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A Short-Filament Mutant of *Anabaena* sp. Strain PCC 7120 That Fragments in Nitrogen-Deficient Medium

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Strain 129 is a fragmentation mutant of the filamentous cyanobacterium *Anabaena* sp. strain PCC 7120. Growing with fixed nitrogen, this mutant forms filaments that are much shorter than wild-type filaments. Following starvation for fixed nitrogen, strain 129 becomes nearly unicellular and forms few heterocysts, although electron microscopy suggests that proheterocysts form while fragmentation occurs. Starvation for sulfate, phosphate, iron, and calcium does not cause this fragmentation. The affected gene in strain 129, *fraC*, was cloned by complementation and characterized. It encodes a unique 179-amino-acid protein rich in phenylalanine. Insertional inactivation of the chromosomal copy of *fraC* results in a phenotype identical to that of strain 129, while complementation using a truncated version of FraC results in only partial complementation of the original mutant. Heterocysts could be induced to form in N-replete cultures of strain 129, as in wild-type cells, by supplying extra copies of the *hetR* gene on a plasmid. Thus, FraC is required for the integrity of cell junctions in general but is apparently not directly involved in normal differentiation and nitrogen fixation.

Anabaena sp. strain PCC 7120 is a filamentous nitrogen-fixing cyanobacterium. A complex developmental program is initiated in a minority of cells along the filament when fixed nitrogen becomes growth limiting. These differentiated cells, called heterocysts, form a simple pattern within 24 h following nitrogen limitation. This pattern is temporally consistent, in that heterocysts continue to form as growth occurs, by the conversion of specific vegetative cells in the longest intervals between heterocysts along the filament. In addition to the formation of heterocysts at these intercalary sites, heterocysts also form at the termini of filaments. Mature heterocysts do not divide. When heterocysts die, their connections to vegetative cells break and the filaments fragment. Additional heterocysts then develop on the ends of the filaments when the terminal cells differentiate.

Other factors contribute to filament length as well. Older, poorly growing cultures contain mostly short filaments, and long-term starvation for some nutrients such as sulfate also leads to extensive fragmentation (2). It is likely that at least some types of filament fragmentation are a genetically programmed response to adverse conditions. For instance, filament shortening following nutrient stress may aid in dispersal of cells to a more hospitable environment. Filament fragmentation during nitrogen fixation may serve to improve the efficiency of this process. When a culture is starved for fixed nitrogen, a sticky outer layer of polysaccharide forms on developing heterocysts, and filaments often hundreds of cells in length break into shorter lengths that in many *Anabaena* and *Nostoc* strains form dense surface mats of cells where O_2 and nutrients may be more effectively managed (10).

Mutants that either fail to fragment or fragment extensively would be useful. Either type could lead to genes involved in the fragmentation process itself, genes regulating the process, or genes involved in sensing environmental conditions. Several filament fragmentation mutants of cyanobacteria have been reported. Some of these mutants fragment in response to fixed nitrogen deprivation (9, 13). One is an apparent unicellular mutant of the filamentous strain *Anabaena* sp. strain PCC 7118 (15). Genes that complement these mutations have not yet been isolated. Earlier we described a set of mutants of *Anabaena* sp. strain PCC 7120 impaired in aerobic nitrogen fixation (5). One of these mutants, strain 129, showed a short filament phenotype under nitrogen-replete (N^+) and nitrogen-free (N^-) conditions. Here we describe this strain more extensively and characterize the gene containing the mutation.

MATERIALS AND METHODS

Materials. All restriction endonucleases and the large (Klenow) fragment of DNA polymerase I were purchased from New England Biolabs, Inc. (Beverly, Mass.) or Boehringer Mannheim Biochemicals (Indianapolis, Ind.). Deoxynucleoside triphosphates (dATP, dCTP, dTTP, and 7-deaza-dGTP) and dideoxynucleoside triphosphates (ddATP, ddCTP, ddGTP, and ddTTP) were purchased from Pharmacia (Piscataway, N.J.). α-^{35}S-dATP was purchased from Dupont, NEN Research Products (Boston, Mass.). Antibiotics were purchased from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals were reagent grade and available commercially.

