Anabaena sp. Strain PCC 7120 Gene *devH* Is Required for Synthesis of the Heterocyst Glycolipid Layer

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In response to deprivation for fixed nitrogen, the filamentous cyanobacterium *Anabaena* sp. strain PCC 7120 provides a microoxic intracellular environment for nitrogen fixation through the differentiation of semiregularly spaced vegetative cells into specialized cells called heterocysts. The *devH* gene is induced during heterocyst development and encodes a product with characteristics of a *trans*-acting regulatory protein. A *devH* mutant forms morphologically distinguishable heterocysts but is *Fox*[−], incapable of nitrogen fixation in the presence of oxygen. We demonstrate that rearrangements of nitrogen fixation genes take place normally in the *devH* mutant and that it is *Fix*⁺, i.e., has nitrogenase activity under anoxic conditions. The *Fox*[−] phenotype was shown by ultrastructural studies to be associated with the absence of the glycolipid layer of the heterocyst envelope. The expression of glycolipid biosynthetic genes in the mutant is greatly reduced, and heterocyst glycolipids are undetectable.

Anabaena sp. strain PCC 7120 is a filamentous cyanobacterium in which, in response to deprivation for fixed nitrogen, vegetative cells at semiregular intervals differentiate into nitrogen-fixing cells called heterocysts. Nitrogenase, the enzyme that catalyzes the reduction of atmospheric dinitrogen (N₂) to ammonia, is rapidly and irreversibly inactivated in the presence of oxygen. The heterocyst provides a microoxic intracellular environment for nitrogenase through the development of a thick envelope, an alteration of photosynthetic activity, and an increased rate of respiration (26).

The heterocyst envelope is composed of an inner laminated glycolipid layer that greatly reduces penetration of oxygen and an outer polysaccharide layer that protects the glycolipid layer (17, 22). Studies of cyanobacterial mutants with heterocyst defects have led to the identification of a number of genes involved in the development of the heterocyst envelope. Biosynthesis of the polysaccharide layer is regulated by the products of *devR* (4) and *hepK* (28), which encode part or all of a two-component regulatory system (27). The *hepA*, *hepB*, and *hepC* genes are involved in the synthesis of the polysaccharide layer (14, 25, 28). Synthesis of the glycolipid layer involves a *hglE* gene (5) and the cluster of genes *hglD*, *hglC*, and *hglB* (*hetM*) (1, 3). The *hglK* (2) and *devBCA* gene cluster (8) are implicated in glycolipid transport and/or assembly.

The *devH* gene was identified in a screen for sequences up-regulated during heterocyst development (6). The steady-state level of *devH* transcripts increases fivefold upon nitrogen starvation of *Anabaena* sp. strain PCC 7120. In the absence of

fixed nitrogen, a *devH* mutant strain (A57) forms heterocysts but is incapable of fixing nitrogen in the presence of oxygen (the *Fox*[−] phenotype) (12). The DevH protein is most closely related to the cyanobacterial transcriptional regulator NtcA, with a high degree of identity in the helix-turn-helix motif presumed to be involved in DNA binding (12). The similarity of DevH and NtcA and the phenotype of the *devH* mutant suggest that DevH plays a role as a *trans*-acting regulatory protein with a role in heterocyst function. Here we provide initial characterization of DevH protein expression and show that the *Fox*[−] phenotype of the *devH* mutant is associated with defective heterocyst envelopes that lack the glycolipid layer.

MATERIALS AND METHODS

Strains and culture conditions. Strains used in this study are listed in Table 1. Liquid cultures of cyanobacteria for protein or nucleic acid isolations were grown in modified Kratz and Myers medium supplemented with 5 mM NH₄NO₃ (K&M, N⁺) as described previously (12). The *devH* mutant (A57) (12) was grown in 40 μg of neomycin sulfate/ml. For the developmental time course, cells were grown essentially as described previously (12) in medium lacking a source of combined nitrogen (K&M, N[−]). Cells collected at various times after the removal of fixed nitrogen (nitrogen stepdown) were used for total DNA or RNA isolation or for Western analysis. For nitrogenase assays, liquid cultures were grown in BG-11 medium as described previously (12) (see “Nitrogenase activity assays,” below). In preparation for electron microscopy, *Anabaena* sp. strains PCC 7120 and A57 were grown for 5 days in AA/8 + N (15) plus, for A57, 80 μg of neomycin sulfate/ml at 30°C in the light at ca. 140 μE m^{−2} s^{−1} (Li-Cor quantum radiometer/photometer, model LI-185A; Lincoln, Nebr.). Cells were then sedimented, washed three times with AA/8 (15), and plated on a Nuclepore Rec-85 membrane atop AA agar for 3 days.

