Nitrogen Deprivation of *Anabaena* sp. Strain PCC 7120 Elicits Rapid Activation of a Gene Cluster That Is Essential for Uptake and Utilization of Nitrate

YUPING CAI[†] AND C. PETER WOLK*

MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824-1312

Received 26 June 1996/Accepted 23 October 1996

A transposon bearing *luxAB*, encoding luciferase, as a reporter of transcription was used to identify genes that are activated rapidly upon deprivation of *Anabaena* sp. strain PCC 7120 of fixed nitrogen. The three transposon-marked loci that were identified as responding most rapidly and strongly are closely linked and situated within *nirA* and *nrtC* and between *nrtD* and *narB*, genes whose products are responsible for uptake and reduction of NO_2^- and NO_3^- . A strain bearing a transcriptional fusion of *narB* to *luxAB* was constructed. Luminescence catalyzed by LuxAB was used to report on the expression of the interrupted genes. Whether these genes are regulated only coordinately is discussed.

Cyanobacteria are ecologically and geographically diverse photosynthetic prokaryotes that are principal components of the food chain in many aquatic ecosystems. Their similarities to chloroplasts of eukaryotic plants, including their use of H₂O as a photosynthetic electron donor and similarities identified by sequencing, have led to the conclusion that cyanobacteria were the evolutionary predecessors of chloroplasts (10, 18). Nitrate, often the most abundant source of fixed nitrogen available for assimilation into microorganisms and plants, is reduced to nitrite, which in plants is further reduced to ammonium within chloroplasts (16). The uptake and utilization of nitrate by the nondiazotrophic, unicellular cyanobacterium Synechococcus sp. strain PCC 7942 have been well studied (16, 37). Nitrate and its catabolite, nitrite, are actively transported into cells by a membrane transporter complex that is encoded by the gene cluster nrtABCD (28, 30, 38, 39). This cluster is flanked at its 5' end by the gene nirA, which encodes a ferredoxin-dependent nitrite reductase (29, 44), and at its 3' end by the gene *narB*, which encodes a ferredoxin-dependent nitrate reductase (3, 41). The nirA-nrtABCD-narB sequence is transcribed as a single operon, whose expression is regulated by the availability of nitrogen (38, 44). Luque et al. (28) interpreted the observation that insertional mutants of nirA showed basal levels of nitrate reductase activity as indicating that narB can be driven to a limited extent by one or more constitutive promoters present within this cluster.

Even cyanobacteria that can fix dinitrogen use fixed nitrogen preferentially, perhaps because of the metabolic expense of maintaining a microaerobic environment for nitrogenase (13, 17). Anabaena sp. strain PCC 7120, a filamentous cyanobacterium that can fix N_2 in differentiated cells called heterocysts, has become a model organism for studies of prokaryotic cellular differentiation and pattern formation (8, 50). We have used derivatives of transposon Tn5 that contain *luxAB* as a transcriptional reporter to identify genetic loci that are transcriptionally activated in response to nitrogen deprivation (49). Three of the mutants isolated bear a transposon within the *nirA-nrtABCD-narB* gene cluster that is required for nitrate uptake and utilization. We have studied certain aspects of the transcriptional regulation of this cluster of genes.

MATERIALS AND METHODS

Anabaena sp. strain PCC 7120 and derivatives of it were grown under the conditions described by Hu et al. (23). Mutants were grown in the presence of neomycin sulfate (Sigma Chemical Co., St. Louis, Mo.) at 20 µg ml-1 in liquid medium supplemented with nitrate or nitrite or at 10 µg ml⁻¹ in the absence of those nitrogen sources and at 200 μg ml⁻¹ in agar-solidified medium without supplemental nitrogen or at 400 μg ml⁻¹ in such medium with supplemental nitrogen. Plasmid pRL1472a, which provided an endogenous source of aldehyde as substrate for measurements of luciferase activity in the presence of different nitrogen sources and for photon-counting microscopy (14), was selected with 5 μ g of erythromycin ml⁻¹. Fixed nitrogen was added, where specified, as 2 mM ammonium chloride plus 5 mM N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid buffer, pH 7.5, or as equimolar concentrations of the Na⁺ and K⁺ salts of nitrate (unless otherwise noted, totaling 5 mM in liquid or 10 mM in agar) or nitrite (totaling 2.5 mM in liquid or 5 mM in agar). Petri dishes with NH₄⁺- containing agar were prepared within 2 days of use. Cells in NH₄⁺-containing medium were sedimented and resuspended in fresh medium no less frequently than once every 2 days, and filters bearing cells atop NH_4^+ -containing agar were equally frequently transferred to fresh petri dishes. L-Methionine-DL-sulfoximine (MSX) was used at 5 µM. Nitrate reductase was measured by the method of Herrero et al. (20, 22).

DNA was manipulated by standard procedures (31). Suicide plasmid pRL796 was constructed for site-directed interruption and transcriptional reporting of the narB gene. To make this plasmid, the 0.9-kb HpaI-MunI internal fragment of narB (see Fig. 1 and 2) was fused at its MunI end to an EcoRI-SstI fragment from the polylinker of pUC19 (52) and the resulting fragment was cloned between the SmaI and SstI sites of pRL561 (12). Plasmids were transferred to Anabaena cells by triparental mating (11). Transposon Tn5-765 (in plasmid pRL765; GenBank accession number U55819) was constructed by addition of a BamHI fragment from pRL488 (12) containing Vibrio fischeri luxAB to the BamHI site of transposon Tn5-764 in plasmid pRL764SX. That plasmid differs from pRL1058 (49) in the following two ways: (i) deletion of a SmaI fragment within the transposon eliminates the bleomycin resistance and streptomycin resistance determinants, reduces the length of the transposon, and leaves, in addition to a unique XbaI site, an adjacent unique BamHI site for the insertion of reporter genes; and (ii) elimination of a SmaI site from a polylinker in the oriT region of the plasmid permits facile replacement of the kanamycin resistance determinant between XmnI and SmaI sites with an alternative selective marker. Mutants generated upon transfer of Tn5-765 to Anabaena sp. strain PCC 7120 were screened (49) for rapid activation of luminescence in response to nitrogen stepdown. Samples for pulsed-field gel electrophoresis were prepared and gels were run as described by Kuritz et al. (24).

^{*} Corresponding author. Phone: (517) 353-2049. Fax: (517) 353-9168. E-mail: 22333cpw@msu.edu.

[†] Present address: Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3206.

Changes of transcriptional activity upon transfer of cells between different media were measured by quantitating the luminescence of homogeneous spots of cells (7, 49). The results presented are averages from three or four independent experiments. The luciferase activities of individual filaments were recorded as described by Elhai and Wolk (12).

Nucleotide sequence accession numbers. The nucleotide sequences of *nirA* and *narB* were obtained by automated sequencing (Applied Biosystems, Inc., Foster City, Calif.) of overlapping fragments from both strands of DNA of



FIG. 1. Maps of the chromosomal region (thick line) bearing insertions of Tn5-765 in strains TLN10, TLN12, and TLN21 and of plasmids derived from that region. All sites for *Bsp*14071 (B), *Cla*I (C), *Eco*RI (E), *Eco*RV (V), *Hind*III (H), *Hpa*I (P), *Mun*I (M), and *Xba*I (X) are shown for the region from 2.9 to 17.4 kb. Plasmid pRL822 was recovered with *Ase*I (A); plasmids pRL824 and pRL829 were recovered with *Bsp*14071; plasmids pRL802, pRL810, and pRL821 were recovered with *Cla*I; plasmids pRL825 and pRL830 were recovered with *Eco*RI; plasmids pRL810R and pRL828 were recovered with *Eco*RV; and plasmid pRL823 was recovered with *Xba*I. Each site of insertion of Tn5-765 is represented by a triangle; the L end of the transposon that bears *luxAB* as reporter is to the left in each case. Regions sequenced on only one strand are shown as open boxes, and regions with complete, overlapping sequencing on both strands are shown as solid boxes. *nirA* and *narB* genes encode nitrite reductase and nitrate reductase, respectively. *nrtA*, *nrtB*, *nrtC*, and *nrtD* are genes that encode nitrate- and nitrite-permease proteins identified and here positioned on the basis of similarities of deduced partial amino acid sequences with corresponding genes from *Synechococcus* sp. strain PCC 7942 (38, 39).

transposon-bearing plasmids (see Fig. 1) and have been submitted to GenBank under accession numbers U61496 and L49163, respectively.

RESULTS

Forty-six independent colonies derived from transposition of Tn5-765 appeared initially to show increased luminescence when transferred on a filter from solid medium containing NH_4^+ to the same medium lacking a source of fixed nitrogen. Of these, seven gave rise to strains (TLN9, -10, -12, -14, -17, -21, and -38) that consistently showed rapid induction of luminescence. TLN17, which showed much lower luminescence upon induction than did the others, was not further studied. Upon transfer from NH_4^+ to N_2 (see below), TLN9 showed 2.7-, 5.5-, 8.0-, 8.7-, and 9.4-fold induction in 1, 2, 3, 4.5, and 6 h; TLN14 showed 8.8-, 17.8-, 24.8-, 28.3-, and 27.8-fold induction during the same intervals; and TLN38 showed 5.9-, 9.3-, 10.3-, 7.0-, and 5.2-fold induction during the same intervals (the results for TLN9, TLN14, and TLN38 are in each case averages from four experiments). The transposon was excised from TLN14 with ClaI and EcoRV separately. The resulting plasmids (pRL826 and pRL827, respectively) were sequenced outwards from the transposon on one strand, and an open reading frame was identified. Upon BLAST searching (1), it was found that the intercepted genetic sequence appeared to encode a protein which, over a stretch of 120 amino acids, showed approximately 41% identity (58% similarity) to the Escherichia coli regulatory protein KdpE (48) (see Fig. 3e). The chromosomal insertion in TLN14 was localized tentatively by pulsed-field gel electrophoresis. Upon cleavage with BlnI (an isoschizomer of AvrII) plus SphI, band AvrA was replaced with two bands, one about 20 kb larger than AvrH and one of about 254 kb; the latter was labeled upon hybridization with a fragment from the right end of the transposon. After digestion with PstI and hybridization, a band of approximately equal size

was labeled and a new band of ca. 170 kb was observed. Tn5-765 lacks sites for *SalI*; hybridization to SalA was observed.

Because of the much greater induction of luciferase activity in TLN10, TLN12, and TLN21 upon transfer of those strains from NH_4^+ to N_2 (see below) and the clustering of the corresponding sites of transposon insertion (see below), the remainder of our experimentation was restricted to those three strains and to a fourth, related mutant. The chromosomal insertion in TLN10 was localized by pulsed-field gel electrophoresis as follows: upon cleavage with a combination of BlnI plus SphI, the AvrE band was cut to yield fragments of ca. 358 and 146 kb, with the latter labeled in a Southern blot upon hybridization with a DNA fragment from the right end of Tn5-765. Fragment SalD was labeled. Although electrophoresis should have resolved any fragment of 800 kb or less, the PstI digest resembled that of DNA from wild-type Anabaena sp. strain PCC 7120. Digestion of DNA from TLN21 with BlnI plus SphI gave rise to new bands of ca. 365 and 141 kb.

