

Transfer of a Genetic Marker from a Megaplasmid of *Anabaena* sp. Strain PCC 7120 to a Megaplasmid of a Different *Anabaena* Strain

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The 410-kb α megaplasmid of the heterocyst-forming cyanobacterium *Anabaena* sp. strain PCC 7120 was found to bear the *nucA* gene that encodes a sugar-nonspecific nuclease. That gene was mutated by insertion of a cassette that confers resistance to neomycin. The resulting strain, AMP2, was mated with a streptomycin-resistant derivative of *Anabaena* sp. strain PCC 7118, a strain that does not form heterocysts. Cells resistant to both neomycin and streptomycin that were derived from such matings were found to bear the neomycin resistance cassette of the donor strain in a larger megaplasmid characteristic of the recipient strain and did not form heterocysts. This is the first example of transfer of a genetic marker directly between strains of cyanobacteria in which incontrovertible physical evidence of transfer has been obtained. DNA sequences homologous to the *nucA* gene were present in 13 heterocyst-forming cyanobacteria that were tested but in none of six diverse unicellular strains that were examined.

The cyanobacteria are phototrophic eubacteria that can carry out oxygenic (plant-type) photosynthesis. Many filamentous cyanobacteria are able to fix dinitrogen in specialized cells called heterocysts that develop at semiregular intervals along the filaments. We have recently shown that *Anabaena* sp. strain PCC 7120, a heterocyst-forming cyanobacterium, produces an exocyttoplasmic sugar-nonspecific nuclease that is able to degrade linear or covalently closed circular double-stranded DNA as well as single-stranded DNA or RNA (16). The gene, *nucA*, encoding this enzyme has been cloned and sequenced (16). In this report, we show that *nucA* is located on the α megaplasmid, whose presence in strain PCC 7120 had been reported previously (4). A cassette conferring resistance to neomycin (NM), when introduced into the *nucA* gene of strain PCC 7120, could thereupon be transferred to a larger megaplasmid of a streptomycin (SM)-resistant derivative of *Anabaena* sp. strain PCC 7118. We have also found that the *nucA* gene is present in numerous heterocyst-forming cyanobacteria.

MATERIALS AND METHODS

Organisms and culture conditions. Cyanobacterial strains were grown photoautotrophically at 30°C in BG11 medium (18) with shaking. Where specified, BG11 medium was solidified by the addition of 1% separately autoclaved Bacto Agar (Difco). For testing growth on dinitrogen, BG11 lacking NaNO₃ was used. For growth of derivative AMP2 of *Anabaena* sp. strain PCC 7120, liquid BG11 medium was supplemented with 5 μ g of NM per ml and solidified BG11 medium was supplemented with 25 μ g of NM per ml. Derivative NW18 of *Anabaena* sp. strain PCC 7118 was grown in BG11 medium

supplemented with 20 μ g of SM per ml. Nm^r Sm^r derivatives of strain NW18 were grown in BG11 medium supplemented with a combination of 20 μ g of SM per ml and NM either at 5 μ g/ml in liquid medium or 25 μ g/ml in solid medium. Chlorophyll *a* was determined in methanolic extracts (13). *Escherichia coli* DH5 α , used for all plasmid constructions, was grown in Luria broth as described previously (14).

Preparation and manipulation of DNA. Total DNA from cyanobacteria was isolated as described by Cai and Wolk (6). DNA of high molecular weight from *Anabaena* sp. strains PCC 7120 and PCC 7118 and their derivatives was prepared and cut with restriction endonucleases, and the resulting plasmids were subjected to pulsed homogeneous orthogonal field gel electrophoresis (PHOGE) (2) as described by Kuritz et al. (11). Bands with a size of less than 50 kb were measured by using *Hind*III digests of coliphage lambda DNA as standards in pulsed-field gels (11).