Escherichia coli strains were grown as described previously (12). *E. coli* strain DH5α (Bethesda Research Laboratories, Gaithersburg, Md.) was the host for most plasmids, and *E. coli* strain BL21(DE3)pLysS was the host for protein expression.

Isolation and analysis of nucleic acids. DNA was isolated as described previously (12). Total RNA was isolated with the RNeasy reagent (Ambion) following the manufacturer's instructions. Southern, Northern, and slot blot preparations were performed as described previously (6, 12).

Probe fragments were obtained by PCR amplification of *Anabaena* sp. strain PCC 7120 genomic DNA or by isolation of plasmid inserts (Table 1). The probe fragments used were the following: (i) for *nifD*, a 3.0-kb HindIII fragment from pAn256; (ii) for *nifH*, a 1.9-kb HindIII fragment from pAn154.3; (iii) for *mpB*, a

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TABLE 1. Bacterial strains, plasmids, and oligonucleotides used in this study

Strain, plasmid, or oligonucleotide	Characteristics ^a or sequence (5' to 3') ^a	Source
<i>Anabaena</i> sp. strains		
PCC 7120	Wild type	R. Haselkorn
A57	PCC 7120 <i>devH</i> ::pBN1-239B, Nm ^r	12
<i>E. coli</i> strains		
DH5 α	F ⁺ recA, p80dlacZdM15	Bethesda Research Laboratories
BL21(DE3)pLysS	F ⁺ <i>omp</i> <i>T</i> <i>hsdS</i> _B (r _B ⁺ m _B ⁺) <i>gal dcm</i> (DE3)/pLysS	Novagen
Plasmids		
pBluescript KS(+); pBS	Cloning vector, Ap ^r	Stratagene
pET30a	Expression vector, Km ^r	Novagen
pET23930a	700-bp <i>devH</i> open reading frame in pET30a, Km ^r	This study
pAn154.3	1.9-kb HindIII fragment containing <i>nifH</i> gene in pBR322, Ap ^r Tet ^r	19
pAn256	3.0-kb HindIII fragment containing <i>nifD</i> element recombination site in pBR322, Ap ^r Tet ^r	19
pRNAseP	602-bp EcoRI genomic PCR fragment of <i>mpB</i> in pBS, Ap ^r	12
Oligonucleotides		
239-W	TGGAATTCATGCAATCTCCATCCTC	This study
239-Xe	ATGAATTCCTAGCTAGCTCTGTGTA	This study
HgIB-F1	ATTCGGCACAGGCAATGG	This study
HgIB-R2	TTGTTTCATCAGACCAGCG	This study
HgIC-F2	GGTATGGCATTGACGAGC	This study
HgIC-R2	CTCTAATAGGCTTGTCGG	This study
HgID-F1	GGCTTCCTACTCAACTGG	This study
HgID-R1	TCGTCAAACCTACCATCC	This study
HgIE-F1	TTAGCGGAGTTAGAAAAGC	This study
HgIE-R1	TCTTGTAACGTCCATCGC	This study
HgIE-F5	GCCCTCAATCAAGTTTGG	This study
HgIE-R7	GAGTATTAAAGGTCACCC	This study
Fdx-F3	CTTACACTATCACCAGCC	This study
Fdx-R4	CAGCAACTTGTTCTCTCG	This study

^a Ap^r, ampicillin resistance; Bm^r, bleomycin resistance; Km^r, kanamycin resistance; Sm^r, streptomycin resistance; Nm^r, neomycin resistance; Tet^r, tetracycline resistance.

602-bp EcoRI fragment from pRNAseP; (iv) for *hglB*, a 1,225-bp fragment amplified with primers HgIB-F1 and HgIB-R2; (v) for *hglC*, a 1,327-bp fragment amplified with primers HgIC-F2 and HgIC-R2; (vi) for *hglD*, a 761-bp fragment amplified with primers HgID-F1 and HgID-R1; (vii) for *hglE1* (*alr5351*), a 1,104-bp fragment amplified with primers HgIE-F1 and HgIE-R1; (viii) for *hglE2* (*all1646*), a 1,360-bp fragment amplified with primers HgIE-F5 and HgIE-R7. The *hglE* probes were shown to be specific to each of the two copies of the *hglE* gene (*all1646* or *alr5351*) by hybridization to Southern blots of *Anabaena* sp. strain PCC 7120 genomic DNA.