Culture conditions. *Anabaena* sp. strain PCC 7120 was grown in modified Kratz and Myers medium C (K&M) or BG-11 medium (16, 18). In place of Na_2HPO_4, 1.125 mM Na_2HPO_4 and 1.125 mM K_2HPO_4 were added to K&M. Throughout this report, K&M will refer to medium without an added source of combined nitrogen (N^- conditions). The nitrogen source added for N^+ growth was either 2.5 mM $(NH_4)_2SO_4$ (K&M-NH_4) or 17.6 mM $NaNO_3$ (K&M-NO_3). Plates contained K&M or BG-11 medium with 1.3% agar (BBL purified) and 17.6 mM $NaNO_3$ if a nitrogen source was included. Cultures were grown photoautotrophically under cool white fluorescent lighting (30 to 40 $\mu E/m^2/s$) at 25 to 30°C in the presence of 2% CO_2 (large-scale cultures were bubbled with a 2% CO_2–air mixture). Mid-log-phase cells refer to cultures containing 2 to 6 μg of chlorophyll per ml, corresponding to 0.7×10^7 to 2.0×10^7 cells per ml. For selective growth of *Escherichia coli* DH5α, antibiotics were used at concentrations of 100 μg/ml (ampicillin) and 50 μg/ml (kanamycin).

Construction of pCCB110 and pCCB11aa. pCCB110 is a derivative of a shuttle vector based on pRL25c (8) which has the polylinker from pIC20R (17) ligated into the *Eco*RI site. The *Bam*HI site in pRL25c and the *Bgl*II site located between the promoter and the neomycin phosphotransferase open reading frame (ORF) were end filled and destroyed. The vector structure is shown in Fig. 1.

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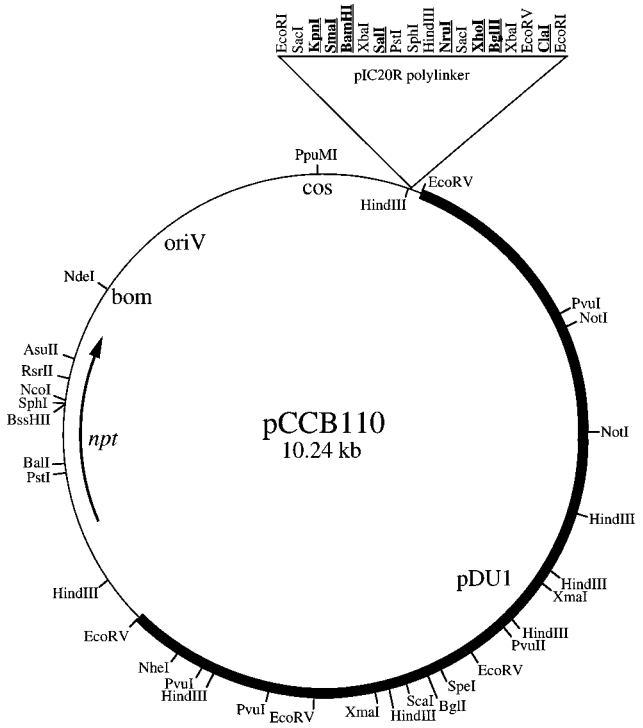

FIG. 1. Physical map of the complementation vector pCCB110. Unique restriction sites in the polylinker region are in boldface and underlined. Details of the construction are provided in the text.

pCCB110 has the *bom* site of pBR322 and can be transferred via conjugation into *Anabaena* sp. strain PCC 7120 as described previously (22).

The vector pCCB111aa is basically a modified version of the vector pCCB110 in which the *Anabaena* sp. strain PCC 7120 origin of replication (pDU1) has been removed by using the *Eco*RV sites surrounding the origin of replication and within the polylinker. In addition, a 63-bp promoter fragment from the *psbA* gene of the chloroplast of *Amaranthus hybridus* (7) was inserted into the *Bgl*II site of the parent plasmid of pCCB110 immediately upstream of the neomycin phosphotransferase gene to provide strong expression in single copy. The *Bgl*II site in the polylinker was removed by end filling.