Tn5-765 was excised from strain TLN10 with ClaI and EcoRV; from strain TLN12 with those two enzymes, EcoRI, and Bsp1407I; and from TLN21 with Bsp1407I, ClaI, EcoRI, and (excluding the left end of the transposon) AseI and XbaI. Restriction mapping indicated that the resulting plasmids overlapped extensively (Fig. 1). Sequencing of portions of the recovered plasmids (open and closed boxes in Fig. 1) and comparison with the amino acid sequences of the proteins encoded by the nirA-nrtABCD-narB region of the chromosome of Synechococcus sp. strain PCC 7942 (3, 29, 36, 39, 44) (Fig. 2, 3a through d, 4, and 5) suggested that (i) a sequence of genes that corresponds closely to the series in Synechococcus sp. strain PCC 7942 is present in Anabaena sp. strain PCC 7120 and (ii) the transposons in TLN10, TLN12, and TLN21 had inserted within *nirA*, within *nrtC*, and between *nrtD* and *narB*, respectively. Figure 4 also compares the predicted sequence of the

GTAGTCATTAGTCAACAGTCAACAGTCATCAGTCTATTTTCTTCCCCCCCTGCTCACTACCCCCACTCTT 1 68 23 134 T N R D S Q G N P T W R V R G D K A H P S S ACAAATCGAGATAGCCAAGGAAATUCAACTISCCGGGTCCGCGCTCATAAAGCCCATCCATCTAGC 50 200 73 PMVRESLDQEFRRVSNDEAFDI. CCAATGGTACGGGAATCACTTGATCAAGAGTTCCGCCGTGTCAGTTGGGATGAAGCTTTTGACCTC 95 332 398 46 N F D A N S R L C M S S A V S G Y I Q S F G 161 AATTTIGATGCTAAGTCTCGGGCTATGTATGTGGGG 530 A D G P P C C Y E D L E L T D C A F L I G T 183 GCIGATGGGCCTCCCIGCTGTGAAGATTGGAGTTACTGACTGTGCATTTTAATIGGGACA 591 N T A E C H P I V F N R L E K Y H R K N H K 205 AATACCGCCGAATGTCACCGGATGTTTTTAACCGCCTGGAGAAGTGCCACAGAAAAACCCATAAG 662 V K M I V V D P R R T P T A E A A D L H L A 227 GTAAAAATGATGTGGTGATGCTGAGGCACCCCACGCGAAGCAGCTGATTTACATTTGGGG 728 I K P G T D I D L L N G I A H I, L M R W N M 249 ATTAAACCGGGTACAGATATTGACTGTTAAAfgGAATTGCTCATTGTTAATGCGTTGGAACATG 794 I D V G F I D D C T R N F S A Y A E V T R H 272 ATAGATGTCGGTTTCATCGATGACCTGTACCCAGAACTTTTCGGGCTTACGCTGAGCTGAGTCACTCGCCAC 860 Y S P E V V A R Q C G I T I E D L E T A S R 293 TATTEGEOGGAGGTAGTAGCTOGTCATGTGGTATCACCATCGAAGATTTAGAAACCGCCTCCCGT 926 992 TAKVRTIINLHLMTGQVGKPGA 337 ACAGCCAAAGTCAGAAGGAGTTATTAACCTGCATTGATGACGGGACAAGTCGGAAAACCAGGGGCT 1058 1124 L L P G Y R L V K N P Q H R A E L E A F W G 381 1190 TTATTACCCCGTTATCGATGGTGAAAAATCCCCCAACACCGGGCAGAATTAGAAGCATTTTGGGGA 1256 D G N V G L L W I A A T N P A V S M P D L E 425 GACGGTAACGTGGGITTACTGTGGGATTGCGGCTACCAGCTGGAGTAGCAGATTGCGAA 1322 R T K K A L L R 5 P F T I Y Q D A Y H P T E 447 CGGACGAAAAAGGCATTATIGCGATCGCCTTTCACCATCTACCAAGATGCTTACCACCCCACAGAA 1188 ТТАҮАНVLL РААОЮ G E K T G I M T 469 1454 ACTACAGETTATGECCATGTTACCAGEAGECCAGEGGGGGGAAAAAACTGGTATCATGACT N S F, R R V T L C Q A F R Q P P R E A K P D 491 AACTCAGAACGTCGAGTFACCCTATGTCAAGCCTTCCGCCAACCACCAGGAGGTAACCAGAT 1520 W E I F A E V G R R L G F E D K F A F T N S 513 1586 TGGGAAATCTTCGCAGAAGTGGGACGCAGATTAGGTTTGAGGACAAGTTCGCCTTTACTAACTCA A Q V Y A E F T Q L T K G R P C D M S G I S 535 GCCCAAGTATACGCCGAGTTGACCAAGGGTCGCCCTTGTGATATGTCTGGTATCAGC 1652 H Q Q L Q A Q G P T Q W P H S S 1 N I E T Q 557 CATCAACAATTGCAAGCCCCACTCCAATGGCCCCACTCCTATAAATATCGGAAACACAA 1218 К Р Т О Н Р Т V N S Q Q S T V T S Q Q S K R 579 Алассалс<u>тсалсастсалсастсялсастсялсастсялсастсялса</u> 1784 I. Y T D L R F H T P D G R A R F C A Y Y S K 601 1850 CTCTACACTCATTTACGTTFTCATACCCCTGATGGGCGCGCCTCGATTTGGCGCATATTATTCTAAG G L A E P P D P S Y P F V L T T G R I Y G H 623 GGATTAGCAGAACCACCAGACCCTAGTTATCCTTTTTGTGTTGACTACTGGACGATTATACGGACAT 1916 W R T Q T R T G R I E K I R A M H P E P F I 645 TGGCACACCCCGAACCCGTACIGCTCGCATGAAAAATCCGCGCCCTGCACCCCGAACCATTTAIC 1982 R R G S A K F P A K V T K A J S P G T V F V 689 UGTCGAGGTACCCCCAAGGTICCCCCAAGGTCACAAAAGCTAITICCCCAGGTACAGTITTFGTG 2114 P M H W G K L W A D D A E A N A L T H S E S 711 CCTATGCACTGGGGTAAACTCTGGGCAGAAGCGAGAAGCTAACGCCCTCACCCATTCAGAATCT C P D S L Q P F L K A C A V Q L I P I S V E 733 TGECCEGATTEACTGEAACCAGAATTAAAAGCETGTGEAGTECAATTAATAECAATETEEGTAGAA N T A Q N Y Q L Q S S Q W * ANCACAGCCCAAAATTATCAACTCCAGTCATCTCAGTGGTAAGCTGTTAUGUATTIAAATTGTATA 2112 ΜΑΤΑCRΑCCARARTERICARCICARCICARCICLARICICARIGUE ARGENTITIAGUATITIANI 2116 ΠΤΟ COCACICACOTIGICAAAGITCAAAGITCAAAGITCAAGCARACCTCACACCATTACACCCTTACTACT 2444 GACTETGGACACAGATTGAAGGCARAATAGTGATTTTAGGCGCGTACTCACCCTTACACCAT 2516 <u>GAACTTCAAAGITCCAAAAGITCCAAAAA</u>AACCGTATTTCCGGCTTTATACACCATTGACGAAAGITCGAAGGAAA 2502 ATACTGGGATACCAAAGATGCGACGACAATTGGGTGCAGCAATGAGGAAAATATTAGGCAAT 2574 TAACGAAACTACTAACGTCGACGAATTTAGTGGCGATCCATATGAGAAAGITGTGGTATTAA 2574 TAACGAAGATTTTTATCACCCATTACACCAATTTACACCAATTTTACTGCTGTTTTAAT 2574 TAACGAAGATTTTTATCACCGCCTCAATTTAACCACATTTTAAACTCTTTGTTTATT 2540 TTTAAAATCCCATTGCCCTATGAAAACCGACGATACGACGATCACTATTTAAACTCCTTGTTTTATT

FIG. 2. Nucleotide sequence of *narB* and contiguous DNA and amino acid sequence of NarB by translation. The first 9 bp shown constitute the sequence repeated upon insertion of transposon Tn5-765 in mutant TLN21 and include the trinucleotide TAG that by comparison of the product of translation of the approximate 5' sequence (determined by sequencing of a single DNA strand) with the sequence of NrtD of *Synechococcus* sp. strain PCC 7942 (Fig. 3d)

NirA protein from *Anabaena* sp. strain PCC 7120 with all or portions of the sequences of nitrite reductases from certain other organisms and with portions of the predicted sequence of a bacterial sulfite reductase. Figure 2 presents the complete sequence of *narB*; as indicated in that figure legend, the nonanucleotide sequence duplicated upon insertion of the transposon in TLN21 appears to contain the stop codon of *nrtD*. The predicted amino acid sequence of NarB from PCC 7120 is compared with corresponding sequences from *Synechococcus* sp. strain PCC 7942 and from other sources in Fig. 5. Because the *nirA-nrtABCD* series of genes from PCC 7120 had already been cloned and were being characterized (15, 16), we sequenced the entirety of only *nirA* and *narB*.

Mutant TLN10 did not grow consistently and was at best sickly on either NO_2^- or NO_3^- . Mutant TLN12 grew healthily on NO₂⁻ and NO₃⁻ and showed a heterocyst frequency approximating that of the N₂-grown wild-type strain in the presence of up to 80 mM NO_3^{-7} . Mutant TLN21 grew healthily in the presence of 5 mM NO_3^{-7} , forming no heterocysts in older cultures and only occasional heterocysts, e.g., one or two per filament of at least 50 cells, in young, dilute cultures and thus evidently expressing limited nitrate reductase activity. Singlecrossover recombination of pRL796 with the chromosome of PCC 7120 produced strain SR796, which lacked an intact copy of narB, as established by Southern hybridization (data not presented). SR796 grew healthily on NO₂⁻ and NO₃⁻; heterocysts did not form on NO_2^- but formed abundantly on NO_3^- . Whereas the means of several measurements of nitrate reductase activity of permeabilized cells of wild-type Anabaena sp. strain PCC 7120 grown on NO₃⁻ and N₂ were 0.74 \pm 0.24 and 0.55 ± 0.04 nmol of NO₂⁻ produced μ g of chlorophyll a^{-1} min⁻¹, respectively, the corresponding mean activity of SR796 grown in the presence or absence of nitrate and presence or absence of 10 μ g of neomycin sulfate ml⁻¹ was maximally about 1% as great and probably not significantly different from zero. When grown on \overline{NH}_4^+ as the sole nitrogen source and at low cell density (Table 1), TLN10, TLN21, and SR796 (all bearing pRL1472a) showed low levels of luminescence (Table 1). The values increased greatly during growth on N_2 and to a lesser extent in the presence of NO_2^- or NO_3^- (Table 1). The activity of LuxAB in vegetative cells of N2-grown TLN10 and much more faintly in N2-grown SR796 was visualizable by photon-counting microscopy; varied spatial gradients of luminescence were observed along the filaments (Fig. 6).

Mutants TLN10, TLN12, TLN21, and SR796 responded to transfer from NH₄⁺ to N₂ with increased expression of *luxAB* within 0.5 h (Fig. 7a and b). The expression of *luxAB* increased the most rapidly initially (with some variation at 1 h; compare Fig. 7a and b) and over the 6-h duration of the experiments monotonically in TLN10, the expression of *luxAB* by TLN12 and TLN21 increased only a bit more slowly initially but peaked and decreased after 2 to 3 h, and the expression of *luxAB* by SR796 increased the most slowly and then also decreased. Blockage of assimilation of NH₄⁺ by addition of MSX in the continued presence of NH₄⁺ led to qualitatively similar but quantitatively less pronounced activation of *luxAB* expression (Fig. 7d). The expression of *luxAB* by TLN10, TLN21, and SR796 decreased with modestly differing time courses upon

represents the termination (*) codon of PCC 7120 *nrtD*. The *HpaI* site (GTTA AC) and *MunI* site (CAATTG) used for insertional mutagenesis are in bold. The following five series of direct repeats are shown (doubly underlined except for singly underlined variant nucleotides): four repeats of GTCAACA, four repeats of GTCAACA, sight more repeats of GTCAACA, and two sets of four repeats of GTCAAAA.

