhoxUR (B) (see Table 1). Total RNA was isolated from *Nostoc* sp. strain PCC 7120 cultures grown in air or from cultures induced in dark, anaerobic conditions for 24 h, and a total amount of 10 μg RNA was used for each lane. The *rnpB* gene was used to standardize the loading and transfer of the RNA. Numbers on the left side of the panels indicate sizes in nucleotides (nt).

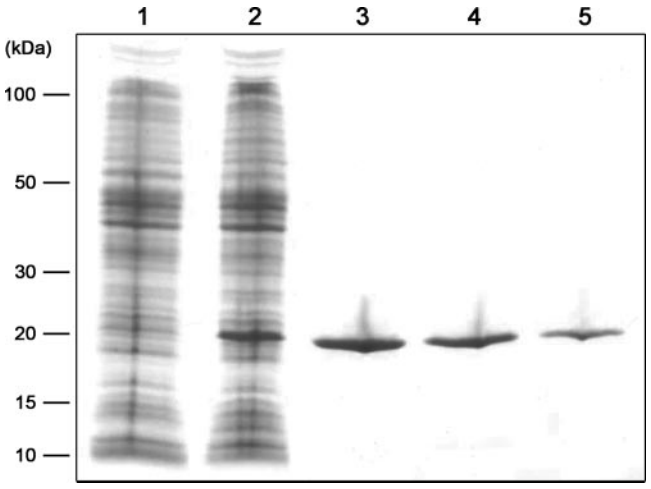

FIG. 4. Overexpression and purification of *Nostoc* sp. strain PCC 7120 LexA. Lanes: 1, noninduced crude extract; 2, induced crude extract; 3 through 5, eluates of purified *Nostoc* sp. strain PCC 7120 LexA.

32.83 ± 2.50 nmol H_2 evolved $\cdot$ h^{-1} $\cdot$ μg chlorophyll a^{-1} under dark, anaerobic conditions), in agreement with earlier physiological studies (19, 20). This also suggests that the regulations of the *hox* genes, located on two different operons, are similar with respect to O_2.

LexA interacts with the promoter regions of the *hox* genes. The sequence GGTACTCTGGTTCG found 63 to 76 bp upstream from the TSP of alr0750 (Fig. 2A) corresponds to the previously defined motif RGTACNNNDGTWCB, described as a LexA box in cyanobacteria (27). Recently, LexA was shown to interact with the promoter region of the bidirectional hydrogenase in *Synechocystis* sp. strain PCC 6803 (30). Additional studies were therefore carried out in order to clarify if this novel role of LexA is specific for *Synechocystis* sp. strain PCC 6803 or if its role can be broadened to other cyanobacteria. A 499-bp fragment containing part of the upstream region of alr0750 (herein referred to as the alr0750 fragment), harboring the putative binding site, was used in EMSAs with purified His-tagged LexA from *Nostoc* sp. strain PCC 7120 (Fig. 4). Increasing amounts of LexA produced a retardation which increased in intensity in relation to the amount of protein, while an unrelated DNA fragment produced no retardation (Fig. 5A). To stress the specificity in the binding, competition assays were carried out with either unlabeled specific fragments or unlabeled nonspecific fragments: it was clearly shown that a nonspecific fragment does not compete with the fragment harboring the LexA binding site (Fig. 5B). Since the five *hox* genes in *Nostoc* sp. strain PCC 7120 are separated on two independent transcripts, it would be expected that they are regulated in a similar manner. However, the LexA box located upstream from alr0750 could not be found upstream from *hoxU*. In a recent report, the LexA-binding sequence of *Bdellovibrio bacteriovorus* was identified, and it was further suggested that the cyanobacterial LexA is probably a common ancestor of the *B. bacteriovorus* LexA (11). In fact, EMSAs using *Nostoc* sp. strain PCC 7120 LexA showed that it could recognize the LexA box of *B. bacteriovorus* (11). The consensus sequence TTACNNNGTAA used in the same work to identify additional LexA binding sites in the genome of *B. bacteriovorus* (11) shows similarity to a stretch of 11 bp upstream from *hoxU* (Fig. 2B). A 535-bp fragment harboring this site (herein referred to as the *hoxU* fragment) was therefore used to elucidate whether LexA interacts with the promoter region of the second cluster as well. The results clearly demonstrate a specific interaction between the *hoxU* fragment and LexA (Fig. 5C and D), and we conclude that LexA can in fact interact with the promoter regions of the two *hox* clusters.