Location of complementing gene. pCos129a, which complements mutant 129 (5), was subjected to partial *Sau*3AI digestion, and DNA fragments of 0.7 to 1, 1 to 2, and 2 to 3 kb were isolated from a 0.7% Tris-borate-EDTA (TBE) gel and electroeluted into dialysis tubing. Each size class was cloned into the *Bam*HI site of pCCB110. Hundreds of resulting colonies were obtained from each fraction and pooled. Mid-log-phase cultures of the pooled *E. coli* transformants were grown in SOC medium (19) containing 50 μg of kanamycin per ml and then subjected to alkaline lysis to isolate plasmid DNA. One hundred micrograms of each pooled fraction of plasmid DNA was used to transform a donor strain of MC1061 containing pRK24 and pRL528. Transformants were again pooled and grown in SOC medium containing 50 μg of kanamycin, 50 μg of ampicillin, and 10 μg of chloramphenicol per ml. The cells were washed and resuspended in one-fifth the volume of LB to 5×10^9 cell per ml. A liquid mid-log-phase culture of mutant 129 was centrifuged and resuspended in K&M at approximately 5×10^7 cells per ml. Then 400 μl of the plasmid-containing *E. coli* cells and 400 μl of the mutant 129 cells were mixed and spread onto filters on K&M-NO_3 plates with 5% LB and allowed to grow for 2 days under normal room lighting. The filters were then transferred to K&M plates containing 30 μg of neomycin per ml and incubated for 2 to 3 weeks until green colonies appeared above the yellow (dead cell) background. These colonies were picked and streaked onto the same medium and allowed to grow for an additional 2 weeks. Plasmid DNA was isolated from the complemented colonies and used to transform *E. coli* MC1061. Plasmids from these cells were retested for complementation essentially by repeating the procedure described above.

DNA sequencing. pCCB4 contains a 1.7-kb *Sau*3AI partial fragment, cloned into the *Bam*HI site of pCCB110, that complements mutant 129. pCCB6 was constructed by subcloning the 1.7-kb *Pst*I-*Kpn*I fragment of pCCB4 into pUC19 digested with *Pst*I and *Kpn*I. Sequencing deletions were constructed in accordance with the directions provided with the Erase-a-Base kit (Promega Biotec), using the enzyme exonuclease III. Deletions in both directions were constructed in pCCB6, using the *Sac*I and *Sma*I sites for one strand and the *Sph*1 and *Sal*I sites for the other. Sequences were determined by the dideoxynucleotide chain termination method on CsCl-purified double-stranded plasmid DNA, using Sequenase (United States Biochemical Corporation). Sequencing reactions were run on a TBE salt gradient gel by using 3 M sodium acetate as described previously (20). Both strands were completely sequenced. To complete the sequence of the ORF that continued past one end of pCCB6, a larger complementing fragment of 2.1 kb from the 2- to 3-kb pool cloned into pCCB110 was selected (pCCB22). pCCB22 was digested with *Xba*I, and a 600-bp fragment was subcloned into *Xba*I-digested pUC19 in both orientations to make pCCB24 and pCCB25. These plasmids were sequenced as described above. The physical map of pCCB22 is shown in Fig. 3, and the sequence of the 2.1-kb insert is shown in Fig. 4.

Determination of the minimal complementing fragment. Selected deletion plasmids made from pCCB6 were digested with *Pvu*II, and the inserts were subcloned into the *Sma*I site of pCCB110. Each plasmid was then tested for complementation of the 129 mutant, using the methods described above.

Determination of genetic lesion. PCR primers were made to a portion of *fraC* thought to contain the genetic lesion. They were 5′ TATTC TTGCT TTCGC CTACC 3′ (positions 1192 to 1173 in Fig. 4) and 5′ ATGCG ATCGC CGTCA ACAGC 3′ (positions 780 to 799). Other primer pairs were made to the rest of the minimal complementing fragment; these were 5′ CCCAA CGCTG CTTGT GACTT ACC 3′ (positions 253 to 275) plus 5′ GCTGT TGACG GCGAT CGC 3′ (positions 782 to 799) and 5′ CCATC GATGT TGAGT GTCTA TGTTT GAAG 3′ (positions 626 to 647) plus 5′ CCATC GATCC CTATA TCTTC ACTTA AGGTG 3′ (positions 1000 to 1022). The latter two primers also have a short 8-base 5′ extension containing a *Cla*I site. PCR amplification of *Anabaena* sp. strain PCC 7120 and mutant 129 chromosomal DNA by using these primers resulted in the expected sizes of 380, 400, and 360 bp, respectively. PCR using these synthetic primers was carried out by using standard protocols found in the GeneAmp kit (Perkin-Elmer Cetus). After amplification, the PCR products were run on a 0.7% TBE gel, cut out, and electroeluted into dialysis bags. The products were then precipitated, and approximately 0.2 μg of the double-stranded product was amplified with one oligonucleotide and sequenced with the other oligonucleotide directly after phenol-chloroform extraction and isopropanol precipitation. Sequence was determined for both strands of the mutant and wild-type PCR products.

RNA isolation. Large-scale cultures of *Anabaena* sp. strain PCC 7120 were synchronously induced to form heterocysts by transfer of vegetative cells from K&M-NH_4 to K&M. One-liter cultures harvested at 6-h intervals were used to prepare total RNA as described previously (11), with the exceptions that aurintricarboxylic acid was substituted for vanadyl ribonucleoside complexes, and a 4 M LiCl precipitation of RNA to remove DNA and polysaccharides was added (6).