(a) 1 MTHVSRRKFLFTTGAAAAASILVHGCTSNGSQSATTGEQAPSAAPAANVSAANAPKVETT An: 60 : **: * :** *: ***** * * ***:: :: * ** * : *** 1 MSOFSRRKFLLTAGGTAAAALWLNACGSNNSSTDTTGSTS.TPAPSGT.SGGDAPEVKGV 58 Ss: 61 KAKLGFIPLTDAAPLIIAKEKGFFAKYCMTDIEVIKQKSWPVTRDNXKIGSSGGGID 117 An: **** ******:*** *** **** * *:** ** ***** :** **** 61 T..LGFIALTDAAPVIIALEKGLFAKYGLPDTKVVKQTSWAVTRDNLELGSDRGGID Ss: 116 (b) 1 VDPIFQVLRTVPPLAWLPISLAAFQQANPSAIFVIFITSIWPILLNTTVGVQQIPQDYIN 60 An: 119 LDPVIOVLRTVPPLAWFPISLMVFODANTSAIFVIFITAIWPIIINTAVGINQIPDDYNN 178 Ss 61 VAKVLRLKGVKYFFKIVFPATVPYIFTGLRIGIGLSWLAIVAAEMLVGGVGIGSFIWDAY 120 An: * *: *:***:* **** :**:******** ****** *** ***** 179 VARVLKLSKKDYILNILIPSTVPYVFAGLRIAVGLAWLAIVAAEMLKADGGIGYFIWDAY 238 Ss: 121 NTTTETNLSEIILALIYVGLVGLLLDRLVGFVASKV 156 An: : : *:***: ****** ***** :* 239 NAGGDGSSSQIILAIFYVGLVGLSLDRLVAWVGRLV 274 Ss: (C) 1 LLKNIIDMVGTSLTANXRPSELSGGMKQRVAIARALATRPKLLLLDEPFGALDALTRGSLQEQL 64 An: ::: **:** 118 IIEETIDLVGLRAAADKYPHEISGGMKQRVAIARGLAIRPKLLLLDEPFGALDALTRGNLQEQL 181 Ss: 65 MKICNEHQITCVMVTHDVDEALLLSDRVVMLTNGPEAHIGQILEVPISRPRQRLEVVKHPSYY 127 An: **** * ************************ ********* 182 MRICOEAGVTAVMVTHDVDEALLLSDRVVMLTNGPAAQIGQILEVDFPRPRQRLEMMETPHYY 244 Ss: (d) 1 KIYPTPEGPYTVLDGIDLKVREGEFVCLIGHSGCGKSTLLNMISGFNTPSEGVVLLQDKP An: 60 24 KTFPTPRGPYVAIEDVNLSVQQGEFICVIGHSGCGKSTLLNLVSGFSQPTSGGVYLDGQP 83 Ss 39' 1' HLAMVGLTEAAEKKPXPDFRGDETTSGDRRALSIRPQVL 61 ITEPGPDRMMVF 72 Aπ ** :***** *:* * : ****** * ********* 84 IQEPGPDRMVVF 95 133 HLELVGLTEAQHKRPDQLSGGMKQRVAIARALSIRPEVL 171 Ss 40'ILDEPFGALDAITKEELQEELLQIWSDHQVTVLMITHDIDEALFLADRVVMMTNGPAAQI 99 Aπ 172 ILDEPFGALDAITKEELQEELLNIWEEARPTVLMITHDIDEALFLADRVVMMTNGPAATI 231 Ss 100'GEILDIPFDRPRNRRRIMEDPKYYDLRNYALDFLFNRFAHNE 141' Aπ 232 GEVLEIPFDRPREREAVVEDPRYAQLRTEALDFLYRRFAHDDD 274 Ss (e) 1 IVXGNPHLRSLLGXHLQQVEYRVHQXASIYQAREAFLSHQPTLVILDADLPDGDGIEFCR 60 An: :* * *: **:::: : :* *:*** ******** ** 6 IVEDEQAIRRFLRTALEGDGMRVFEAETLQRGLLEAATRKPDLIILDLGLPDGDGIEFIR 65 Ec: 61 WLHRQQQPLILMLSARTNEADIVAGLKAGADDYLXKPFGMQEFLARVEALIRRKRTPTAP 120 An: :** *** 66 DLRQWSRVPVIVLSARSEESDKIAALDAGADDYLSKPFGIGELQARLRVALRRHSATTAP 125 Ec:

FIG. 3. Partial results of BLAST searches using products of translation of incompletely defined nucleotide sequences from a region 3' from *nirA* of *Anabaena* sp. strain PCC 7120 (An) compared with the NrtA protein from *Synechococcus* sp. strain PCC 7942 (Ss; 36) (BLAST score, 200; 54% identical [*] and 65% similar [:]) (a), a region between *nirA* and the site of insertion of the transposon in mutant TLN12 compared with the NrtB protein from strain PCC 7942 (39) (BLAST score, 529; 65% identical and 76% similar) (b), a region adjacent to the transposon in mutant TLN12 compared with the NrtC protein from strain PCC 7942 (39) (BLAST score, 465; 73% identical and 82% similar) (c), two regions between the sites of insertion of Tn5-765 in mutants TLN12 and TLN21 compared with the NrtD protein from strain PCC 7942 (39) (BLAST score, 502; 67% identical and 81% similar) (d), and a region adjacent to the transposon in mutant TLN14 compared with the *NrtD* protein KdpE (Ec; 48) (BLAST score, 202; 54% identical and 58% similar) (e). Except in panel a, the numbering of the *Anabaena* sequence assigns amino acid position 1 arbitrarily.

	* ** * ***				
An	MTDTVTTPKASLNKFEKFKAEKDGLAIKSELEKIASLGWEAMDATDRDHRLKWVGVFFR.PVT	62			
Ph	MTDTLAAPTLNKFEKLKAEKDGLAVKAELEHFARLGWEAMDETDRDHRLKWLGVEFR. PVT	60			
Se	MAGATATTEKI, NKEEKI, KLEKDGLAVRDOTOHFASIGWEAMDPGDREHRLKWLGIEWR, PVT	61			
So	69 KFEFESCINDAEKVKIEKDPMKLEIEDGISDLATLSMEEVDKSKHNKDDDVRLKWLGLEHBRKHH	134			
50		121			
	* ** * * * * * * * * *** * * * * * *****				
Αn	PGK FMMRMRMPNG I LTSDOMRVLAEVVORYGDDGNAD I TTRONIOLRG I RIEDLPHI FNK FHAVGLTSVO	132			
Ph	PGKFMLEMRVPNGI ITSGOTRVIGETI OBVGDDGNADITTEONFOLBGIRIEDLPEIFRKFDOAGLTSIO	130			
Ss	PGREMARLE I PSGTLOSOOLNALANFLORYGDOAS ID ITTRONLOLBGLILEDTPEFLERLHAVGLTSVO	131			
So	VGREMMBLKLPNGVTTSEOTRYLASVIKKYGKDGCADVTTRONWOIRGVVLPDVPEIIKGLESVGLTSLO	204			
st	81 IJECRIPGGVITTTOWOATDKFAADNTIYGSIRITNROTFOFHGILKKNVKPVHOMLHSVGLDALA	146			
	**** ** * * * * * * * * * * * * * * * *				
An	SGMDNIRNITGDPIAGLDADELYDTRELVQQIQDMLTNKGEGNREFSNLPRKFNIAIAGGRDNSVHAEIN	202			
Pb	SGMDNVRNITGSPVAGIDADELIDTRGLVRKVQDMITNNGRGNSSFSNLPRKFNIAIAGCRDNSVHAEIN	200			
Ss	SGMDNVRNITGSPVAGLDAAELFDTRSLIQALQDDLTAAGQGNSEFTNLPRKFNIAIEGGRDNSIHAEIN	201			
So	SGMDNVRNPVGNPLAGIDPHEIVDTRPFTNLISOFVTANSRGNLSITNLPRKWNPCVIGSHDLYEHPHIN	274			
st	TANDMNRNV 155 212 LPRKFKTTVVIPPQNDIDANLH	233			
	— — —				
	* * ** *				
An	DLAFVPAFKEGIGDWVLGNGEESSTYQKVFGFNVLVGGFFSAKRCEAAIPLNAWVTPEE.VLPLCRAILE	271			
Pb	DIAFVPAFKDGVAVCEAILT	252			
Ss	DLAFTPAYQDGTLGFNVWVGGFFSSTRVAPAIPLNAWVPADHSVIRLSRAILE	254			
So	DLAYMPATKNG	326			
St	<u>DMNFVAIAENGELVGFNLLVGG</u> GL <u>S</u> 258 280 EHTLAVAEAVVT	291			
-		220			
An	VYRDNGLRANRLKSRLMWLIDEWGIDKFRAEVEQRLGKSLEPAAPKDEIDWEKRDHIGVYRQAQEGL	220			
ЧЧ	VYRNLGLRANRQKARLMWLIDEMGLEPFREAVEKQLGYAFTPAAAKDEILWDKRDHIGHAQKQPGL	212			
SS	VFRDNGSRGNRQKTRLMWLIDEWGIERFRQVVSEAYGAPLAA.AAPELMDWEKRDFLGVHPQKQAGL	320			
So	AFRDLGFRGNRQKCRMMWLIDELGMEAFRGEVEKRMPEQVLERASSEELVQKDWERREYLGVHPQKQQGL	396			
St	TQRDWGNRTDRKNAKTKYTLERVGLETFKAEVERRAGIKFEPIRP 336				
	**** ***** *** ** ** ** ** ** ** ** **				
An	NYVGLHTPVGRLYARDMFELARIADVVGSGETRMTVEONIIIPNITDSRLRTLLTDPLL. ERFSLDPGAL	407			
Ph	NYVGLHVPVGRLVAODLEDLARTAEVVGSGEIRLTVEONVLIPNVPDSRVSALLREPIV, KRESIEPONL	388			
Se	NEVEL HVP/GPLTTEDLYELABLADTYGOGEVPLTYFONVILTHIPDAOLPTLLAEPLL. TRESPOPAPL	389			
So	SEVEL 1 PVCRLOADEMEELARTADVYGSGELRLTVFONILIPNVENSKIDSLLNEPLLKERYSPEPPIL	466			
St	388 GEFRITANONLITASVPESO 407	100			
00					
	* *** * * **** ** *********************				
An	TRSLVSCTGAOFCNFALIETKNRALEMIKGLEAELTFTRPVRIHWTGCPNSCGOPOVADIGLMGTKAR.K	476			
Pb	SRALVSCTGAOFCNFALIETKNRAVALMOELEODLYCPRPVRIHWTGCPNSCGOPOVADIGLMGTKVR.K	457			
Ss	SRGTVSCTGSOYCNFALIETKORAIAIAOSLEAELDLPRPVRIHWTGCPNSCGOPQVADIGLMGAKVR.K	458			
So	MKGLVACTGSOFCGOAIIETKARALKVTEEVORLVSVTRPVRMHWTGCPNSCGOVOVADIGFMGCMTRDE	536			
st	ENSMACVSFPTCPLAMAEAERFLPSFTDKVEA 460 477 TGCPNGCGRAMLAEIGLVG 495				
	* ** * ** ** ** ***				
An	NGKAVEGVDIYMGGKVGKDAHLGSCVQKGIPCEDLHLVLRDLLITNFGAKPRQEALVTSQ	536			
Pb	b DGKTVEGVDLYMGGKVGKHAELGTCVRKSIPCEDLKPILQEILIEQFGAR 507				
Ss	DGQMVEGVDIFLGGKVGYDAHLGEKAMTGVACEDLPDVLRQLLIERFGAQARSH	512			
So	NGKPCEGADVFVGGRIGSDSHLGDIYKKAVPCKDLVPVVAEILINQFGAVPREREEAE	594			

FIG. 4. Comparison of the deduced NirA sequence from *Anabaena* sp. strain PCC 7120 (An) with the first 507 amino acids of the sequence of NirA from the filamentous, N_2 -fixing but nondifferentiating cyanobacterium *Plectonema boryanum* (Pb; 42) (BLAST score, 1,150); NirA from *Synechococcus* sp. strain PCC 7942 (Ss; 29, 44) (BLAST score 768); all but the first 69 amino acids of the precursor of *Spinacia oleracea* (spinach) NirA (So; 5) (BLAST score, 416); and portions of the (NADPH-dependent) hemoprotein component of *Salmonella typhimurium* sulfite reductase (St; 40) (BLAST score, 99). Amino acids common to all the NirA sequences shown for a particular position are indicated by asterisks; those also common to *S. typhimurium* sulfite reductase are underlined. The cysteines thought to be involved in binding of the Fe₄S₄ and siroheme prosthetic groups (40) are in bold print, and their asterisks are highlighted. Dots indicate gaps introduced to enhance the alignment.

transfer of those strains from N₂ to NH₄⁺ (Fig. 7c) (see Discussion). TLN21 showed an extensive increase in *luxAB* expression upon transfer from NH₄⁺ to either NO₂⁻ or NO₃⁻ (Fig. 8a) but little or no increase when NH₄⁺ was supplemented with but not replaced by NO₂⁻ or NO₃⁻ (Fig. 8b).