Additionally, LexA from the *Synechocystis* sp. strain PCC 6803, another cyanobacterial strain, was further used in EMSAs, making use of the same alr0750 and *hoxU* DNA fragments, to determine whether the identified LexA binding sites found in *Nostoc* sp. strain PCC 7120 can be recognized by a close phylogenetic relative. These experiments clearly demonstrated that there is indeed an interaction between LexA from *Synechocystis* sp. strain PCC 6803 and the promoter region of alr0750, the region containing the cyanobacterial LexA binding box (Fig. 6). Moreover, the signal decreased when increasing amounts of unlabeled alr0750 fragment were added, indicating a specific binding, and no shift was observed when an unrelated DNA fragment was used (Fig. 6). However, no shift was observed when the DNA fragment covering the promoter region of *hoxU* (data not shown) containing the LexA binding site similar to that of *B. bacteriovorus* was used.

The LexA box is widespread in the *Nostoc* sp. strain PCC 7120 genome. The *Nostoc* sp. strain PCC 7120 LexA box

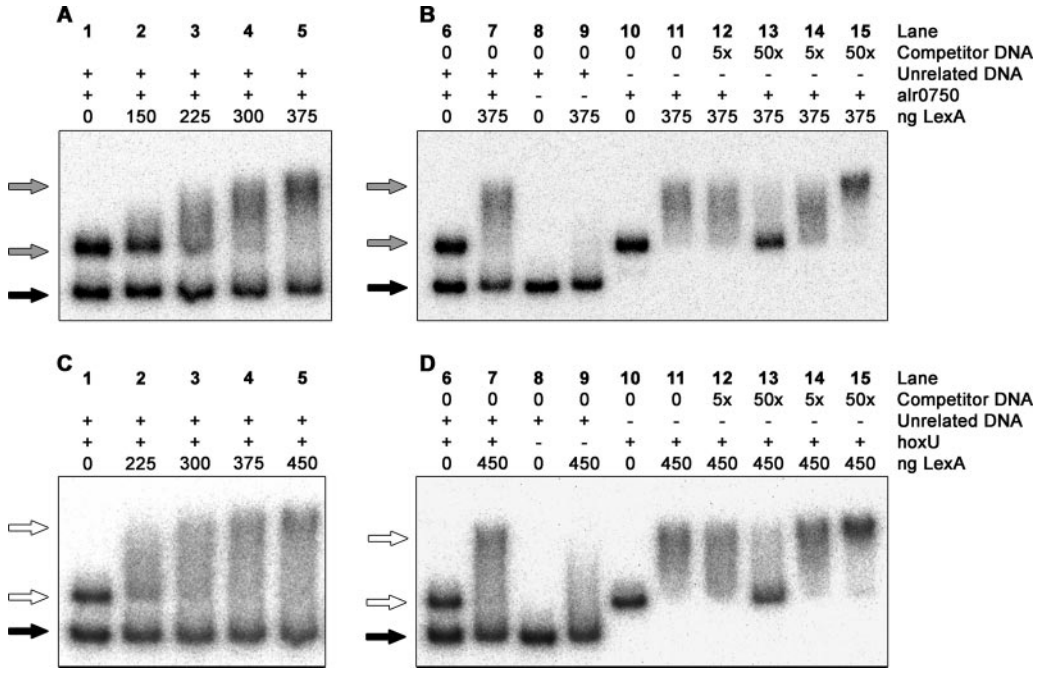

FIG. 5. EMSAs with purified LexA from *Nostoc* sp. strain PCC 7120 and the two regulatory *hox* operon regions. The alr0750 (A and B) and *hoxU* (C and D) DNA fragments were generated by PCR using genomic DNA from *Nostoc* sp. strain PCC 7120, and the unrelated DNA fragment was generated by using pQE-30 vector (QIAGEN). The positions of the DNA fragments relative to the regulatory regions of the *hox* operons are shown in Fig. 2. EMSAs were first carried out with the specific alr0750 (A) and *hoxU* (C) fragments, incubated together with the unrelated DNA fragment without protein (lane 1) or together with increasing amounts of LexA (lanes 2 through 5). The unrelated DNA is indicated with a black arrow and the alr0750 and *hoxU* fragments and their retardations are indicated with gray and white arrows, respectively. EMSAs were carried out to demonstrate the specificities in the binding between *Nostoc* sp. strain PCC 7120 LexA and the alr0750 (B) and *hoxU* (D) fragments. The unrelated DNA was incubated with and without LexA, alone or together with the alr0750 or *hoxU* fragment (lanes 6 through 9). The alr0750 and *hoxU* fragments were also incubated together with a 5× or 50 × excess of either unlabeled specific (lanes 12 and 13) or nonspecific (lanes 14 and 15) DNA. The amount of LexA (in ng) used for each lane is indicated in the figure. A plus sign indicates that the fragment was included in the assay and a minus sign indicates that the fragment was excluded from the assay.