Effect of *hetR* on strain 129. A plasmid containing the *hetR* gene that can replicate in *Anabaena* sp. strain PCC 7120, pWB216S2.4, was introduced into mutant 129, using the plate mating method described above. Exconjugants were selected with 50 μg of neomycin per ml on K&M-NO_3. Resulting neomycin-resistant colonies were streaked onto fresh K&M-NO_3 with 50 μg of neomycin per ml and grown for 1 week. A portion of each streak was collected, washed twice, replated on K&M, and incubated for 3 days. Microscope slides of these streaks were then prepared and phenotypically scored.

Insertional mutagenesis. Insertional mutagenesis was accomplished with the suicide vector pCCB111aa containing a fragment mostly internal to the ORF of the *fraC* gene. This single insertional recombination method has been described previously (1). The 360-bp PCR product described above was cloned following restriction with *Cla*I into the *Acc*I site of pUC19 to form pCCB28, which was then fused to pCCB111aa at each plasmid's unique *Bam*HI site. After conjugation, single recombinants that interrupted gene function were selected by using 100 μg of neomycin per ml. Southern analysis to confirm complete chromosomal replacement used the PCR product as a probe.

Electron microscopy. Sample preparation and electron microscopy were performed according to established procedures (see reference 12 for general techniques). Samples were prepared by pelleting the cyanobacterial filaments in a clinical centrifuge and resuspending them in half-strength Karnovsky fixative (2% formaldehyde and 2.5% glutaraldehyde in 3 mM $CaCl_2$–50 mM cacodylate buffer [pH 7.4]) (14). Fixation was for 1 h at room temperature and then for 2 to 16 h at 4°C. The cells were then washed with 50 mM cacodylate and postfixed in 2% $KMnO_4$ or 1% OsO_4 buffered with 50 mM cacodylate (pH 7.4) overnight. The samples were washed and then dehydrated through a graded ethanol series (two changes of 15 min each in 70, 85, and 95%; three changes of 20 min each in 100%) and two 30-min incubations in propylene oxide. The samples were infiltrated with a 1:1 mixture of propylene oxide and Epon 812 for 4 h followed by a 1:3 mixture of propylene oxide and Epon overnight. The samples were incubated in pure Epon under vacuum for 4 h at room temperature and then embedded in fresh degassed Epon in BEEM capsules. The samples were left to harden at 55°C overnight. Thin sections were collected on Parlodion-carbon-coated grids and stained with 3% aqueous uranyl acetate for 30 min followed by Reynolds' lead citrate for 30 s. These were examined in a Siemens 101 electron microscope operated at 80 kV.