DISCUSSION

A transposon bearing *luxAB*, encoding luciferase, as a reporter of transcription was used to identify genes activated rapidly upon deprivation of *Anabaena* sp. strain PCC 7120 of fixed nitrogen. The three loci (in strains TLN10, TLN12, and

TLN21) that were identified as responding most rapidly and strongly proved to be structurally closely linked and situated within a series of genes whose products are responsible for assimilation of NO_2^- and NO_3^- . The (virtual) absence of nitrate reductase activity in cells of SR796 and the continued presence of heterocysts in that strain in the presence of NO_3^- but not NO_2^- substantiate the idea, based on sequence similarity (Fig. 5), that NarB is the nitrate reductase of *Anabaena* sp. strain PCC 7120. The nitrate reductase activity of TLN21 may be driven by a weak promoter situated in IS50R of Tn5-765. The poor and inconsistent growth of TLN10 in the presence of NO_2^- and NO_3^- is interpretable as being due to the

h	VERSET CONCOURCE EVERPENT WE STUDIE OF DEVERSED AND SOCIAL STREET	57
na na	WINDOWS COVCOURS STATES CREWN BOOC PUWKVOGDER SSGRVCV	57
2-	MEDI PUTT BUTT BUTT BUTT AN ANT AN	21
22	AFPESRIEFVIIFERIDIAR BERGERGERGENERVIER GURARITARI GARI ANDRE AL	10
ES Ver	MER POTODVCAVCAVIAS BADHADVS	aa
кр	MIETRI CEICEGEGUIAS, METROZVO, TTTTTTTE CELQUEAN ON CO	
3 -	TO SHIT S POL DAWNEL WY DAVD FOR DOFFUDY RADES FOR ITTS I DOWN FTOGARA I CHYGSGOFOTEDYYIA	128
00	TO ATTATCH WORLD' VOW OF SUDE OF DYENDEN! NATURE IOTVEFTOGAEAICNYG8GOFOTEDYYVA	128
50	TO ATVA FRUST SPIKTPHERASI DOPFTE I BUDEAL DRICORIO TO ADVGKDGICK YGBGOFOTEDYYIA	142
BE	KG. MNAHOHAINSSEITEPILKKNGEFMPYHWEEALNHIKDOVTMIOTENGUDAMAVYGSASITNEEAYLL	100
KD	KGA., ALGET. VGLEGENLEPEVDGE., RATWPOAGGGREAPAGDYRSANG., OAVRETBGOLLTEDYYAA	108
··F		
	± * * * * * * *	
٨n	OKLMRGCLGSNNFDANSRLCMSSAVSGYIOSFGADGPPCCYEDLEL.TDCAFLIGTNTAECHPIVFNRLEK	198
0c	OKLMKGCLGTNNFDANSRLCHBBAVAAYIQSFGSDGPPCCYDDLDL.TDCAFLVGTNTAECHPIIFNRLRK	198
Ss	ORLVKGCLGTNNFDTNSRLCMSEAVSAYSLCLGSDGPPACYEDLOL . ADCLLIVGSNTAECHPILFNRYRK	212
Bs	GKFARVCLQTKY1DYNGRLCHBAAATAANQTFGADRGLTNPLSOLPHTRVIILAGTNIAECQPTIMPYFEK	171
Кр	NKLMKGFIGAANIDINSRLCHSSAVIGYKRARWCIVVPCSYEDVENSDLVV.LVGSNAAWAHPVLYQRLAQ	176
	*** * ** ** * * * * * * * * * * *	
Ал	YHRXNH . KYKMIVVDFRRTFTAÉAADLHLAIKPGTDIDLLNGIAHLLKRWNHIDVGFIDDCTRNFSAYAEV	268
0c	HHKKNNCKYRMVVVDPRRTATAEAADLHLAIRPGTDIDLFNGMAYLLHRWGKIDTIFIDECTSNFPAYCEV	209
Ss	RHKQCCTNL., IVVDPRCTPTAEVADLHLALKPGSDVALLNGLGWLLYQMGYVKKDFIANQTEGFEDWLA1	281
BS	AKENGAYFIAIDPRETATTKIADLHLKIKPGTDAALANGLVKIIIDEQLINEDFIQSETNGFELLKQH	239
ĸр	AKRDNPQMRV.VVIDPRRTATCDIADRHLALAPGSDGGLFVGLENAIAASRRISDDFNDAQRA	243
-		110
An	IRRYSPEVVARQCGITIEDLETASKIWGESGKVLSLWARGVAGSBEGIARVAIIINUHTEQVGKFGAG	140
UC .	IRRIPPLYVARACGITYELDETACKIWARATAYIDADAGIGUDAGIGARAALDIAAAAAA	152
55	TED I FFURTA E LI CLAVAE DA RAMA DA VERA MARIA DA CARA CARA CARA CARA CARA CARA CARA	310
55	IDSIM AND TARGES USED OF A REAL AND A REAL A	N 4
ъþ	ADBULDKYAGI CODING TABI TABI TABI ANTAL TATI MUTAGASABASA ATTA BANGATI DI DI DI	
10	PELTCODENEMCEPISCILLITERYSLUCENPOREICLEFEGPOPGOISPHPGLTAWDHITGLEDGNVGLL	410
OC .	TRITCOPNANGGREAGGLSHLLPGYRVVENARBRSEVEKFWGLPVGRIAPERGLTVWEMILGLEMGKVGLV	411
Se	TSI-TGOPNANGGRETGGLAHLLPGYRKVIDPOHRADVETIWGLPMGSIBPOPGRTAWOHIEGLEDGAVGFL	423
ßs	CALTGOGNCOGAREHGOKADOLPGYRSIENEENRAHIAKVWGIHOUDI,PRKGVSAYEVNEKINDGDIKGLF	381
Кр	FALTGOPNANGGREVGGLATMLAAHMNF, EPDDLRRPARFWG., SERLAQTGGLTGLELFAAIGRGEVKAV	3 B 2
	···· · · · · · · · · · · · · · · · · ·	
An	wiratnpavempdlertxkallrspftiyqdayhptettayahvllpaaqwgektgintnserrytlcqaf	481
0c	WIAATNPAVBHPDLERTKKALSRSPFTVYQDAYYPTETANYAHVLLPAAQWSEKTGVHTNSERRITLCQAF	462
SS	WVAATNPAVBLPDVKRAQAALKRSPFTVLQDAYHPTETTTYAHLLLPAAQWSEKTGIMTNSERRVTLCQAF	494
88	LMC. SNPAVSSPNANLVKKALRRLTFFVAIDI.FI.SETAKYADVILPASSYLEDEGTHTNVEGRVTLREAS	450
Кр	WIMCTNAMVSLDGTSHAVTEDWPVSLVIISDVAD.TDTGRFAHIRFPALAWGEKSGTVTNSERRISLQRAF	452
	*** * ****	
An	ROFPREAKPDWEIFAEVGRRLGFEDKFAFTNSAQVYAEFTOLTKGR.PCDKSGISSQQLQAQGPTONP	548
oc	RDRPCEAKADWEIFAEVGRRLGFVEQFNFANBAEVYAEFTQLTKGR.PCEQTGICHQRLGADGPLQWP	549
SS	TO AND A TANKA YARA TANA TANA TANA TANA TANA TANA TANA T	
BS	ROFPGEARADWOIFAEVGRRLGFAFOTIDAAAVTAETVOVIRGR.LCDLBGLBAEDDAQAGTQG	559
	RCPFGERKHDWQIICDLASALGKGRYFSYTSAEDIFNELREASROG.IADYBGIBYGRLAREGGIHWP	559 517
кр	RGPFGERRADHQIFA EVGRIUGF.AFOTIDAAVYAETVQITAMK.LODGLGBELDAGGGIGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	559 517 523
Кр	RUPPOLARADWQIFALVGRAUGY:AFU/IDAAVEREFUVINGA.LOUBGUBACLDAUMAGAU RECFOLARIDWQIICDLASAUGAGIFYISADIFIALEEASAGGIIDDAGGIGAURAAIGA RECFOLARIDQIICDLASAUGAGIFYISADIFIALEEASAGGIISADWRIGAATWIALEEVKWP REFFOLARAAGGISPAGRGLASALPGSIRTRCSANTPRFQQMKTWASGRLISADWRIGAATWIALEEVKWP	559 517 523
кр	RUFPEDERKADWUIFADVGKRUFFOITDARAVKETUTINGK.EDUGUBHELDARAGGUMP REFOREARHOUILCDLSALGKGETYSTSANDIFNELERASGG.IADVGGIGYGKERRGGUMP NPPPGEARAAGGLSPAGRGIASALPGSIRTRCSANTPRFQCMKTMASGRLISADWFISAATWIALEPVRMP	517 523
Kp An	HQPPLOARADDQLFALVGRLDYAFOIIDAAVYRETQLIARALCDUGDSGLDSGLDAQAGQU RCPCJCARHDQLLCDLSALGXQFYYSTADDIFHELEASSGG INDEGGLSGLDAQAGQU RPPFGLNAAGGLSPAGRGLASALDGJIFTRGSANPFRQARKINASGRLISADFGLSANVHILEVKRP HSSINIETQRPTQHPTVRSQCFRLIVENQUFRLIVEPRQARKINASGRLISADFDPSYPPULTGG	517 523 619 601
Kp An Oc	HQFPGEARADWQIFAEVGKRLGF:.NFOITGARAYRAFTQTINGR.LCDLGGLGBHELDAQAGQUF RCFCGEARHOQIICDLGAALGKGFYSTARDIFNELERASRG.IADWGISACHERGGIGUF NFPFGEARAAGGLSPAGRGIASALPGJIRTRCSANTPRFQCMKTMASGRLISADWFISAATWIALEPVRHP HSSINIETQRFTQHFTYNGQQSTYTSQQSKRLYTDLRFXFPDGRARFGAY9SKGLAEPFDPSYPVLTTGR CFEEAGENNAALYTDLRFXFPDGRARFAAY8SKGLAEPFDPSYPVLTTGR CFEEAGENNAALYTDLRFXFPDGRARFAAY8SKGLAEPFDPSYPVLTTGR	517 523 619 601
Kp An Oc Ss Bc	RUPPLORADADULTA EVGRLUP: .AFOIIDAAVKRYTQUINDA.: LOLGADAGUSALLARADAGUSAL REPECTARKINGUILCDLASALUKGEYEYİSADDITHELERASKGI.IDDIGGIYEYRAKINGALIMP REPECTARKINGUILCDLASALUKGEYEYİSADDITHELERASKGI.IDDIGGIYEYRAKINTALEVKMP HSGINIETQRPTQHPTVMGQOTVTBQQIKTLYTDLERYFPDGRARAFGAYYBKGLAEPPDBYYPVL CPEEDAGENKARLYTDLERYFPDGRARAFQYHKGGLAEPPDDPYYPVL CPEEDAGENKARLYTDLERYFPDGRARAFQYPHLGVAEFPDDFYPUTUTGA CPEEDAGENGLETIEKRLYKHIRAYADGRARQOFALIYDVEG	559 517 523 619 601 612 569