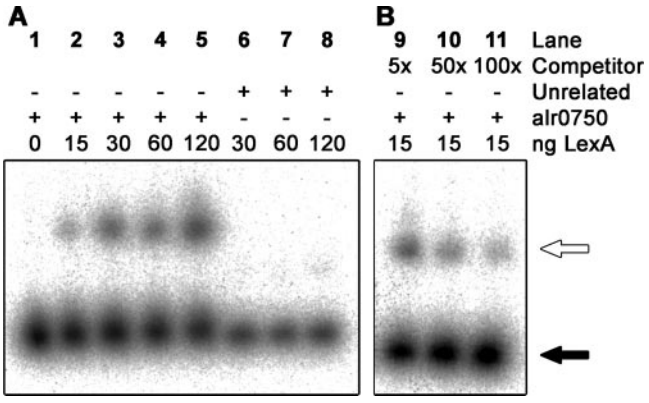

FIG. 6. EMSAs with purified LexA from *Synechocystis* sp. strain PCC 6803 and the *Nostoc* sp. strain PCC 7120 regulatory *hox* operon region covered by the alr0750 DNA fragment. The fragments used were generated by PCR using genomic DNA from *Nostoc* sp. strain PCC 7120 (lanes 1 through 5 and 9 through 11) or pQE-30 vector from QIAGEN (unrelated DNA, lanes 6 through 8). The position of the DNA fragment relative to the regulatory region of the *hox* operon is shown in Fig. 2. The assays were carried out using 20 fmol of each labeled fragment incubated with 0, 15, 30, 60, or 120 ng purified LexA (lanes 1 through 5). Competition experiments were carried out with 15 ng LexA in the presence of 5×, 50 ×, and 100 × molar excesses of the unlabeled alr0750 fragment as competitor DNA (lanes 9 through 11). White arrows indicate unbound DNA fragments, and black arrows indicate a shift, i.e., LexA bound to the DNA fragment.

RGTACNNNDGTWCB (27) was used in a whole-genome search, using a Perl script developed in-house, to identify other genes associated with the binding sequence. To broaden the search, two more variations of the sequence were included. The C in the first part of the motif was therefore exchanged for a T (RGTAC→RGTAT) to include the LexA binding site found upstream from *recA* in *Nostoc* sp. strain PCC 7120 (27). The first part of the motif was also altered by replacing the T with an A (RGTAC→RGAAC), a change made so the motif would resemble the closely related gram-positive LexA binding site CGAACRNRYGTTYC (55). LexA has previously been shown to interact with both these motifs in *Nostoc* sp. strain PCC 7120, although it interacts with the latter with a lower efficiency (27). A total number of 216 putative binding sites were found in the genome of *Nostoc* sp. strain PCC 7120; most of them, though, were located within annotated ORFs. A selected part of the identified putative binding sites found is listed in Table 2. Functional LexA binding sites have been previously reported to be located within ORFs (4, 33), and a few interesting putative binding sites located within ORFs are therefore also included in Table 2.