Alkaline phosphatase-labeled probes (all except *nifH*) were prepared using the AlkPhos Direct kit (Amersham Biosciences), and hybridizations were conducted according to the manufacturer's recommendations. Hybridization signals were detected by using ECF substrate (Amersham Biosciences), followed by scanning on a Storm instrument (Molecular Dynamics). Bands were quantitated using ImageQuant software (Molecular Dynamics). A ³²P-labeled, randomly primed *nifH* probe was prepared using a Rediprime kit (Amersham Biosciences) according to the manufacturer's instructions and hybridized as described previously (12). Rearrangement of the *fdxN* gene was assayed by amplification of DNA isolated from differentiated and undifferentiated filaments by using primers Fdx-F3 and Fdx-R4.

Expression and purification of His-tagged DevH recombinant protein and production of antibodies. The *devH* gene was PCR amplified from *Anabaena* sp. strain PCC 7120 genomic DNA using oligonucleotides 239-Xe and 239-W and cloned into the EcoRI site of vector pET30A (Novagen, Madison, Wis.) to produce plasmid pET23930a. This plasmid encodes recombinant DevH (rDevH) bearing a hexahistidine (His₆) tag.

An overnight culture of *E. coli* BL21(DE3)pLysS containing pET23930a was inoculated into 10 ml of 2YT medium supplemented with chloramphenicol (50 μ g/ml) and kanamycin (50 μ g/ml) and incubated at 37°C with shaking (200 rpm). When the optical density at 600 nm (OD₆₀₀) of the culture reached 0.6 to 1.0, isopropylthiogalactopyranoside was added to a final concentration of 1 mM. Cells were incubated further for 3 h and harvested by centrifugation at 5,000 $\times$ g for 5 min. The pellet was resuspended in 600 μ l of 10 mM Tris-HCl (pH 7.5), and the cells were lysed by cavitation with a Fisher Scientific Co. sonic dismembrator, model 300, for 4 min at 4°C. Soluble fractions were separated from cell

debris and inclusion bodies by centrifugation at 12,000 $\times$ g for 10 min. The protein concentration in the supernatant solution was determined using the Bio-Rad protein assay, with bovine serum albumin as the standard. Soluble fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

For purification of rDevH, frozen pellets of 100 ml of induced *E. coli* BL21 (DE3)pLysS cells carrying pET23930a were thawed at 4°C in binding buffer (5 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl; pH 7.9). Cells were disrupted by sonication for 4 min at 4°C with the sonic dismembrator, and cell debris was separated by centrifugation at 23,000 $\times$ g for 20 min. Further purification of rDevH from the soluble fraction was conducted with a His•Bind resin (His•Bind kit; Novagen), according to the manufacturer's instructions. Eluted fractions were analyzed by SDS-PAGE. The band corresponding to rDevH (35 kDa) was excised from an SDS–12% polyacrylamide gel and used for polyclonal antibody production in rabbits (Cocalico Biologicals, Inc., Reamstown, Pa.).

Western analysis. Cells collected at 0, 24, and 48 h after nitrogen deprivation were resuspended in 10 mM Tris-HCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF; pH 8.0) and stored at –80°C until further analysis. Frozen samples were thawed, and cells were disrupted by cavitation for 7 min at 4°C, followed by vortexing with glass beads for 5 min. Cell debris was eliminated by centrifugation at 4°C, 10,000 $\times$ g, for 20 min. Total protein (40 to 50 μ g) was fractionated by SDS–10% PAGE and electroblotted onto a nitrocellulose membrane (NitroBind; Osmonics) with transfer buffer (25 mM Tris-HCl, 192 mM glycine, 0.1% SDS, and 20% methanol) (21). Membranes were pretreated in TBST (20 mM Tris, 137 mM NaCl, 0.1% Tween; pH 8.0) containing 5% nonfat dry milk for 1 h at room temperature. Incubations with the DevH immune serum or a dinitrogenase reductase immune serum (provided by P. Ludden) were at a 1:5,000 dilution in TBST. A secondary hybridization with donkey anti-rabbit antibody linked to horseradish peroxidase (Amersham Biosciences) was at a dilution of 1:5,000. Detection was performed using the ECL Plus system (Amersham Biosciences) followed by scanning on a Storm instrument (Molecular Dynamics). Bands were quantitated using ImageQuant software (Molecular Dynamics). Triplicate analyses were conducted for wild-type and A57 cells.