Kp An Oc Ss Bs Ko	RUFPEDARADWULFAL. EVGRLOF: AFOITOARAV KEFUUTURAL LOUBGUBSLDAWARGUNF REFORMANDUILDUSALUKGYFYSTAADIFHRULERASRG.IDAVGAGURFRGGINF RFFFGENARAGGLSPAGRGLASALHGUIRTKCSANTPRFQGNKTMASGRLISADWRISAATWIALEPVRWF HSSINIETQRPTQHPTWNSQQSFKUYTDLRFHTPDGRARFAAYUSKGLAEPFDPSYPYVLTTGG .CFEEAGENTAALLYTDLRFHTPDGRARFAAYUSKGLAEPFDPSYPYVLTTGG .CFEEAGENNAALLYTDLRFHTPDGRARFAAYUSKGLAEPFDPSYPYVLTTGG .CFEEAGENNAALLYTDLRFHTPDGRARFAAYUSKGBAEPFDPFYVLTTGG .CFESDIFGTGRLHYSHHFAXUDRARFAOFTRATTVASILIALADWFILTTGG	559 517 523 619 601 612 569 569
Kp Oc SS BS Kp	RUPPOLARADOLIA. BYORLOY .AFOITOARYYARYANAR.LOUGUBACLDAOROGYHUP REPFORANDAGIIDIARAUKARYYYTSADDIFHELEASAGGIIDYBGIYGYARARAGAGI HYPFOLANAAGGIYAAGAGIASALUGGIRTYTBOGYARYYPEGARAFAYSGRLISADYHJALEVRWP HSGINIETQRPTQHPTVNSQQBTVTBQQBKRLYYDLGRYFPDGRAFAYYMSGLAEPPDBYPFVTTG .CPEEDGENTAARLYYDLGRYFPDGRAFAYYMSGLAEPPDDFYTUTG .CPEEDGENTAARLYYDLGRYFPDGRAFAYYMSGLAEPPDBYPFYTUTG .CPEEDGENTGACHTISAHNORGAALSYYTPAFPYFREPPFYFLFTTGA .VSRBEAMSYHKGWHRDGKLRMYPVAPQPTRATTVABIALUAADQ	517 523 619 601 612 569 569
Kp Oc Ss Bs Kp	RUFPEDARADOLIADVGRLDY.AFOITOARVYREFUUTURALLODGUBALDAMAGURA REPECENANDULLCDLSALGKOFFYSTAADOITABLEASSGG LIDAGGISURARRAGURA REPECENANGULSPAGRGLASALPGSIRTKCSANTPRFQGNKTMASGRLISADWISAATWIALEPVRWP HSBINIETQRPTQHPTVNSQQSTVTSQQSKRLYTDLRFHTPDGRARFAAYYSKGLAEPFDPSYPFVLTTGR .CPEERGENNAARLYTDLRFHTPDGRARFAAYYSKGLAEPFDPSYPFVLTTGR .CPEERGENNAARLYTDLRFHTPDGRARFAAYYSKGLAEPFDPSYPFVLTTGR .CPEERGENNAARLYTDLRFHTPDGRARFAAYYSKGLAEPFDPSYPFVLTTGR .CPEERGENNAARLYTDLRFHTPDGRARFAAYYSKGLAEPFDPSYPFVLTTGR .CPEERGENNAARLYTDLRFHTPDGRARFAAYYSKGLAEPFDPSYPVLTTGR .CPESDIFPTCRLFTSHAFDGKAALSVTPNEPFVFKEKFTADYPLYLTTGR	559 517 523 619 601 612 569 569
Kp Oc Ss Bs Kp An	RUPPEDARADWELTA BYGREDYRFOITARVYRETONIAR.ECDESDBACLDAGMAGYINP REPETERMENTELICDLASADURGEYFSIARDITHELEASIGG INDYRGIBYERGKREGATURP NEPFOLANAAGGISYAGRGIASADUGSIRTKESANYPREQORKTMASGRLISADWELTARVETIL HSSINIETQRPTQHPTVNEQQETVTBQQBKLLYTDLEFXFPDGRARAFVANSGLAEPPDFYPFURT CPEEDGENTARLYTDLEFXFPDGRARAFVANSGLAEPPDPFYFURT CPEEDGENTARLYTDLEFXFPDGRARAFVANSGLAEPPDPFYFURT CPEEDGENTARLYTDLEFXFPDGRARAFVANSGLAEPPDFYFUTUTG CPESDIPGTGNCLTESSAHBORGALAISYTDREPPFFKERFTAPPFLYLIFTGA VSRBLAMSUHKGWHRDGKLENYPVAPOPTRATTVABIAILMADPQ LYGHWETOTRTGRIEKIRAMPEPFIEIFPFEFIFI	559 517 523 619 601 612 569 569 569
Kp Oc Ss Bs Kp An Oc	RUPPOLARADOLIA BYORKLOY AFOI IDARY KAF YUTURA LODGUBALDAGASULARAGU YA REPOPLARADOLIA DISALGKOFY KYTAADI FINLERSING I IDAGGI YORKIR REGI YA REPOPLARADGU ISPAGRG LASALPGSI RETKESANTPREQCHKTMASGRLI SADVRI SAATWI AL LEVENAP HSSINI ETQRPTQHPTVNSQQSKTUYTQQSKRLYTDLRFHTPDGRARPGAYYSKGLAEPPDPSYPFVLTTGR CPEEAGENNAARLYTDLRFHTPDGRARPGAYYSKGLAEPPDPSYPFVLTTGR CPEEAGENNAARLYTDLRFHTPDGRARPGAYYSKGLAEPPDPSYPFVLTTGR CPEEAGENNAARLYTDLRFHTPDGRARPGAYYSKGLAEPPDPSYPFVLTTGR CPEEAGENNAARLYTDLRFHTPDGRARPGAYYTSKGLAEPPDPSYPFVLTTGR CPEEAGENNAARLYTNHFAY NDGRARPAYYTHLGVAEPPODFYTUTTGR CPEEAGENNAARLYTNHFAY NDGRARPAYTYASI AL UMADPO VSRBEAMSYHKGWRBGKLENVTVAPOPTRATTVASI AL UMADPO LYGHWETQTRTGR IEKIRAMPEPFI EINPRDAAKLGI TDNYVVEVRGRBGGAKPPAYYTAI SOTVF	517 523 619 601 601 569 569 569 569 569
Kp OC SS BS Kp An OC SS	RUFPEDARADWUITA EVGRLOFFOITUADAV REFUTINGR. LODEGUBSELDAVAGUER REPCFORAMUNGIICDLSALGKGEFYSTSANDIFNELERSING. I.DAVGGIEVGRLEREGGIEN NPFPGEANAAGGLSPAGRGLASALPGLIRTRCSANTPREQGNETMASGRLISADWEISAATWIALEPVNH RESINIETQRPTQHPTVNSQQSFRUYTDQRFRTPDGRARFAAYUSKGLAEPFDPSYPVLTTGG 	517 523 619 601 612 569 569 688 670 681
Kp Oc Ss Bs Kp An Oc Ss Bs	RUPPOLARADOLIA BUGKLDYRUDIIDANY REFUGIIRAR.LUDIGUBALDAURAGUIN REPORDARHOUILCDLASALGKOUPYYSYSADDIPHELEBASKGG.LDDIGGUSYGARRAGUIN REPORDARHOUILCDLASALGKOUPYYSYSADDIPHELEBASKGG.LDDIGGUSYGARRAGUIN HEBINIETQRPTQHPTVNSQQSKRLYTDLRFHTPDGRAFFAAYASKGLAEPPDPSYPYLTTGR CPEEAGENNAAKLYTDLRFHTPDGRAFFAAYASKGLAEPPDPSYPYLTTGR CPEEAGENNAAKLYTDLRFHTPDGRAFFAAYASKGLAEPPDPSYPYLTTGR CPEEAGENNAAKLYTDLRFHTPDGRAFFAAYASKGLAEPPDPSYPYLTTGR CPEEAGENNAAKLYTDLRFHTPDGRAFFAAYASKGLAEPPDPSYPYLTTGR CPEEAGENNAAKLYTDLRFHTPDGRAFFAAYASKGLAEPPDPSYPYLTTGR CPEEAGENNAAKLYTDLRFHTPDGRAFFAAYASKGLAEPPDPSYPLYLTTGR CPEEAGENNAAKLYTDLRFHTPDGRAFAAYASKGLAEPPDPSYLTYAGI.ALGAAF LYGHWATGTRTGRIEKIRAHPEPFIEIHPRDAKGLGIDDWYVEVRGRRGAKRPAAYYAAIASGYLF LYGHWATGTRTGRIEKIRAHPESTMEIHPRDAKSKISTDDEAVENUKEBRRGARRPAAYYAAIASGYLF	517 523 619 601 612 569 569 569 688 670 681 638
Kp An Oc Ss Bs Kp An Oc Ss Bs Kp	RCFCDEARADWCITADVGRLDYAFOITOANAYAR FOUTUAR LODGEDBELDAWAGCI RU RCFCCEARHOUILCDLSALGKOEPYSTSADCIFNELEEASRGC.LDBEGLBECLBAWRGCIRF RFFFGEARAAGGLSPAGRGLASALDGLIRTRCSANTPRFQGNKTMASGRLISADWRISAAYWIALEPVNW HSSINIBTQRPTQHPTVNSQQSKRLYTDLRFHTPDGRARFAAYUSKGLAEPFDPSYPTVLTTGG CFEEAGENNAALLYTDLRFHTPDGRARFAAYUSKGLAEPFDPSYPTVLTTGG CFEEAGENNAALLYTDLRFHTPDGRARFAAYUSKGLAEPFDPSYPTVLTTGG CFEEAGENNAALLYTDLRFHTPDGRARFAAYUSKGLAEPFDPSYPTVLTTGG CFEEAGENNAALLYTDLRFHTPDGRARFAAYUSKGLAEPFDPSYPTVLTTGG CFEEDIFGTGNLFISSHAHFDGKAALSYTDPRAFTVASISL.DYPVLYLTTGG VSRBEAAWSVHKGWHRDGKLENVTVAPAFPTATTVASIALIAUFDTYLYLTGG LYGHNETQTRTGRIEKIRGHNPEPFIEIHPRDAKLGITDNYVEYRRBRGAAKPPAYTYAISPGTVF LYGHNETQTRTGRIEKIRGHNPEPFIEIHPRDAKKIGITDDVVEYRRBRGACFPAVTTAISPGTVF LYGHNETGTGRIEKIRGNHPEPFIEIHPRDAKKIGITDDVVEYRRBRGACFPAVTTAISPGTVF UNGHNUTGYCRKISALAAHFESTNMEIHPGTAATVHIEDFUJVIEPPGBITVKGLAFGIEKOTVF	557 523 619 601 601 569 569 569 569 569 688 670 681 638 638 640
Kp OC SS BS Kp An OC SS BS Kp	RUPPOLARADOLIADVGRLDY.AFOINAVARY KAPJUNAR.LODGUBALDAUAGUIN REPORDARADOLICDLGAAGUGUYYYSADDIPHOLARAFGUNAR.LODGUGUARRAGUIN REPORDARAGU SPAGRGIASALDGSIRTRCSANTPROGARTASGRI SADVATIALEVNAP HEBINIETQRPTQHPTVNSQQBTVTSQQBKRLYTDLRFHTPDGRARFAAYYSKGLAEPPDPSYPYULTGR CPEEAGENNARLYTDLRFHTPDGRARFAAYYSKGLAEPPDPSYPYULTGR CPEEAGENNARLYTDLRFHTPDGRARFAAYYSKGLAEPPDPSYPYULTGR CPEEAGENNARLYTDLRFHTPDGRARFAAYYSKGLAEPPDPSYPYULTGR CPEEAGENNARLYTDLRFHTPDGRARFAAYYSKGLAEPPDPSYPYULTGR CPEEAGENNARLYTDLRFHTPDGRARFAAYYSKGLAEPPDPYULTTGR CPEEAGENNARLYTDLRFHTPDGRARFAAYYSKGLAEPPDPYULTTGR CPESOUPOICKITSSFAAHDGKAALSYTPHEPYVEKERTADYPULTTGR LYGHWTTLTTGRIEKIRAHPEPYIEBIHPRDARKIGIFDDDVEVRGR&GAKRPPARYTKAISGOVF LYGHWTTLTTGRIEKIRAHPEPYIEBHPRDARENISEQAVURGR&GAKRPPARYTKAISGOVF QRADPRYSMAHHOPHPAVRIMCHISKGARNISEQAVURGR&GUCEPRAVYTAIGSOVF QRADPRYSMAHHOPHPAVRIMCHINFVEVVPPAEPORYHLEGELARVRSPKCVMVARVTGRANGGSLF	557 523 619 601 601 569 569 569 569 569 569 569 569 569 569