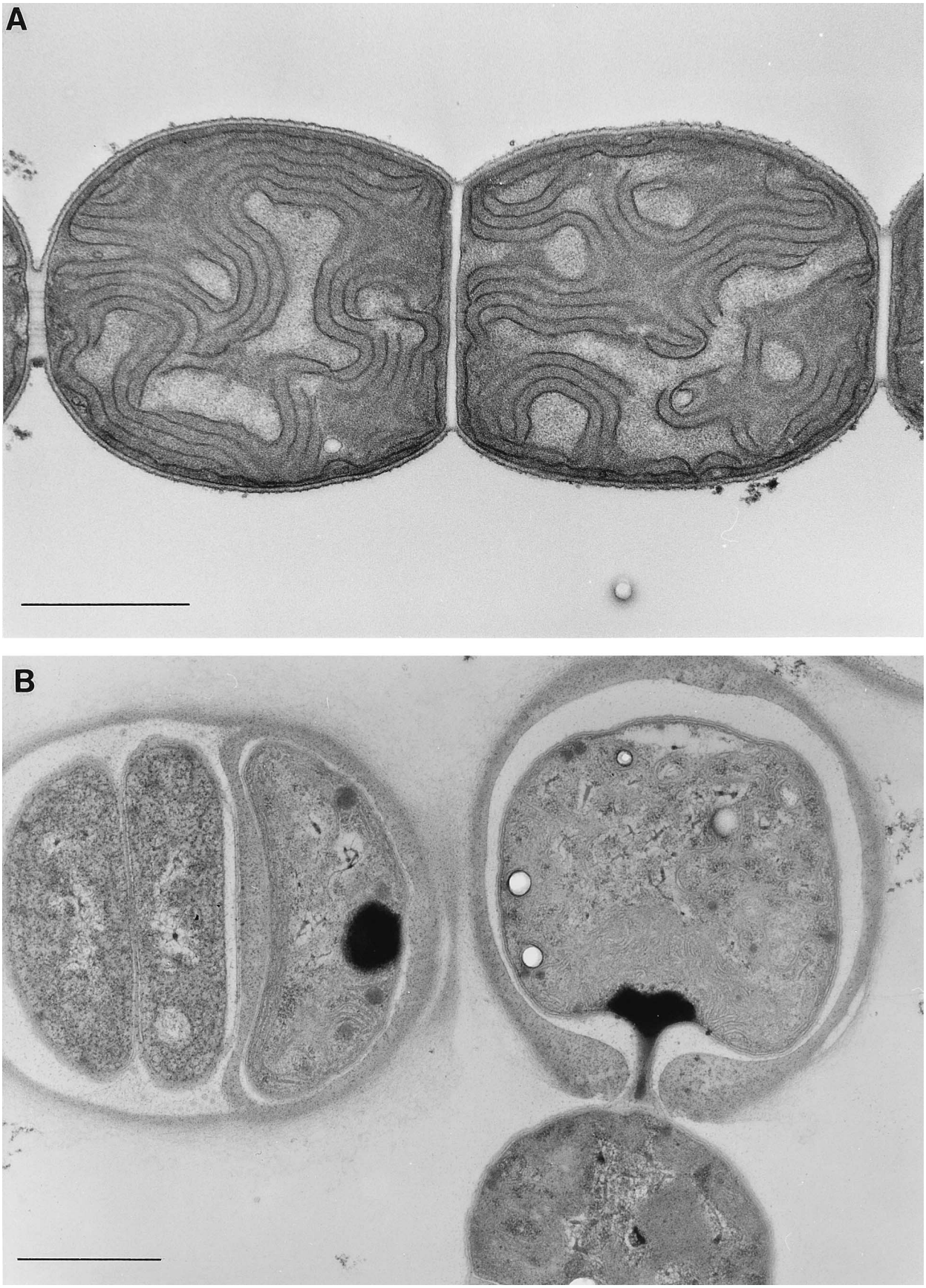
A
B

FIG. 2. (A) Strain 129 grown under N^+ conditions. All of the morphological features are those seen for wild-type cells grown under the same conditions. Postfixation was with $KMnO_4$. The bar represents 1 μm. (B) Strain 129 following transfer to N^- medium for 2 days. Postfixation was with OsO_4. The right side of the micrograph shows a rare heterocyst attached to a single vegetative cell. The left side shows a short filament of abnormal cells surrounded by a diffuse polysaccharide envelope. The rightmost cell in the filament is a heterocyst that appears to have been separated from adjacent cells on the right side at some prior time, as seen by the remains of a darkly stained polar body. This cell also has a second, more closely apposed polysaccharide layer, indicating that it is the product of a second differentiation. The two vegetative cells on the left of the filament are abnormally flattened and probably arose following division of a regressed proheterocyst. The bar represents 1 μm.

RESULTS

Strain 129 was originally isolated as a mutant of *Anabaena* sp. strain PCC 7120 unable to grow aerobically on N_2 as a nitrogen source (5). Under N^+ conditions, the mutant grows as short filaments that rarely exceed 15 cells. After transfer to N^- conditions, the filaments rapidly fragment to mostly single cells, although some pairs and higher multiples are present. The fragmentation occurs within 24 h and is accompanied by the formation of proheterocysts, but few mature heterocysts are observed. Under N^+ conditions, starvation for sulfate, phosphate, iron, or calcium does not result in such extensive fragmentation within this time frame. The average cell diameter of strain 129 ranges from 2 to 4 μm under either N^+ or N^- growth conditions, somewhat smaller than the 3 to 6 μm diameter of wild-type cells (1a).

Morphology. Electron micrographs of strain 129 growing under N^+ conditions appear very similar to those of the wild type aside from difference in cell size (Fig. 2A). Micrographs of strain 129 following 2 days of induction in N^- medium provide evidence of rapid fragmentation and partial differentiation (Fig. 2B). Many cells display a surrounding polysaccharide layer that is typical of proheterocysts and heterocysts. Often two or three cells are found within a single or double sheath, probably polysaccharide, indicating multiple rounds of partial differentiation, fragmentation, reversion, division, and redifferentiation. Many of the cells also display changes in their photosynthetic membranes from the normal vegetative cell parallel configuration to the differently organized, curved structures characteristic of heterocysts.