Nitrogenase activity assays. For comparison of nitrogenase activity under oxic conditions, wild-type and A57 cultures were grown in BG-11 N⁺ to an OD₆₀₀ of

FIG. 1. Western analysis of DevH and dinitrogenase reductase in *Anabaena* sp. strain PCC 7120 (WT) and its *devH* mutant, A57. Protein samples (50 µg) from the wild-type strain and strain A57 were collected after 0, 24, or 48 h of nitrogen deprivation under oxic conditions, fractionated by SDS-10% PAGE, and stained with Coomassie brilliant blue R250 (A) or blotted onto a nitrocellulose membrane and detected by immunostaining with antiserum against DevH (B) or dinitrogenase reductase (C).

0.4 and transferred to BG-11 N⁻ as described previously (12). The cultures were constantly shaken and bubbled with air. After nitrogen stepdown, 3-ml samples were periodically transferred to 25-ml tubes and incubated for 30 min at 30°C in the presence of 10% (vol/vol) acetylene in air. Ethylene in the gas phase was measured using gas chromatography as described previously (12). Chlorophyll *a* was analyzed by standard methods (16). Nitrogenase activity was expressed as the nanomoles of ethylene produced per microgram of chlorophyll *a* per hour.

The nitrogenase activity of strain A57 under oxic or anoxic conditions was compared following the methodology of Ernst et al. (7) with slight modifications. Cultures were grown in BG-11 N⁺ to an OD₆₀₀ of 0.4 and then incubated for 2 days in BG-11 N⁻ in air to ensure sufficient glycogen accumulation for nitrogenase expression under anoxic conditions (20). Following this step, cultures were concentrated approximately sixfold. For determination of nitrogenase activity under oxic conditions, 3-ml aliquots were transferred to 25-ml tubes fitted with serum stoppers. To the remainder of the concentrated cell suspension, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was added to a final concentration of 20 µM. Samples (3 ml) of the DCMU-treated cell suspension were transferred to 25-ml tubes fitted with serum stoppers, and the gas phase was replaced by argon (anoxic conditions). After 0 and 22 h at 30°C, acetylene was added to a final concentration of 10% (vol/vol) to duplicate samples of the cell suspensions under oxic (air) or anoxic (argon) conditions. Ethylene chromatographic peaks were measured 4 h after acetylene addition and continued incubation at 30°C.

Electron microscopy. Cultures were prepared for electron microscopy according to the method of Black et al. (2).

Glycolipid analysis. Wild-type and A57 strains that had been deprived of fixed nitrogen for 0 or 48 h were extracted with chloroform-methanol (2:1, vol/vol). The organic phases were concentrated, and glycolipids were separated on a silica gel 60 plate, using as a mobile phase chloroform, methanol, acetic acid, and water in a ratio of 85:15:10:3.7 (by volume) (18). Plates were developed by charring with 25% sulfuric acid at 200°C for 5 min (24).

RESULTS AND DISCUSSION

DevH levels increase after nitrogen starvation. To facilitate studies of DevH, the protein was overexpressed in *E. coli* as

rDevH. A protein of the expected size was expressed (35 kDa) and used to raise polyclonal antibodies.

In cell extracts of *Anabaena* sp. strain PCC 7120 grown in air in the presence or absence of fixed nitrogen, rDevH antiserum detected a protein of 29 kDa (Fig. 1B). This molecular mass corresponds well with that predicted from the *devH* coding region. In extracts of *devH* mutant strain A57, a protein of about 33 kDa was detected (Fig. 1B). The 33-kDa protein is a DevH fusion protein resulting from the insertional inactivation used to generate the *devH* mutant (12), in which the last nine codons of the *devH* open reading frame are replaced by 43

FIG. 2. Southern analysis of the *nifD* rearrangement (A) and Northern analysis of *nifH* transcripts (B) in *Anabaena* sp. strain PCC 7120 (WT) and its *devH* mutant, A57. (A) Genomic DNA isolated from the wild-type strain and strain A57 that had been collected at 0 and 48 h after nitrogen stepdown under oxic conditions was digested with HindIII, fractionated, blotted, and hybridized with a *nifD* probe. Sizes in kilobases are indicated at the left. The *nifD* rearrangement occurs only in heterocysts (~10% of the cells in differentiated filaments; 48-h sample) and generates HindIII fragments of 1.8 and 2.1 kb. (B) Northern analysis of *nifH* transcripts during a time course of heterocyst development in wild-type and A57 strains. Samples of total RNA (5 µg) from the wild-type strain and strain A57 cells deprived of fixed nitrogen for 0, 6, 24, or 48 h under oxic conditions were fractionated, blotted, and hybridized with a *nifH* probe. Hybridization to an *mpB* probe was used as a loading control. The sizes (in kilobases) of the three stable *nifHDK* operon transcripts are indicated on the left.