Kp OC SS BS Kp An OC SS BS Kp	RUFPOEARADWEITA EVGRADUTFUTURATURATURATURATURATURATURATURATURATUR	557 523 619 601 601 569 569 569 569 688 670 688 638 640
Kp OCSSBS BS An SS BS Kp An SS SS Kp	RUPPOLARADULT. SVGRLDY .AFOINASY RAF UNDRE LOUDSDBELDAUMAGUING RECPOLARADULT. SVGRLDY .AFOINERS	5577 523 619 601 612 569 569 688 638 640 746
Kp OCSSBS BS Kp An SS BS Kp An CS SS Kp	RUPPOLARADOLIADVGRLDY.AFOIIDARY REFUTIARE LOUGHSLDSRLDAWAGUNG REPCFOLARHOUILCDLSALGKOUPYSTARDIFNELESSEG.IADYGGIYORGURARGGING REPCFOLARHOUILCDLSALGKOUPYSTARDIFNELESSEG.IADYGGIYORGURARGGING REPCFOLARHOUILCDLSALGKOUPYSTARDIFNELESSEG.IADYGGIYORGURARGGING REPCFOLARHOUILCDLSALGKOUPYSTARDIFNELESSEG.IADYGIYOLTAG CPEERGENNAALYTDLRFMTPDGRARFARYSKGLAEPFOPSYPYLTTG CPEERGENNAALYTDLRFMTPDGRARFARYSKGLAEPFOPSYPYLTTG CPEERGENNAALYTDLRFMTPDGRARFARYSKERTADYPLYLTTG CPEERGENNAALYTDLRFMTPDGRARFARYSKERTADYPLYLTTG CPESOLFOTGKLITSSTAHPOGKAALSYTDVENERSEGSAKPARYTKAISPGTVP LYGUMTITGKGIEXTKGRHPOPTEINFRAGKGURDENUENWEVERSEGSAKPARYTKAISPGTVP LYGUMTITGKGIEXTKINHPOPTEINFRAGKURTEDENVENERSEGGAKPARYTKAISPGTVP LYGUMTITGKGIEXTKINHPOPTEINFRAGKURTEDENVENERSEGSAKPARYTKAISPGTVP UPPOMTOTRKGIEXTANPESTMEINFORANTISEGGAVURGERGECEPRAVTTAISPGTVP QRADPHSMAHHOPHPAVRIMOHINEPVVEVPPAETORYNISEGGAVURGESGIFWXXKFTIG.RGNGRSLF 	5577 523 619 601 612 569 569 569 688 638 640 746 7175
Kp An Oc Be Kp An Oc Se B Kp An Oc Se Kp	RUPPOLARADOLIADVGRADUTPOLIDARVIKEY PIELEVIARE.COLGUSTARIAGULARAGOLUM REPORTARIAGULARADOLIADVGRADUTPOLIDARVIKEY PIELEVIARE.COLGUSTARIAGULARAGOLUM REPORTARIAGULSPAGHGIASALDGISHTRGSANTPREQCHETHASCHLISADHSLEDAVIGULARAGOLUM REPORTARIAGULSPAGHGIASALDGISHTRGSANTPREQCHETHASCHLISADHSLEDAVIGULARAGOLUM CEPERGENNAAHLITDLAFFTDGRANFRADAYSKGLAEPPDBEYPYULTUTG CEPERGENNAAHLITDLAFFTDGRANFRADAYSKGLAEPPDBEYPYULTUTG CEPERGENNAAHLITDLAFFTTDGRANFRADAYSKGLAEPPDDEYPJULTUTG CEPERGENNAAHLITDLAFFTTDGRANFRADAYSKGLAEPPDDEYPJULTUTG CEPERGENNAAHLITBLAFATHDGRANFVANHONDOLUMUTTGR CEPERGENNAAHLITBLAFATHDGRANFVANHONDOLIMUTTGR CEPERGENNGAAHSVHKGMHBOKLANFVANOPTRATTAIAILILALADO USBELANASVHKGMHBOKLANFVANOPTRATTAIAILILALADO LYGHWITLATGRIEKIKOMHÇOFISIHHRDARKIGIFDEDWVEYRBRGHARPPANYTKAISDOTYP LYGHWITLATGRIEKIKOMHÇOFISIHHRDARKIGIFDEDWVEYRBRGHARPPANYTKAISDOTYP LYGHWITLAFGRIEKIKOMHÇOFISIHHRDARKIGIFDEDWVEYRBRGHARPPANYTKAISDOTYP QRADPHSMAHHOHPAVRINGININFESIMENTRADARVINISGGANIJBBAGGOCEPANYTAISDOTYP SANAHLOHPAVRINGI HENDENGENDELQDEKRACANGU VIABBAGGOSEFVANYTAISSQUY VENNILITAVGRIENALGER KACANGU VIADINAAHLEFAKSBKISATFSY VPHMGKIMADSAENNAHTBFBAGGBALQDEKRACANGU VIADINAAHLIFAKSBKISATFSY VPHMGKIMADSAENNAHTBFBAGGBALQDEKRACANGU VIADINAAHLIFAKSBKISAFTSY VPHMGKIMADSAENNAHTBFBAGGBALQDEKRACANGU VIADINAAHLIFAKSBKISAFTSY	5577 523 619 601 601 569 569 569 569 569 688 6670 688 6640 7466 7717 7266
Kp An SSS BK An SSS BK An SSS An SSS An SSS BK	RUPPOLARADOLIADVGRLDYAFOIIDARY REFUGIARE.LDARGEDBELDARGEGUM REPCFOLARHOULICDLSALGROUPYSYTSADIFHELERSSGG.LDARGEDBELDARGEGUM REPCFOLARHOULICDLSALGROUPYSYTSADIFHELERSSGG.LDARGEDBELDARGEGUM RESINIETQRPTQHPTVNSQQSTVTSQQSKRLYTDLRFHTPDGRARFAAYYSKGLAEPFOPSYPYLTTGR CPEERGENNAALYTDLRFHTPDGRARFAAYYSKGLAEPFOPSYPYLTTGR CPEERGENNAALYTDLRFHTPDGRARFAAYYSKGLAEPFOPSYPYLTTGR CPEERGENNAALYTDLRFHTPDGRARFAAYYSKGLAEPFOPSYPYLTTGR CPEERGENNAALYTDLRFHTPDGRARFAAYYSKGLAEPFOPSYPYLTTGR CPEERGENNAALYTDLRFHTPDGRARFAAYYSKGLAEPFOPSYPYLTTGR SSBEAMSVHKGWRBGKLAWYVASPOPTRATTVASIAIIASCTV LYGUMHTQTRTGRIEXKGNHPOPTEINFBARKIGGTEDWSVGRSRGSAEPAXYTAISDCTVP LYGUMHTQTRTGRIEXKINHPESFNELMENGTANINIEGDAVUTERGRGGGAEPAXYTAISSCVLP VBSYLTGVDYKKSALAAHPFSYNELMPERDRYNISEGQAVUTERGRGGCGFPAXYTAISSCVLP QRADPHSYGAHOPHPAVRIMQHINFPVVEVPPAEPQRVHIEGGAAVIRGBRUKKEVTGERARGSLF 	5577 523 619 6612 569 569 569 569 569 569 569 688 638 640 746 717 729 674 711
Kp An OC SS BS Kp An CS SS BS Kp An CS SS BS Kp Kp	RUPPOLANADALIA. BYGRADY .AFOI IDARYTRFYUTIAR LODGUSBALDAUMAGUNG REPORTANDULL. DISALGKOUP, YISADDIPHELEBASKGG INDIGGISTORGAN RAGUNG REPORTANDULL. DISALGKOUPYSYSADDIPHERBASKGG INDIGGISTORGAN RAGUNG HESINIETQRPTQHPTVNSQQSKTUTDQQSKRLYTDLRPHTPGGANFRANYSKGLAEPPDBYPYUTIG .CPERGENNANLYTDLRFHTPGGANFRANYSKGLAEPPDDYPYUTIG .CPERGENNANLYTDLRFHTPGGANFRANYSKGLAEPPDDYPYUTIG .CPERGENNANLYTDLRFHTPGGANFRANYSKGLAEPPDDYPYUTIG .CPESGUPOTGKLTISTANDGKANJYTNGFANTAOPTNITUTUTG .VSRELANSVHKGMHBGKLANVTVAPTRGFNATUNGPTRTTVAILILLKAND LYGUNGTTRTGRIEKIKGNHPQPTISIHPRDAKLGIFDLNVVVEYRGBGGAKAPPAYVTKAIS. POTVP LYGUNGTTRTGRIEKIKGNHPQPTISIHPRDAKLGIFDLNVVVEYRGBGGGANYTRGRAECTPANTTLAI. DOTVP LYGUNGTTRTGRIEKIKGNHPQPTISIHPRDAKLGIFDLNVVVEYRGBGGGCFANYTTAIS. POTVP LYGUNGTURKSALANHPENTNIIPPACAGUPTATINIEGORAVVIRGBGGGCFANYTTAIS. POTVP LYGUNGTURKSALANHPENTNIIPPACAGUPTATINIEGORAVVIRGBGGGCFANYTTAIS. POTVP UNSHILTSVGFNSALANHPENTNIIPPACAGUPTATINIEGORAVVIRGBGGGCFANYTATAS. POTVP UNSHILTSVGFNSALANHPENTNIIPPACAGUPTATINIEGORAVVIRGBGGGCFANYTATAS. POTVP UNSHILTSVGFNSALANHPENTNIIPPACAGUPTATINIEGORAVVIRGBGGGCFANYTATAS. POTVP UNSHILTSVGFNSALANDFENTNIIPPACAGUPTATINIEGORAVVIRGBGGGCFANYTATAS. POTVP UNSHILTSVGFNSALANDFENTNIIPPACAGUPTATINIEGORAVVIRGBGGGCFANYTATAS. POTVP UNSHILTSVGFNSALANDFENTNEIPPACAGUPTATINIEGORAVVIRGBGGEGFANYTAGASLS. KOTVP UNSHILTSVGFNSALANDFENTNEIPPACAGUPTATINIEGORAVVIRGBGGTAVVIRGTAGANGGSLF YMMMALMGDRFZANALTFPACTIGGEPELRACAQUPTATONIKATHSILFIANSHVIRGAMUSATFSV YPMIMGALMGDRFZANALTFPACTIGGEPELRACAQUPTATINIKATHSILFIANSHVIRGANGSLSANATFSV YPMIMGALMGDRFZANALTFPACTIGGEPELRACAQUPTATINIKATHSILFIANSHVIRGANGGRUNANTIGANANJANANANJAN	5577 523 619 661 6612 569 569 569 569 569 688 640 746 638 640 746 7777 724 676 711
Kp An OCSBER An CSBER An CSBER An CSBER An CSBER	RUPPOLARADOLIA LYOKRADY FOI IDANY RAF VIEW RUMAR LODGAD BARLADAMAGUNG RECFORMANDULL DASALGKOFY KYTSADCIFIELEASSGG DADGGIFY GARAR RAGING RECFORMANDULL RECFORMANDULL REGINIETQRPTQHPTVNSQQSTVTSQQSKRLYTDLRFHFDGRARFGAYYSKGLAEPFDPSYPYLTTGR 	5577 523 619 661 563 569 569 569 569 569 688 638 638 638 638 638 638 638 638 639 746 7777 729 7275 711 782
Kp An OCSB BS Kp An OCSS SS Kp An OCSS SS Kp Kp	RUPPOLANADALIA. BYGRADY .APOILOAAVYARYAQAINAR LOUGABALDAANAQIA REPCOLANADALIA. BYGRADY .APOILOAAVYARYAQAINAR LOUGABALDAANAQIA REPCOLANADALIA REPCOLANADALIA CHEADALIA	5577 523 619 6612 569 569 569 569 688 6670 6688 6670 6688 6640 7467 7129 676 7457 7851