The *fraC* gene. One of the cosmids, pCos129A, previously identified as complementing strain 129 for growth on N^- medium (5), was mapped with several restriction enzymes, with the results shown in Fig. 3. To identify the specific region necessary for complementation, the cosmid was subcloned into the shuttle vector pCCB110, using size-fractionated DNA from a *Sau*3AI partial digest (4). The subclones were pooled and transferred via conjugation into strain 129, and complementing plasmids were selected. Two of the complementing plasmids with the smallest inserts were chosen for further study. pCCB4 and pCCB22 contain inserts of 1.7 and 2.1 kb, respectively, and were shown to complement strain 129 at a high frequency when reintroduced by conjugation. Complemented strain 129 is indistinguishable from the wild type by light microscopy.

The DNA sequence of the insert of plasmid pCCB4, as well as a 600-bp partially overlapping fragment of pCCB22, was determined. The sequence of this region and the ORFs are presented in Fig. 4. Selected sequencing deletions were chosen, subcloned into pCCB110, and tested for complementation

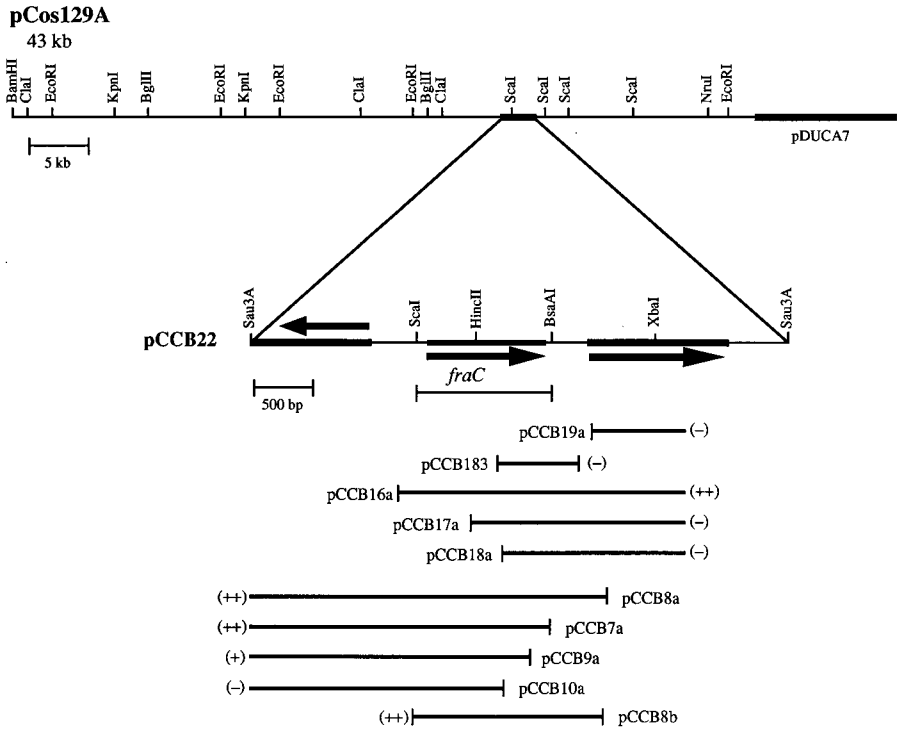

FIG. 3. Restriction map of the *fraC* region and positions of subclones tested for complementation. The upper part shows the map of cosmid pCos129A and the position and map of a 2.1-kb partial *Sau*3AI subclone, pCCB22, that complements strain 129 for growth on N_2. Thickened lines in pCCB22 denote ORFs. The lower part shows the positions of subclones used to determine the minimum region required for complementation, shown as a bar below *fraC*. The symbols (++), (+), and (−) refer to full, partial, and no complementation, respectively.