Plasmid isolation from *E. coli*, transformation of *E. coli*, restriction, and ligation with T4 DNA ligase were performed by standard procedures (1, 14). DNA fragments were purified from agarose gels with the GeneClean kit (Bio 101, Inc.). The *nucA*-containing hybridization probes were either plasmid pRLA1, which carries 12 kb of strain PCC 7120 genomic DNA including the *nucA* gene and flanking sequences, or a 473-bp *Hind*III-*Bst*NI fragment internal to *nucA*, isolated from pCSAM29 (16). The neomycin phosphotransferase (*npt*) probe used was cassette C.K3 (7) excised with *Bam*HI. DNA probes were ³²P labeled with [α -³²P]dCTP by using a nick translation kit (Boehringer Mannheim) or with [α -³²P]dATP by using a randomly primed DNA labeling system kit (Bethesda Research Laboratories) or were digoxigenin labeled with a Genius II kit and the signal was enhanced by Lumi-Phos 530 (Boehringer Mannheim). Southern blotting of total DNA from cyanobacteria to Hybond-N+ membranes (Amersham) was performed under alkaline conditions (14). Hybridization was carried out under low-stringency conditions at 55°C as described previously (1) or under high-stringency conditions at

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65°C as described in the recommendations of the manufacturer of Hybond-N+ membranes by using 5 mM KH₂PO₄ buffer (pH 7.2) containing 1 mM EDTA and 0.2% sodium dodecyl sulfate (SDS) to wash the filters. PHOGE-resolved fragments of DNA were blotted to GeneScreen membranes (DuPont), and hybridization was carried out as described previously (4, 11). Plasmid RP-4 was digested with *EcoRV* before labeling with ³²P. Hybridization of ³²P-labeled RP-4 with *HindIII* or *EcoRV* digests of genomic DNA from strains AMP2 and PCC 7120 was carried out under high-stringency conditions.

Matings between cyanobacteria. Cells of strain NW18 and of a donor strain carrying the *npt* gene integrated in its genome were harvested and washed with BG11 medium by low-speed centrifugation, mixed, and spread on Nuclepore REC-85 filters set atop plates of solid BG11 medium. The donor strain used in most experiments was AMP2 (16), but strain EF116(pRL52) (9) was also tried. An amount of cells containing about 4 µg of chlorophyll of the donor strain and 8 µg of chlorophyll of strain NW18 was inoculated per plate (1 µg of chlorophyll corresponds to ca. 3.3×10^6 cells of these cyanobacteria). After 48 h of incubation under growth conditions, the filters were transferred to plates of BG11 medium supplemented with 25 µg of NM per ml and 20 µg of SM per ml and further incubated for 2 to 4 weeks.

Nuclease assays. Nuclease assays in DNA-containing SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gels were performed essentially as described previously (19) but with modifications (16). Samples were subjected to electrophoresis in gels containing 15 µg of calf thymus DNA (type IV; Sigma) per ml. After electrophoresis, proteins were renatured by gentle agitation of the gels in nuclease buffer, and gels were incubated overnight in the same buffer to allow enzymatic degradation of embedded nucleic acids. The gels were then stained with ethidium bromide and photographed under UV illumination.

For preparation of cell extracts of cyanobacteria, cells (ca. 200 µg of chlorophyll) were suspended in 0.25 ml of 10 mM Tris-HCl buffer (pH 7.5) containing 1 mM phenylmethylsulfonyl fluoride and then subjected to freezing in liquid air in a prechilled mortar and grinding. The homogenate was centrifuged at $10,000 \times g$ for 10 min. The resulting supernatant solution constituted the cell extract. Protein concentration in cell extracts was estimated as described previously (5). Protein concentration in cell suspensions was estimated by the method of Markwell et al. (15).