FIG. 3. Ultrastructural analysis of heterocysts of *Anabaena* sp. strain PCC 7120 (WT) and its *devH* mutant, A57. Shown are transmission electron micrographs of ultrathin sections of heterocysts of the wild-type strain (A and B) and of A57 (C and D). Boxed regions in panels A and C are shown at higher magnification in panels B and D, respectively. The laminated layer of glycolipids (GL), present only in the heterocysts of the wild-type strain, is indicated in panel B. Bar, 0.5 μm (A and C) or 0.1 μm (B and D).

codons derived from vector sequences. In wild-type DevH, the helix-turn-helix motif is 24 amino acids from the carboxy terminus of the protein; in the mutant DevH protein from A57 there are 58 amino acids between the helix-turn-helix domain and the carboxy terminus. Although the A57 fusion protein is primarily wild type in sequence and maintains the helix-turn-helix domain, the protein is apparently nonfunctional, perhaps because the extension interferes with DNA binding.

In wild-type *Anabaena* sp. strain PCC 7120, a low level of *devH* transcripts is detected in undifferentiated filaments. By 24 h of nitrogen stepdown, there is a fivefold increase in the steady-state levels of *devH* transcripts (12). DevH protein is detectable in undifferentiated filaments (0 h) of wild-type *Anabaena* sp. strain PCC 7120 and induced by 24 h after nitrogen starvation, although the magnitude of induction ($\sim$ twofold) is less than that for the *devH* transcript (Fig. 1B). The DevH

fusion protein from mutant A57 also shows a pattern of induction (Fig. 1B), although the protein level in undifferentiated filaments (0 h) on average is higher than that observed for the wild-type protein and the induction by 24 h after nitrogen starvation is lower. Because the mutant and wild-type proteins differ in sequence, differences in stability of the transcripts, or in translation or stability of the proteins may affect the relative levels of the proteins in the wild-type and mutant strains. Alternatively, the regulation of expression of DevH may be altered in the mutant. Preliminary studies have demonstrated that rDevH binds specifically to the *devH* promoter (unpublished data), suggesting that *devH*, like *ntcA* (reviewed in reference 13), may have autoregulatory activity.

The *nifHDK* operon rearranges normally in A57, but *nifHDK* transcripts are undetectable. In response to nitrogen limitation, strain A57 forms morphologically distinguishable cells at

FIG. 4. TLC analysis of glycolipids from *Anabaena* sp. strain PCC 7120 (WT) and its *devH* mutant, A57, collected at 0 or 48 h after nitrogen stepdown. The arrows indicate the heterocyst-specific glycolipid fractions.

semiregular intervals that bind a heterocyst-specific stain but lack the polar bodies characteristic of mature heterocysts (12). In the absence of fixed nitrogen, strain A57 is unable to fix nitrogen in the presence of oxygen (12) and, thus, has a Het^+ Fox^- phenotype (7).

The inability of strain A57 to grow diazotrophically could result from impairment of some aspect of nitrogen fixation. Expression of nitrogen fixation (*nif*) genes in *Anabaena* sp. strain PCC 7120 is preceded by three programmed heterocyst-specific DNA rearrangements. One of these removes an 11-kb interruption in the *nifD* gene of the *nifHDK* operon that encodes nitrogenase and dinitrogenase reductase (11), and a second eliminates a 55-kb element from the heterocyst-specific ferredoxin gene, *fdxN* (10). *Anabaena* sp. strain PCC 7120 mutants that lack NtcA, the protein most closely related to DevH, are impaired in the *nifD* and *fdxN* gene rearrangements and lack *nif* gene expression (9, 23).

The *nifD* rearrangement occurs normally in strain A57 under oxic conditions (Fig. 2A). The *fdxN* rearrangement was also observed (data not shown); however, *nifHDK* transcripts were undetectable by Northern analysis (Fig. 2B). Consistent with this result, dinitrogenase reductase (NifH) was undetectable by immunostaining with antiserum against the enzyme in strain A57 after nitrogen starvation in the presence of oxygen (Fig. 1C).