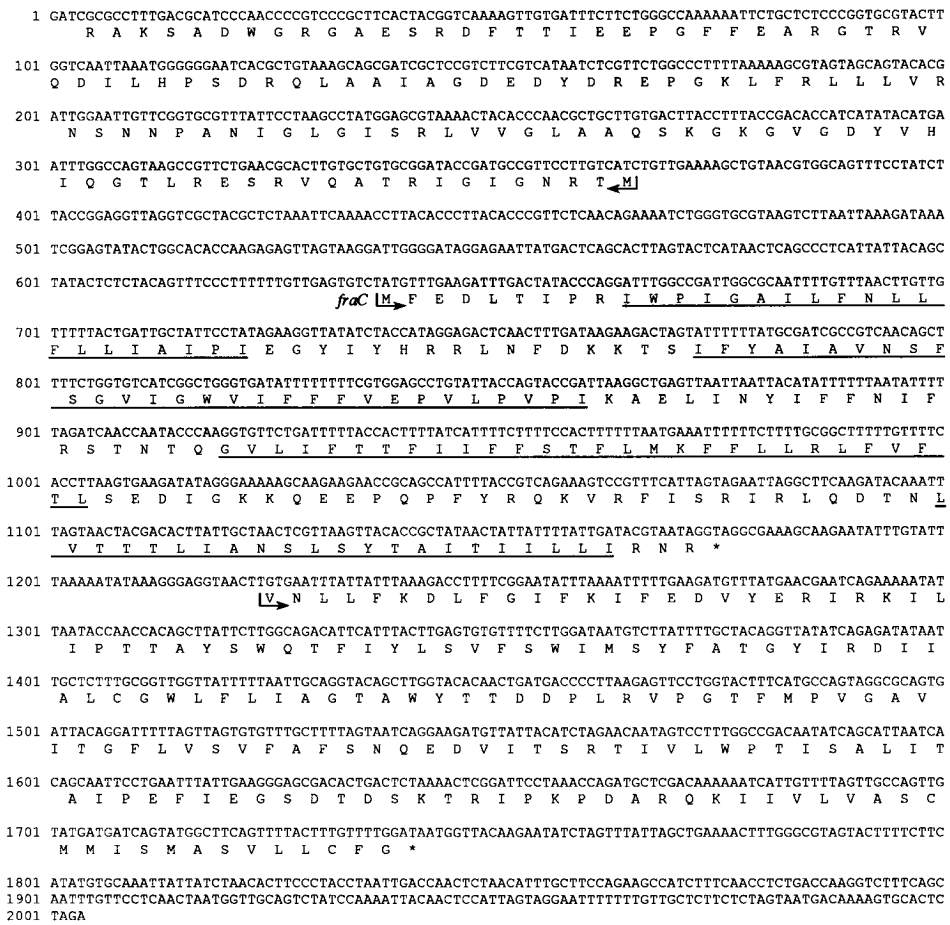

FIG. 4. DNA sequence and translation of ORFs in the sequenced region containing *fraC*. *fraC* is located in the center of the figure and spans bases 638 to 1177. Note that the incomplete ORF upstream of *fraC* is transcribed from the opposite strand from *fraC*. Underlined regions denote potential membrane spans.

of strain 129. These results are presented in Fig. 3. A 600-bp fragment containing a single ORF of 179 amino acids, named *fraC*, was sufficient for complementation. One deletion subclone that lacks the last 30 codons of *fraC*, pCCB9a, was able to partially complement strain 129. Partially complemented colonies were olive green in N^- media and grew more slowly than the wild type. Heterocysts were present in this strain, and the average filament length was only 6 to 15 cells. These complementation results indicate that the mutation in the *fraC* gene in strain 129 is responsible for the fragmentation phenotype and that the mutation is not polar on the downstream ORF (see below).

The sequence of the *fraC* gene gives few clues to its function. No significant matches could be found following searches of GenBank with the protein or DNA sequence of *fraC* or either flanking ORF. Hydrophobicity plots suggest the presence of several membrane-spanning regions spaced throughout the protein, indicated by underlining on the sequence shown in Fig. 4. There is an unusually high phenylalanine content in FraC, approximately 13%, and in the downstream ORF, about 8%. These ORFs have poor ribosome-binding sites: GAG for *fraC* at −9 to −6 and the stronger GGAGG for the downstream ORF, but the latter is at −11 to −7, a bit distant from the start of translation for general prokaryotic ribosome-binding sites. The upstream ORF (note that it is transcribed from the opposite strand) has no ribosome-binding site at all.

To verify that mutation in the *fraC* gene is responsible for the phenotype of strain 129, insertional mutations were generated via single recombination of a homologous PCR-generated fragment of the gene, cloned in the mobilizable but nonreplicating vector pCCB111aa. This 360-bp PCR fragment extends from 12 bp upstream of the ATG to 152 bp upstream of the stop codon. Single recombination of this fragment generates two defective *fraC* copies flanking the inserted vector sequences, one with an intact ORF but lacking any promoter sequences and the other lacking the terminal 51 codons. Four independent colonies were isolated and tested on N^- media. Growth characteristics and light microscopy showed each to have a phenotype identical to that of strain 129.