DISCUSSION

The results demonstrate that the *hox* genes, encoding the bidirectional hydrogenase, in *Nostoc* sp. strain PCC 7120 are

TABLE 2. Putative LexA binding sites in the genome of *Nostoc* sp. strain PCC 7120

Sequence	ORF	Position[a]	Protein annotation[b]	Putative function/comment/reference
AGTACTTTTGTTCC	all0061	−869	Site-specific DNA-methyltransferase	Inside alr0063 and *rbfA*
AGTACTTATGTACT	alr0088	−28	Single-stranded DNA-binding protein	27
AGTACTTTTGTACT	asl0401	−71	Unknown protein	Probable nitroreductase
AGAACCAGAGTTCC	all0687N	−246	[NiFe] uptake hydrogenase large subunit, N end fragment	Inside all0688 (*hupS*), located upstream from all0687N
GGTACTCTGGTTCG	alr0750	−142	Hypothetical protein	Encodes universal stress protein (Usp)
GGAACAGGAGTACT	all0998	+630	Putative catalase	Inside gene, protein contains manganese catalase domain
AGTACTTAAGTACT	alr1041	−175	Fructose-1,6-bisphosphatase	
AGAACTTTAGTTCT	alr1194	−425	Two-component response regulator	
GGTACTGTAGTACG	all1512	−311	Cytochrome b_6/f-complex iron-sulfur protein PetC	Inside asl1513, encodes hypothetical protein
AGAACAATTGTACC	alr1855	−150	Unknown protein	Similar to DNA double-strand break repair *rad50* ATPase gene
GGTACTGATGTTCC	alr1879	+228	Glycogen synthase	Inside gene
AGTACATAAGTACT	alr2104	−59	Probable methyltransferase	
AGAACTTTAGTTCT	alr2481	−265	Two-component sensor histidine kinase	
AGTACAGAAGTTCT	all3374	+654	DNA repair and genetic recombination protein RecF (encoded by *recF*)	Inside gene
AGTACTTAAGTACC	all3503	−109	Probable integrase	
AGTACTATTGTTCT	alr3716	−84	Excinuclease ABC subunit A (encoded by *uvrA*)	27
AGTACCAAAGTTCT	asl3860	−46	Glutaredoxin	
GGTACTTGTGTTCC	all3866	−234	Probable dioxygenase, Rieske iron-sulfur component	Inside alr3867, encodes putative aminotransferase
GGTACTTTTGTTCG	alr4239	−241	Toxin secretion ABC transporter ATP-binding protein	Upstream from alr4240, encodes RTX toxin transporter
GGTACAAATGTACG	all4790	−45	Hypothetical protein	Upstream from all4789, encodes DNA helicase Re cG
AGTACTAATGTTCT	alr4908	−47	SOS function regulatory protein, LexA repressor	Upstream from alr4909, similar to DNA repair gene *rad25* (27)
AGTACTATGGTACG	asr4942	−53	Unknown protein	Upstream from alr4943, encodes putative 4Fe-4S cluster binding protein
GGTACTCCAGTACG	all0475	+528	Probable short-chain dehydrogenase	Inside gene
GGTATTTATGTACT	alr1044	−472	Transcriptional regulator	
GGTATCGCTGTACG	alr1095	+687	GAPDH (encoded by *gap3*)	Inside gene
GGTATCGTTGTACT	alr2780	−276	DNA topoisomerase I	
AGAATTAGTGTACC	all2951	+33	Probable helicase protein	Inside gene
AGTATTATTGTTCC	all5215	−104	Transcriptional-repair coupling factor	Helicase domain
GTATCCAAGTTCC	all3735	−291	Fructose-bisphosphate aldolase class I	Inside all3736, encodes unknown protein
AGTATATCTGTTCT	all3272	−66	Recombination protein RecA (encoded by *recA*)	27