The *devH* mutant is Fix^+ . The absence of *nifHDK* transcripts in strain A57 could result from a defect in the regulatory system that induces transcription of the operon. Alternatively, transcription or transcript stability could be impaired by oxygen that enters the cell as a consequence of heterocyst structural defects. Evaluation of nitrogenase activity by acetylene reduction assays performed in the presence or absence of oxygen can be used to distinguish between these possibilities.

Nitrogenase activities of the wild-type and A57 strains after

nitrogen starvation were measured under oxic (bubbling air) conditions. Acetylene reduction was detected in the wild-type strain by 37 h after nitrogen stepdown and peaked at ca. 46 h after stepdown at 16.4 nmol of ethylene produced per μg of chlorophyll *a* per h. No acetylene reduction by strain A57 was detected. In the presence of fixed nitrogen, when the systems for heterocyst development are suppressed, neither strain reduced acetylene.

The ability of strain A57 to reduce acetylene was tested under oxic and anoxic conditions. Nitrogenase activity was detected in A57, but only in concentrated cell suspensions that were maintained under anoxic conditions. To increase the sensitivity of the assay, the incubation time with acetylene was increased from 30 min to 4 h. Even longer incubation times have been used to detect nitrogenase activity in some Fox^- mutants (7). When assayed starting after 22 h of incubation under argon, the A57 cell suspension produced a total of 5.7 nmol of ethylene, while no ethylene was detected for a similar cell suspension maintained under oxic conditions. The ability of strain A57 to reduce acetylene under anoxic conditions demonstrates that the strain can induce a functional nitrogenase and thus has a Fix^+ phenotype.

The *devH* mutant is Hgl^- . The Fox^- Fix^+ phenotype of A57 suggests that the strain might have heterocyst defects that facilitate the entry of oxygen into the cell. To determine if such defects could be discerned, the ultrastructure of A57 hetero-

FIG. 5. Slot blot analysis of *hgl* gene expression in *Anabaena* sp. strain PCC 7120 (WT) and its *devH* mutant, A57. Samples of total RNA (10 μg) from cells deprived of fixed nitrogen for 0 or 24 h under oxic conditions were transferred to a Hybond N^+ membrane and hybridized with *hglC*, *hglE1* (*alr5351*), or *hglE2* (*all1646*) probes. Hybridization to an *rnpB* probe is presented as a loading control.

cysts was examined by electron microscopy. Figure 3 shows that the laminated layer of envelope glycolipids is absent in strain A57 heterocysts. These results were confirmed by thin layer chromatography (TLC) of lipids from differentiated filaments. The heterocyst glycolipids that were apparent in the wild type after heterocyst induction were absent from the strain A57 profile (Fig. 4).

Expression of glycolipid biosynthetic genes is altered in the *devH* mutant. The absence of heterocyst-specific glycolipids in strain A57 heterocysts suggests that the expression of glycolipid biosynthetic genes may be altered in the mutant. Slot blots of total RNA from wild-type and A57 strains grown in the presence or absence of fixed nitrogen were probed with the heterocyst glycolipid biosynthetic genes *hglB*, *hglC*, *hglD*, *hglE1*, and *hglE2*. Figure 5 shows that transcripts from *hglC* and *hglE1* were induced in strain A57 under oxic conditions, but at 20 to 30% of the wild-type level. Similar results were obtained for *hglB* and *hglD* (data not shown). *hglE2* was induced in the wild type but not detectably expressed in strain A57. If heterocyst envelope glycolipids are synthesized, it is at such a low level that they are undetectable by TLC and insufficient to form a glycolipid layer. Alternatively, there may be hitherto-undetected turnover of those glycolipids. Because DevH is similar to other transcriptional regulators, it could regulate expression of the network of genes required for heterocyst glycolipid formation by direct interaction with their promoters. The effect of DevH also could be indirect and exerted through other gene products. Identification of the genes with altered expression in *devH* mutants, as well as the identification of genomic sites to which DevH binds, should provide a clearer picture of the role of DevH in heterocyst development and function.

Differentiation of a functional heterocyst requires coordinated expression and assembly of components that comprise the polysaccharide and glycolipid layers of the heterocyst envelope. Biosynthesis of the polysaccharide layer is controlled by the HepK/DevR two-component regulatory system (27), and we have shown here that biosynthesis of the glycolipid layer is regulated by DevH. Elucidation of components in the pathways upstream of each of these distinct transcriptional regulators should allow identification of a common control element(s) that coordinates their activation during heterocyst development.

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