Transcription of *fraC*. Northern (RNA) gel analysis to determine the level and timing of expression of *fraC* was attempted. RNA was isolated from wild-type cells at 6-h intervals following transfer to N^- medium. Northern gels with up to 60 μg of total RNA loaded per lane were hybridized with an internal fragment of *fraC*. Exposures of up to 1 month were insufficient to detect any message on these gels (data not shown). Controls with other gene probes showed that the blots had adequate RNA to detect other transcripts.

Interaction with HetR. Because mature heterocysts are so rarely seen in strain 129, we determined whether overexpression of *hetR* could induce the inappropriate formation of heterocysts in N^+ cultures of the mutant as it does in wild-type

cultures (4). A *hetR*-containing plasmid was transferred to strain 129 via conjugation. Heterocysts indistinguishable from those induced in the wild type were seen. Extra copies of the *hetR* gene did not alter the phenotype of strain 129 under N^- conditions.

Strain 129 contains a frameshift in *fraC*. It was of interest to determine the nature of the original mutation found in strain 129. To this end, PCR was used to generate overlapping fragments of DNA from the chromosomal copies of *fraC* from the wild type and the mutant. Sequencing of the entire mutant and wild-type genes from these fragments revealed two base changes. One is a silent change of the third base of a threonine codon (T to G) at position 1108 (Fig. 4). The other is a frameshift at position 770 caused by the deletion of a single base. Since this deletion is found early in the gene, we expect that it creates a null mutation. As mentioned above, mutations created by insertion of vector sequences at amino acid 128 result in strains with the same phenotype as the original mutant strain.

DISCUSSION

The phenotype of strain 129, especially as revealed by electron microscopy, is noteworthy. The presence of multiple cell envelopes surrounding very short two- or three-cell filaments is indicative of incomplete differentiation and proheterocyst reversion. As Wilcox et al. showed (21), proheterocysts that become detached revert to vegetative cells even late in the developmental process. The simplest explanation for the electron microscope images is that upon transfer to N^- medium, heterocysts begin to differentiate on the existing short filaments, become detached before completing the process, and revert to vegetative cells. These cells then divide, forming a small-celled short filament enclosed within the proheterocyst envelope. One of the terminal cells of this filament then attempts to differentiate, and the cycle repeats until the available nitrogen stores are depleted. It seems likely that the weakness of the vegetative cell junctions becomes even more pronounced in a heterocyst junction which is normally more prone to breakage.

The lack of similarity to existing sequences, the absence of recognized structural or functional motifs, and our inability to detect transcripts make assigning a function to *fraC* difficult. It is unlikely that FraC is directly involved in heterocyst formation or function, since strain 129 forms some mature heterocysts under N^- conditions and can be induced to form them under N^+ conditions when supplied with extra copies of *hetR*. In addition, strain 129 has previously been shown to fix nitrogen anaerobically (5). The potential hydrophobic domains argue for a role in a membrane, and the fragmentation phenotype itself suggests an outer membrane location, perhaps in the septum region where the breakage occurs.

The phenotypes of other fragmentation mutants share some characteristics with that of strain 129. A wild-type strain in the Pasteur collection, *Nostoc* sp. strain PCC 7121, grows in complete medium as short filaments of three or four cells. It cannot differentiate heterocysts or fix nitrogen. We have isolated another mutant of *Anabaena* sp. strain PCC 7120 following chemical mutagenesis, called strain 415, that grows normally on N^+ medium but, following transfer to N^- medium, fragments extensively and forms many proheterocysts but few heterocysts (5). It cannot fix nitrogen aerobically and is not complemented by the *fraC* gene. The gene that complements the mutation in strain 415 encodes a very large protein with many potential membrane spans (3). A third mutant of *Anabaena* sp. strain PCC 7120 with a similar fragmentation phenotype was constructed by insertional inactivation of a gene termed *fraH*, an ORF originally detected in a subtracted library of cDNA clones corresponding to RNA present 30 h after transfer to N^- medium (1). FraH is a 289-amino-acid polypeptide with no significant matches in the protein databases but notable for a very high proline content (18%). The *fraH* mutation is less severe than the *fraC* mutation, in the sense that *fraH* filaments are slightly longer than *fraC* filaments in N^- medium. Under these conditions, there are numerous mature heterocysts floating freely in the *fraH* mutant culture and the remaining cells do not die but grow very slowly. Unlike *fraC*, the *fraH* message is easily seen on Northern gels of RNA prepared 12 h or later after transfer to N^- medium. None of these genes appear to be linked in the chromosome. It remains for the future to determine how the abundant, proline-rich FraH, the rare, phenylalanine-rich FraC, and the large protein defective in strain 415 interact in the junctions of vegetative cells and heterocysts.

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