[a] Position of the first base pair in the motif in relation to the TSP of the downstream gene.
[b] According to Cyanobase (http://www.kazusa.or.jp/cyano/cyano.html).

located in at least two separate operons. Two of the three genes encoding the diaphorase part (*hoxE* and *hoxF*) form one operon together with the ORF alr0750. The third diaphorase subunit, *hoxU*, is thus separated from *hoxE* and *hoxF* and located on a second operon together with the two genes *hoxY* and *hoxH*, which encode the hydrogenase part. Interestingly, a similar genomic arrangement and transcription units of the *hox* genes are found in the unicellular cyanobacteria *Synechococcus* sp. strain PCC 7942 (40) and *Synechococcus* sp. strain PCC 6301 (10).

In order to study the transcription of the *hox* genes in *Nostoc* sp. strain PCC 7120, two different techniques were used: RT-PCR and Northern blotting. Both confirmed the existence of two separate operons and, in addition, due to different sensitivities, resulted in additional and complementary information. The very sensitive RT-PCR experiments demonstrated that alr0760 and alr0761 are part of a long transcript together with *hoxU*-alr0763-*hoxY*-alr0765-*hoxH*. However, the very low transcript levels of alr0760 and alr0761 observed by use of Northern blot hybridization (Fig. 3A) indicate that the alr0760-*hoxH* transcript is present only at a very low level, meaning that it is not the main contributor of the *hoxUYH* transcripts to the total mRNA pool. This suggestion is supported by the TSP upstream from *hoxU*. However, a careful examination of the Northern blot hybridizations revealed a rather complex transcription of the polycistronic *hoxU*-alr0763-*hoxY*-alr0765-*hoxH* transcription. In addition to the fainter signal in the form of a band that smears down on the lane (*hoxU*; Fig. 3B), matching to the large transcript, the predominance of shorter transcripts of approximately 1,900 nucleotides can be distinguished. Considering the size of the band and the TSP found upstream from *hoxU*, it is possible to predict that the band corresponds to the transcript consisting of *hoxU*, alr0763, and part of *hoxY*. A similar situation has been reported for *Synechococcus* sp. strain PCC 7942 by Schmitz et al. (40), who used reporter gene constructs and real-time RT-PCRs. In addition, they showed

TABLE 3. Annotations of genes and the ORFs found in the vicinity of the *hox* genes (Fig. 1A) in *Nostoc* sp. strain PCC 7120

Gene/ORF	Protein annotation[a]	Putative function/characteristic of protein	Reference
all0748	Hypothetical protein		
asl0749	30S ribosomal protein S15		
alr0750	Hypothetical protein	Contains UspA domain	
hoxE	NADH dehydrogenase I chain E		42
hoxF	Hydrogenase subunit; HoxF		42
all0753	Hypothetical protein	Toxin of toxin-antitoxin system (*vapC*)	32
all0754	Unknown protein	Antitoxin of toxin-antitoxin system (*vapB*)	32
asr0755	Hypothetical protein		
asr0756	Unknown protein		
asr0757	Unknown protein	Antitoxin of toxin-antitoxin system *(mazE*)	32
alr0758	Hypothetical protein	Toxin of toxin-antitoxin system (*mazF*)	32
all0759	WD repeat-containing protein	Contains WD-40 (Trp-Asp, ~40 amino acids) repeat domains	
alr0760	Hypothetical protein		
alr0761	Unknown protein		
hoxU	Hydrogenase chain U		42
alr0763	Hypothetical protein		
hoxY	Hydrogenase small subunit; HoxY		42
alr0765	Hypothetical protein	Contains CP12 domain	32
hoxH	Hydrogenase large subunit; HoxH		42
all0767	Hypothetical protein	Endoribonuclease domain	
all0768	Hypothetical protein	Acetyltransferase	
all0769	Acetyl coenzyme A synthetase		
all0770	Hypothetical protein	*hoxW* protein; hydrogenase-specific endopeptidase	56