FIG. 5. Comparison of NarB sequences from *Anabaena* sp. strain PCC 7120 (An), *Oscillatoria chalybea* (Oc; 47), *Synechococcus* sp. strain PCC 7942 (Ss; 2), *B. subtilis* (Bs; 33) (in parentheses, a continuation of the translation 5' from the putative start codon), and *K. pneumoniae* (Kp; 26). Amino acids common to all five sequences are indicated by asterisks. Dots and the space after the parenthesis indicate gaps introduced to enhance alignment. Amino acids in common with the *Anabaena* sp. sequence are shown in bold print.

toxicity of exogenous or NO_3^- -derived NO_2^- in the absence of a functional nitrite reductase.

The results of pulsed-field gel electrophoresis indicated that the transposon insertion in strain TLN10 is located ca. 146 kb from an end of AvrE and suggest strongly that it is at ca. 0.71 Mb in the chromosome, oriented clockwise, about 100 kb clockwise from the phycocyanin operon (24). The experimental results are consistent with the interpretation that the transposon in TLN21 maps close to and at a location 3' from the transposon in TLN10. Finally, the transposon in a fourth rapidly activated locus (in strain TLN14) maps at a distant site, apparently at approximately 5.17 Mb, with the corresponding open reading frame oriented counterclockwise. KdpE, which is similar to the predicted polypeptide product of the open reading frame interrupted in TLN14, is thought to regulate an *E. coli* transport ATPase with high affinity for K⁺ that responds to changes in cell turgor (48). The relationship of such a function

TABLE 1. Luminescence of TLN10, TLN21, and SR796 during growth at low cell density with various nitrogen sources^{*a*}

Nitrogen	Luminescence $[10^7 \text{ quanta}/(\mu \text{g of chlorophyll } a \cdot \min)]^b$			
source	TLN10	TLN21	SR796	
NH_4^+	$63 \pm 9 (10)$	88 ± 27 (4)	98 ± 18 (4)	
NO_2^{-}		$310 \pm 50(2)$	$356 \pm 16(2)$	
NO_3^{-}		$758 \pm 241(5)$		
N ₂	8,992 ± 1,803 (4)	$1,234 \pm 266$ (4)	728 ± 94 (9)	

^{*a*} Each strain bore pRL1472a as a source of aldehyde, a substrate of luciferase. Cell density was $<2.1 \ \mu$ g of chlorophyll *a* ml⁻¹.

^b Data are the means \pm standard errors of the means of the number of experiments indicated parenthetically.

to nitrogen deprivation is obscure but could involve a structural similarity of NH_4^+ , whose uptake might be stimulated, and K^+ .

Expression of *luxAB* by transcriptional fusions was used to report on the transcription of genes involved in the assimilation of nitrate. It is notable that there is a decreasing gradient of activation of *luxAB* expression and thus of transcription from TLN10 (in which *luxAB* is fused to *nirA*) to TLN12 (fused to nrtC), TLN21 (fused at the 3' end of nrtD), and SR796 (fusion to narB) (Fig. 7a and b). This result would be expected if these genes form an operon in PCC 7120. How can such a model account for the subsequent reduction in transcription reported for TLN12, TLN21, and SR796, but not for TLN10? Perhaps an additional regulatory influence affects transcription as monitored in the former three strains. Other results also suggest that this cluster of genes is not regulated only coordinately. First, the ratios of steady-state luminescence in the presence of different nitrogen sources differed strikingly for TLN10, TLN21, and SR796 (Table 1). However, the exceptionally high luminescence of TLN10 grown on N₂ (on average, severalfold higher than that of a strain that bears a P_{rbcLS}luxAB fusion [12]) may itself impose a metabolic burden on cells. If so and if such a burden rendered them more nitrogen deprived than are TLN12, TLN21, and SR796, it would be possible to account for the differences illustrated in Fig. 7b and d and Table 1. Second, averaged from four independent experiments for each of these three strains, the decrease in luminescent intensity attendant upon transfer of SR796 from N2 to NH_4^+ appeared to lag the corresponding decreases observed for TLN10 and TLN21 (Fig. 7c). Multivariate repeatedmeasures analysis (27) of the data in Fig. 7c was consistent with the idea (probability of 0.94) that TLN10 and TLN21 were sampled from the same population but gave highly significant values for the hypotheses that SR796 and TLN10 were sampled from the same population (probability of 0.0079) and that SR796 and TLN21 were sampled from the same population (probability of 0.0007). Our data are, therefore, subject to the interpretation that *nirA* and *narB* may be to some extent independently regulated.

MSX, an inhibitor of glutamine synthetase, presumably prevents assimilation not only of exogenous NH_4^+ (when, as in the experiments illustrated in Fig. 7a and b, cells are deprived of that nitrogen source) but also of internally generated NH_4^+ , perhaps accounting for the diminished activation observed in the presence of MSX (Fig. 7d). The increase in expression of *luxAB* by TLN21 upon transfer of that strain from NH_4^+ to either NO_2^- or NO_3^- (Fig. 8a) was nearly completely eliminated when NH_4^+ remained present (Fig. 8b), suggesting that the increase is attributable primarily to the lesser effectiveness



FIG. 6. Representative images of light emission from filaments of TLN10(pRL1472a) (a through d) and SR796(pRL1472a) grown on N₂ (e and f). (b, d, and f) Bright-field video images. H, heterocyst. (a, c, and e) Corresponding images of luminescence recorded during a 20-min exposure, with cell outlines drawn from superimposed bright-field images.

of NO₂⁻ and NO₃⁻ as nitrogen sources in strain TLN21 rather than to induction by these two substrates.

Whereas heterocyst differentiation is first evident by transmission light microscopy after ca. 12 h of nitrogen deprivation, the first indication of cells that can be identified as presumptive heterocysts is localized transcriptional activation of *hetR* after 3.5 h of deprivation (7). Our current results show that cells respond physiologically to nitrogen deprivation within 0.5 h and that the initial activation of *nrtC*, *nrtD*, and *narB* moder-



FIG. 7. Response of strains TLN10 (\blacktriangle), TLN12 (\blacklozenge), TLN21 (\blacklozenge), and SR796 (\blacksquare) to transfer between different environmental conditions. (a and b) Transfer from NH₄⁺ to N₂. The relative luminescence (the mean ± standard error of the mean of the results from the indicated number of experiments, compared with a value of 1.00 at 0 h) for each of these strains was 1.8 ± 0.4 (n = 3), 1.3 ± 0.2 (n = 4), 1.3 ± 0.1 (n = 4), and 1.7 ± 0.2 (n = 3) at 0.25 h and 4.3 ± 0.2, 3.0 ± 0.5, 2.3 ± 0.3, and 2.0 ± 0.2 at 0.5 h (same number of experiments as before). (c) Transfer from N₂ to NH₄⁺. The mean ratio (standard error of the mean/mean) for all data points ≥1 h, averaged over four experiments, was 0.06. (d) Transfer from NH₄⁺ to NH₄⁺ plus MSX.

ates, perhaps in response to mobilization of internal nitrogenous reserves, within 2.5 h. Others have observed that the presence of NO2⁻ or NO3⁻ is essential for all but very lowlevel activity of nitrite reductase and nitrate reductase in Anabaena strains (16, 32, 35; see also references 4, 19, 21, and 43). In contrast, the results presented in Table 1 show that despite abundant availability of cellular nitrogen during growth on N₂, it is under those conditions that the NO₃⁻-assimilatory genes are most strongly promoted in our cultures of Anabaena sp. strain PCC 7120. Moreover, our measurements of nitrate reductase activity confirm that wild-type filaments possess plentiful nitrate reductase activity during growth in the absence of nitrate. Unlike the nif genes, whose expression in aerobic cultures of Anabaena sp. strain PCC 7120 is restricted to heterocysts (12), the genes for nitrite and nitrate reductase were expressed in vegetative cells of filaments grown on N₂ (Fig. 6).

As shown by Fig. 7d, MSX blocks the repressing effect of NH_4^+ on expression of *luxAB* in all four mutant strains examined. Therefore, repression may be affected by glutamine or, as in *Synechococcus* sp. strain PCC 7942 (44), by a product of its metabolism. Heterocysts transfer fixed nitrogen to contiguous vegetative cells partially at least in the form of glutamine (46). We therefore anticipated that in N₂-grown filaments of TLN10



FIG. 8. Response of TLN21 to transfer from NH_4^+ to either NO_2^- (\Box) or NO_3^- (\blacksquare) in the absence (a) or continued presence (b) of NH_4^+ .

and SR796, there might be lesser expression of *luxAB* near heterocysts than in cells farther from heterocysts and that such a gradient of expression could be used as a bioassay to identify substances that might be responsible for regulation. However, in the nine filaments of TLN10 (examples shown in Fig. 6a through d) and six filaments of SR796 (one shown in Fig. 6e and f) that were imaged, no consistent deficiency of transcription of *nirA* or *narB* was observed in cells contiguous with heterocysts.

Repeats of oligonucleotides, often heptanucleotides, are common features of DNA sequences of Anabaena sp. strain PCC 7120 and closely related strains (45); Bauer et al. (6) observed, as we have here, such repeats within the coding sequence of a gene. Andriesse et al. (3) commented that because their sequence of narB predicted two possible start codons, it was unclear whether the NarB of Synechococcus sp. strain PCC 7942 contains 715 or 729 amino acids. The likelihood of similarities with the other sequences that are now available provides a reason to suggest that it is the second methionine in the open reading frame they reported that is the actual start codon (Fig. 5). No classical example of a ribosome binding site is seen 5' from the coding region of the PCC 7120 narB, but on the basis of the sequence of 16S rRNA from that organism (25), either GGAG or GAAG, which is more likely because it is closer to the GTG start codon, may serve. The predicted mass and pI of the polypeptide portion of Anabaena sp. strain PCC 7120 NarB are 83.0 kDa and 7.40, respectively. In the 746 amino acids of PCC 7120 NarB, only 17 cysteines are found; of these (except for a pair, amino acids 167 and 168), only 3 form a spaced cluster, CXXCXXXC (amino acids 9 through 16), which may serve to bind a molybdenum cofactor or nonheme iron of an Fe_xS_x cluster (51). Although the sequence, TXTXCPYCGVGCG, close to the N terminus is highly conserved within cyanobacteria and Klebsiella pneumoniae, no corresponding sequence within the predicted NarB of Bacillus subtilis was reported (33). However, one codon in advance of their proposed initiation codon is a sequence that translates as TQCPFCSMQC, i.e., TXCPXCXXXC. No plausible initiation codon is present between the previous stop codon and this amino acid sequence; therefore, if this series of amino acids is to be considered part of B. subtilis NarB, there would have to be a prior frameshift in the sequence reported. Other regions of high conservation of amino acid sequence among enzymes that contain a pterin molybdenum cofactor correspond to amino acids 142 through 152, 211 through 239, 416 through 422, 441 through 488, and 688 through 693 in the sequence of NarB from Anabaena sp. strain PCC 7120. In a structurally related molecule, E. coli formate dehydrogenase, C-148 (in the Anabaena sequence) is replaced by selenocysteine (51, 53).