[a] According to Cyanobase (www.kazusa.or.jp/cyano/cyano.html).

that the level of expression of *hoxU* is approximately six times higher than that of *hoxH*, even though *hoxU* and *hoxH* are clustered and belong to the same operon. In agreement, we suggest that the bands with stronger signals in our Northern blot hybridizations correspond to partial termination of the transcription and/or mRNA instability within the regions between *hoxY* and *hoxH*. Interestingly, a similar observation can be made for the operon alr0750-*hoxE-hoxF*: the 1,200-nucleotide strong band in the Northern blot hybridizations, occurring when using the alr0750 probe (Fig. 3B), may correspond to the alr0750-*hoxE* transcript. It is also important to notice that the Northern blot hybridizations in the present work are based on double-stranded DNA fragments, denatured at 95°C prior to the hybridization. As a consequence, any presence of antisense RNA, as has been described for the *hox* operons in *Synechococcus* sp. strain PCC 7942 (40), will result in hybridization patterns with more complex interpretations.

The roles of the ORFs transcribed together with the structural *hox* genes as well as the ORFs located around the two *hox* clusters are unclear. For most of them, no obvious connection to either the bidirectional hydrogenase or the hydrogen metabolism can be found. The three ORFs alr0750, alr0763, and alr0765, found on the same transcripts as the *hox* genes, are all physically located in the same position in *Anabaena variabilis* ATCC 29413 (42; see also the U.S. Department of Energy Joint Genome Institute website [http://genome.jgi-psf.org/finished_microbes/anava/anava.home.html]), although in this strain, the *hox* genes are located in one single cluster. Interestingly, alr0750 and alr0765 are also found in *Nostoc punctiforme*, a species lacking the bidirectional hydrogenase (48). Homologues of alr0760 are present in both *Anabaena variabilis* ATCC 29413 and *Nostoc punctiforme*. In Table 3, the annotations of all genes noted in Fig. 1A are listed, and for some of the genes, putative functions and characteristics are proposed.

The first gene in the first *hox* cluster, alr0750, contains an UspA (universal stress protein) domain. UspA in *E. coli* has been reported to be stimulated in response to a large variety of conditions, including, e.g., carbon and nitrogen (25). Nachin et al. (29) proposed that Usp proteins have evolved different physiological functions to reprogram the cell to defense during cellular stress. They further showed that UspA is involved in regulating the capacity for the cells to withstand oxidative agents. Larger proteins in the Usp family in *Archaea*, cyanobacteria, and plants often contain other functional domains (25), and the domain found in alr0750 also includes a part of an Na^+/H^+ antiport motif. There are no obvious putative domains in alr0760, alr0761, or alr0763. Orf3 in *Anabaena variabilis* ATCC 29413 (9, 49), corresponding to alr0765 in *Nostoc* sp. strain PCC 7120, shows homology to a small protein named CP12 (34). This protein has been reported to oligomerize with two of the key enzymes in the Calvin cycle, phosphoribulokinase (PRK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Recent data suggest that under light and dark conditions, the oligomerization of CP12 with PRK and GAPDH regulates the activities of both these enzymes and, thus, the carbon flow from the Calvin cycle to the oxidative pentose phosphate cycle in the cyanobacterium *Synechococcus* sp. strain PCC 7942 (50). Furthermore, the discovery of genes for CP12 in mosses, green algae, and cyanobacteria, together with the demonstration of conserved PRK/CP12/GAPDH complex composition and function in *Chlamydomonas* and *Synechocystis* spp. suggests that the regulation of the Calvin cycle, via an NADPH-mediated dissociation of the complex, is conserved in all photosynthetic organisms (34, 54). Wedel and Soll (54) stated that the detection of proteins containing CP12, or parts of it, shows that these motifs have been used as evolutionary conserved modules for natural genetic engineering, allowing control of different enzymatic activities by

NADP(H). The last step in maturation of NiFe enzymes involves the endoproteolytic cleavage of a C-terminal peptide of the large subunit precursor (12). The gene encoding the endopeptidase responsible for the cleavage in *Nostoc* sp. strain PCC 7120, the *hoxW*, was found to be located in close proximity to the *hox* gene clusters (Fig. 1A) (56). Four of the ORFs located in the 8.8-kb DNA region between the two *hox* clusters, all0753, all0754, asr0757, and alr0758 (Fig. 1A and Table 3), were recently identified as toxin-antitoxin loci (32). These loci seem to be abundant in bacterial and archaeal chromosomes (15), and suggested functions include nutritional stress response elements and/or quality control elements increasing the fitness of free-living prokaryotes (32). Interestingly, toxin-antitoxin loci are also found between the *hox* genes in *Anabaena variabilis* ATCC 29413 (U.S. Department of Energy Joint Genome Institute [http://genome.jgi-psf.org/finished_microbes/anava/anava.home.html]) and *Synechocystis* sp. strain PCC 6803 (32). In *Synechocystis* sp. strain PCC 6803, the two ORFs constituting the toxin-antitoxin locus are part of the *hox* operon (30).

Looking carefully at the promoter regions of the two *hox* operons in *Nostoc* sp. strain PCC 7120, it is possible to observe that the positions and characters of the −10 and −35 boxes show similarity to known promoters from other genes in cyanobacteria (13). The segments of 40 nucleotides of the alr0750 and *hoxU* promoter regions that share a high degree of homology (Fig. 2) harbor the promoter recognition elements, suggesting a regulation common between the different transcripts. During searches for additional recognition motifs upstream from the transcriptional start site of alr0750 and *hoxU*, putative FNR (fumarate and nitrate reduction) binding sites at positions 218 to 231 bp and 208 to 221 bp upstream from the TSP, respectively, showing high similarity to the *E. coli* FNR consensus sequence could be found (43). FNR proteins are global transcription regulators that respond to fluctuations in environmental oxygen, and in *E. coli*, they regulate the response to the transition between aerobic growth and anaerobic growth (23). A putative FNR binding site has been previously reported to be upstream from *hupSL* genes encoding the uptake hydrogenase in *Anabaena variabilis* ATCC 29413 (17). The *hyn* operon of the purple sulfur bacterium *Thiocapsa roseopersicina*, encoding a membrane-associated [NiFe] hydrogenase, is up-regulated under anaerobic conditions, and it was recently demonstrated that an FNR homologue can bind to two proposed sites and initiate transcription (24). Although no obvious FNR protein-encoding gene can be found in the annotated genome of *Nostoc* sp. strain PCC 7120, it is possible to identify ORF proteins of interest; e.g., All4541 shows 22% amino acid sequence identity to FNR of *E. coli* and 31% identity to FNR of *Bacillus subtilis*. Furthermore, All4541 contains a conserved domain of the Fnr/Crp superfamily of transcription factors and has three cysteine residues that resemble the cluster of conserved cysteine residues coordinating the [4Fe-4S] cluster required for function (23, 36).

Interestingly, when cells of *Nostoc* sp. strain PCC 7120 were grown under dark, anaerobic conditions, the expression levels of the *hox* transcripts increased substantially (Fig. 3), a result which is in agreement with physiological data reported earlier (19, 20). It has long been demonstrated that microaerobic/anaerobic conditions influence the bidirectional hydrogenase activity in heterocystous cyanobacteria (6, 19, 20, 39, 44, 46). However, this is the first time that it is demonstrated that the anaerobically induced activity of the enzyme is in parallel with an increase in transcription of the two *hox* operons in *Nostoc* sp. strain PCC 7120.