Whereas NarB from Anabaena sp. strain PCC 7120 shows extensive similarities in amino acid sequence to the nitrate reductases from a diversity of other prokaryotes (Fig. 5), NirA from that same strain shows extensive similarities in amino acid sequence to the nitrite reductases of other cyanobacteria and higher plants (Fig. 4) but very low similarities to the nitrite reductases of heterotrophic bacteria [e.g., a BLAST score of only 52 with the NAD(P)H-dependent nitrite reductase from B. subtilis (34)]. It actually shows greater similarities to bacterial sulfite reductases (one example in Fig. 4), which share with nitrite reductases the use of a siroheme cofactor. Higher-plant nitrite reductase is a nucleus-encoded chloroplast enzyme (9). These observations are consistent with two ideas presented by Flores and coworkers (16, 29). First, cyanobacterial nirA is evolutionarily related, albeit distantly, to bacterial sulfite reductases. Second, when an endosymbiotic cyanobacterium

evolved into a chloroplast, the NirA protein that it encoded remained chloroplast situated, presumably to make use of ferredoxin as a reductant, whereas the gene encoding that protein became relocated to the plant nucleus.

ACKNOWLEDGMENTS

We thank Lei Chen and Yi-Hsuan Lai (Dept. of Statistics and Probability, Michigan State University) for assistance with statistical analysis.

This work was supported by NSF grant IBN-9118152 and by DOE grant DE-FG02-90ER20021.

REFERENCES

- Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403–410.
- 2. Andriesse, A. J., and H. Bakker. GenBank accession number X74597.
- Andriesse, X., H. Bakker, and P. Weisbeek. 1990. Analysis of nitrate reduction genes in cyanobacteria, p. 303–307. *In* W. R. Ullrich, C. Rigano, A. Fuggi, and P. J. Aparicio (ed.), Inorganic nitrogen in plants and microorganisms. Springer-Verlag, Berlin, Germany.
- Avissar, Y. J. 1985. Induction of nitrate assimilation in the cyanobacterium Anabaena variabilis. Physiol. Plant. 63:105–108.
- Back, E., W. Burkhart, M. Moyer, L. Privalle, and S. Rothstein. 1988. Isolation of cDNA clones coding for spinach nitrite reductase: complete sequence and nitrate induction. Mol. Gen. Genet. 212:20–26.
- Bauer, C. C., L. Scappino, and R. Haselkorn. 1993. Growth of the cyanobacterium *Anabaena* on molecular nitrogen—*nifJ* is required when iron is limited. Proc. Natl. Acad. Sci. USA 90:8812–8816.
- Black, T. A., Y. Cai, and C. P. Wolk. 1993. Spatial expression and autoregulation of *hetR*, a gene involved in the control of heterocyst development in *Anabaena*. Mol. Microbiol. 9:77–84.
- Buikema, W. J., and R. Haselkorn. 1993. Molecular genetics of cyanobacterial development. Annu. Rev. Plant Physiol. Plant Mol. Biol. 44:33–52.
- Crawford, N. M. 1995. Nitrate: nutrient and signal for plant growth. Plant Cell 7:859–868.
- Douglas, S. E. 1994. Chloroplast origins and evolution, p. 91–118. *In* D. A. Bryant (ed.), The molecular biology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Elhai, J., and C. P. Wolk. 1988. Conjugal transfer of DNA to cyanobacteria. Methods Enzymol. 167:747–754.
- Elhai, J., and C. P. Wolk. 1990. Developmental regulation and spatial pattern of expression of the structural genes for nitrogenase in the cyanobacterium Anabaena. EMBO J. 9:3379–3388.
- Fay, P. 1992. Oxygen relations of nitrogen fixation in cyanobacteria. Microbiol. Rev. 56:340–373.
- Fernández-Piñas, F., and C. P. Wolk. 1994. Expression of *luxCD-E* in Anabaena sp. can replace the use of exogenous aldehyde for in vivo localization of transcription by *luxAB*. Gene 150:169–174.
- 15. Flores, E. Personal communication.
- Flores, E., and A. Herrero. 1994. Assimilatory nitrogen metabolism and its regulation, p. 487–517. *In* D. A. Bryant (ed.) The molecular biology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Gallon, J. R. 1992. Reconciling the incompatible: N₂ fixation and O₂. New Phytol. 122:571–609.
- Gray, M. W., and W. F. Doolittle. 1982. Has the endosymbiont hypothesis been proven? Microbiol. Rev. 46:1–42.
- Hattori, A. 1962. Adaptive formation of nitrate reducing system in *Anabaena cylindrica*. Plant Cell Physiol. 3:371–377.
- 20. Herrero, A. Personal communication.
- Herrero, A., E. Flores, and M. G. Guerrero. 1981. Regulation of nitrate reductase levels in the cyanobacteria *Anacystis nidulans, Anabaena* sp. strain 7119, and *Nostoc* sp. strain 6719. J. Bacteriol. 145:175–180.
- Herrero, A., E. Flores, and M. G. Guerrero. 1985. Regulation of nitrate reductase cellular levels in the cyanobacteria *Anabaena variabilis* and *Syn*echocystis sp. FEMS Microbiol. Lett. 26:21–25.
- Hu, N.-T., T. Thiel, T. H. Giddings, and C. P. Wolk. 1981. New Anabaena and Nostoc cyanophages from sewage settling ponds. Virology 114:236–246.
 Kuritz, T., A. Ernst, T. A. Black, and C. P. Wolk. 1993. High-resolution
- Kuritz, T., A. Ernst, T. A. Black, and C. P. Wolk. 1993. High-resolution mapping of genetic loci of *Anabaena* PCC 7120 required for photosynthesis and nitrogen fixation. Mol. Microbiol. 8:101–110.
- Ligon, P. J. B., K. G. Meyer, J. A. Martin, and S. E. Curtis. 1991. Nucleotide sequence of a 16S rRNA gene from *Anabaena* sp. strain PCC 7120. Nucleic Acids Res. 19:4553.
- Lin, J. T., B. S. Goldman, and V. Stewart. 1993. Structures of genes *nasA* and *nasB*, encoding assimilatory nitrate and nitrite reductases in *Klebsiella pneumoniae* M5al. J. Bacteriol. 175:2370–2378.
- Littell, R. C., R. J. Freund, and P. C. Spector. 1991. SAS system for linear models, p. 266–272. SAS Institute Inc., Cary, N.C.
- 28. Luque, I., A. Herrero, E. Flores, and F. Madueño. 1992. Clustering of genes

involved in nitrate assimilation in the cyanobacterium *Synechococcus*. Mol. Gen. Genet. **232**:7–11.

- Luque, I., E. Flores, and A. Herrero. 1993. Nitrite reductase gene from *Synechococcus* sp. PCC 7942: homology between cyanobacterial and higherplant nitrite reductases. Plant Mol. Biol. 21:1201–1205.
- Luque, I., E. Flores, and A. Herrero. 1994. Nitrate and nitrite transport in the cyanobacterium *Synechococcus* sp. PCC 7942 are mediated by the same permease. Biochim. Biophys. Acta 1184:296–298.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Martin-Nieto, J., A. Herrero, and E. Flores. 1989. Regulation of nitrate and nitrite reductases in dinitrogen-fixing cyanobacteria and Nif⁻ mutants. Arch. Microbiol. 151:475–478.
- Ogasawara, N., Y. Fujita, Y. Kobayashi, Y. Sadaie, T. Tanaka, H. Takahashi, K. Yamane, and H. Yoshikawa. 1995. Systematic sequencing of the *Bacillus* subtilis genome: progress report of the Japanese group. Microbiology 141: 257–259.
- Ogawa, K.-I., M. M. Nakano, P. Zubar, and K. Yamane. 1994. GenBank accession number D30687.
- Ohmori, K., and A. Hattori. 1970. Induction of nitrate and nitrite reductases in *Anabaena cylindrica*. Plant Cell Physiol. 11:873–878.
- Omata, T. 1991. Cloning and characterization of the *nrtA* gene that encodes a 45-kDa protein involved in nitrate transport in the cyanobacterium *Synechococcus* PCC 7942. Plant Cell Physiol. 32:151–157.
- Omata, T. 1995. Structure, function and regulation of the nitrate transport system of the cyanobacterium *Synechococcus* sp. PCC7942. Plant Cell Physiol. 36:207–213.
- Omata, T., M. Ohmori, N. Arai, and T. Ogawa. 1989. Genetically engineered mutant of the cyanobacterium *Synechococcus* PCC 7942 defective in nitrate transport. Proc. Natl. Acad. Sci. USA 86:6612–6616.
- Omata, T., X. Andriesse, and A. Hirano. 1993. Identification and characterization of a gene cluster involved in nitrate transport in the cyanobacterium *Synechococcus* sp. PCC7942. Mol. Gen. Genet. 236:193–202.
- 40. Ostrowski, J., J.-Y. Wu, D. C. Rueger, B. E. Miller, L. M. Siegel, and N. M. Kredich. 1989. Characterization of the *cysJIH* regions of *Salmonella typhimurium* and *Escherichia coli* B. DNA sequences of *cysI* and *cysH* and a model for the siroheme-Fe₄S₄ active center of sulfite reductase hemoprotein based on amino acid homology with spinach nitrite reductase. J. Biol. Chem. 264:15726–15737.
- 41. Rubio, L. M., A. Herrero, and E. Flores. 1996. A cyanobacterial narB gene

encodes a ferredoxin-dependent nitrate reductase. Plant Mol. Biol. 30:845-850.

- Suzuki, I., H. Kikuchi, S. Nakanishi, Y. Fujita, T. Sugiyama, and T. Omata. 1995. A novel nitrite reductase gene from the cyanobacterium *Plectonema boryanum*. J. Bacteriol. 177:6137–6143.
- Suzuki, I., T. Omata, and T. Sugiyama. 1992. Gene expression and regulation of nitrate assimilating enzymes in *Synechococcus* PCC7942. Res. Photosyn. 4:75–78.
- Suzuki, I., T. Sugiyama, and T. Omata. 1993. Primary structure and transcriptional regulation of the gene for nitrite reductase from the cyanobacterium *Synechococcus* PCC 7942. Plant Cell Physiol. 34:1311–1320.
- Thiel, T. 1994. Genetic analysis of cyanobacteria, p. 581–611. *In* D. A. Bryant (ed.), The molecular biology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- 46. Thomas, J., J. C. Meeks, C. P. Wolk, P. W. Shaffer, S. M. Austin, and W.-S. Chien. 1977. Formation of glutamine from [¹³N]ammonia, [¹³N]dinitrogen, and [¹⁴C]glutamate by heterocysts isolated from *Anabaena cylindrica*. J. Bacteriol. **129**:1545–1555.
- Unthan, M., W. Klipp, and G. H. Schmid. 1996. Nucleotide sequence of the narB gene encoding assimilatory nitrate reductase from the cyanobacterium Oscillatoria chalybea. Biochim. Biophys. Acta 1305:19–24.
- Walderhaug, M. O., J. W. Polarek, P. Voelkner, J. M. Daniel, J. E. Hesse, K. Altendorf, and W. Epstein. 1992. KdpD and KdpE, proteins that control expression of the *kdpABC* operon, are members of the two-component sensor-effector class of regulators. J. Bacteriol. 174:2152–2159.
- Wolk, C. P., Y. Cai, and J.-M. Panoff. 1991. Use of a transposon with luciferase as a reporter to identify environmentally responsive genes in a cyanobacterium. Proc. Natl. Acad. Sci. USA 88:5355–5359.
- Wolk, C. P., A. Ernst, and J. Elhai. 1994. Heterocyst metabolism and development, p. 769–823. *In* D. A. Bryant (ed.), The molecular biology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- 51. Wootton, J. C., R. E. Nicolson, J. M. Cock, D. E. Walters, J. F. Burke, W. A. Doyle, and R. C. Bray. 1991. Enzymes depending on the pterin molybdenum cofactor: sequence families, spectroscopic properties of molybdenum and possible cofactor-binding domains. Biochim. Biophys. Acta 1057:157–185.
- Yanisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene 33:103–119.
- 53. Zinoni, F., A. Birkmann, T. C. Stadtman, and A. Böck. 1986. Nucleotide sequence and expression of the selenocysteine-containing polypeptide of formate dehydrogenase (formate-hydrogen-lyase-linked) from *Escherichia coli*. Proc. Natl. Acad. Sci. USA 83:4650–4654.