The two identified LexA binding sites upstream from alr0750 and *hoxU* that interact with LexA purified from *Nostoc* sp. strain PCC 7120 (Fig. 5) suggest that this novel role of LexA described by Domain et al. (14) may not be restricted to *Synechocystis* sp. strain PCC 6803 but can possibly be expanded to other cyanobacterial strains. Interestingly, when using *Synechocystis* sp. strain PCC 6803 LexA in EMSAs, it was observed that it could interact only with the alr0750 promoter region (which harbors the previously described LexA box) but not with the *hoxU* DNA fragment. This fact is in agreement with the results reported by Mazon et al. (27), in which *Nostoc* sp. strain PCC 7120 LexA recognized the LexA box upstream from the *Synechocystis* sp. strain PCC 6803 *lexA* gene. However, here we show that although the LexA proteins from *Synechocystis* sp. strain PCC 6803 and *Nostoc* sp. strain PCC 7120 can both recognize the box RGTACNNNDGTWCB, they have different DNA recognition capabilities, which might be a consequence of the differences found on the amino-acidic level (14, 27, 30).

In *E. coli*, LexA is well characterized in the SOS response system, where it is accepted as a typical repressor (53). In cyanobacteria, microarray experiments carried out by Domain et al. (14) demonstrated that the expression of a number of genes in a LexA-depleted mutant of *Synechocystis* sp. strain PCC 6803 was either up- or down-regulated. Many of the identified genes are involved in carbon assimilation or controlled by carbon availability, although none connected to the SOS response system (14). Furthermore, Patterson-Fortin et al. (33) reported recently that the expression of the DEAD box RNA helicase, the *crhR* gene product, in *Synechocystis* sp. strain PCC 6803 is negatively regulated by LexA. Interestingly, the expression of *crhR* is regulated in response to conditions which elicit reduction of the photosynthetic electron transport chain (33). In *Nostoc* sp. strain PCC 7120, LexA has been previously shown to interact with a few of the genes, e.g., *recA*, *uvrA*, and *ssb*, involved in the SOS system in *E. coli* (27). The results from the bioinformatics search for additional putative LexA binding sites in the genome of *Nostoc* sp. strain PCC 7120 (Table 2) indicate that a number of genes connected to DNA replication, recombination, and repair may be part of the LexA regulatory network. However, many genes in the *E. coli* LexA regulon do not show any LexA box upstream from its coding sequence in *Nostoc* sp. strain PCC 7120 (27). Moreover, many identified LexA boxes correspond to intergenic regions located upstream from genes encoding either unknown or hypothetical proteins. This may indicate that LexA in *Nostoc* sp. strain PCC 7120 is involved in regulatory networks, so far not described, that definitely deserve further attention. Although the physiological role of the cyanobacterial bidirectional hydrogenase is unclear, the suggestions, e.g., being part of respiratory complex I, involved in fermentation, and functioning as a valve during photosynthesis, are all in agreement with an enzyme system being under redox control. Recently, LexA was suggested to be a mediator of the intracellular redox state (1, 33) in *Synechocystis* sp. strain PCC 6803. Nevertheless, it re-

mains to be clarified what is the specific role of LexA on the regulation of the *hox* genes in *Nostoc* sp. strain PCC 7120 and in which signal transduction pathways LexA is directly involved.

In conclusion, the *Nostoc* sp. strain PCC 7120 *hox* genes, encoding the bidirectional hydrogenase, are separated in two operons that show similar responses to anaerobic induction. The function of the additional ORFs transcribed together with the *hox* genes is not apparently associated with hydrogen metabolism. Two transcriptional start sites, upstream from alr0750 and *hoxU*, have been identified, but it is not possible to exclude the possibility that additional transcriptional start sites are present. LexA interacts with the regulatory region of the two *hox* gene clusters. However, since the transcription of *lexA* does not seem to be clearly affected by anaerobic conditions (data not shown), it is likely that additional transcription factors are involved in the regulation of the bidirectional hydrogenase in *Nostoc* sp. strain PCC 7120 and maybe also in other cyanobacterial strains.

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