RESULTS AND DISCUSSION

Location of *nucA* in the α megaplasmid. Strain AMP2 is a derivative of *Anabaena* sp. strain PCC 7120 in which the *nucA* gene had been inactivated by the insertion of C.K3, a cassette bearing the *npt* gene from Tn5 (16). DNA of high molecular weight was isolated from PCC 7120 and AMP2 and subjected to digestion with *PstI* or *SalI*, and the restriction fragments thus generated were resolved as described previously (2). The pattern of bands (4, 11) obtained in each of the two digestions was identical for PCC 7120 and AMP2, except that the band corresponding to the α megaplasmid (Fig. 1, arrow a), a plasmid of 410 kb present in *Anabaena* sp. strain PCC 7120 that bears a single site for *SalI* (3, 4), was replaced by bands of ca. 390 (Fig. 1, arrow c) and 19 (not seen in Fig. 1) kb in the *SalI* digest of AMP2 because of the presence of a *SalI* site near the end of the *npt*-containing cassette inserted in *nucA* in AMP2. In addition, the α megaplasmid from AMP2 is linearized by *PstI* (Fig. 1, arrow b) because of the introduction of a *PstI* restriction site on the inserted cassette. These results

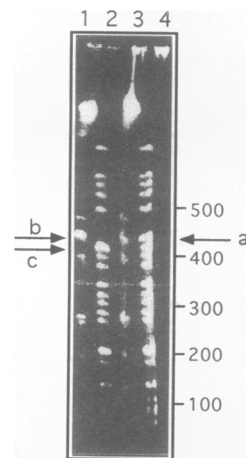


FIG. 1. Alteration of *Anabaena* sp. strain PCC 7120 α megaplasmid by the insertion of cassette C.K3 into the *nucA* gene encoding a sugar-nonspecific nuclease. DNA of high molecular weight from strains AMP2 (lanes 1 and 2) or PCC 7120 (lanes 3 and 4) was subjected to digestion with *PstI* (lanes 1 and 3) or *SalI* (lanes 2 and 4) and resolved by the PHOGE system. Arrow a points to plasmid α (410 kb) in *SalI*-digested strain PCC 7120 DNA, whereas arrow c points to a digestion product of this plasmid in *SalI*-digested DNA of strain AMP2. Arrow b points to plasmid α in *PstI*-digested DNA of strain AMP2. The positions of several size markers (in kilobases), as deduced from a 48.5-kb lambda ladder, are shown to the right of the figure. The effect of DNA concentration on the distance of migration was discussed previously (11).

suggested that the *nucA* gene was located in the α megaplasmid. To test this possibility further, labeled plasmid pRLA1, which carries the *nucA* gene and flanking DNA, was used to probe high-molecular-weight DNA of strain PCC 7120 that had been digested with *BlnI*, *PstI*, or *SalI*, resolved by PHOGE, and blotted. A strong hybridization signal resulted in the band that represents the linearized α megaplasmid (Fig. 2). Megaplasmid α, poorly seen on the PHOGE gels of either *PstI* or *BlnI* digests of DNA from wild-type PCC 7120 (there are no sites for *PstI* or *BlnI* in the α megaplasmid), is visualized in Southern hybridizations because some megaplasmid linearized by nicking enters the gel.

Plasmids ranging in size from ca. 1.5 kb to 1 Mb have been described in the cyanobacteria (10, 17, 26). All cyanobacterial plasmids heretofore studied remain cryptic; i.e., beyond replication functions (20, 24–26, 30), there are no known functions encoded by them except that a normally silent copy of *psbG* has been found to be plasmid localized in *Synechocystis* sp. strain PCC 6803 (22). Our data show that *nucA* represents the first expressed marker for an indigenous cyanobacterial plasmid (11).

Transfer of *nucA*::C.K3 between cyanobacterial strains. Many large, bacterial plasmids are self-transmissible by conjugation (27). Conjugal transfer of plasmid DNA from *E. coli* to *Anabaena* sp. proceeds efficiently (29), but genetic transfer between cyanobacterial cells (see, e.g., reference 23) has not been unequivocally demonstrated previously. The *nucA* gene, and therefore the *nucA*::C.K3 construction present in strain AMP2, represents a marker for the α megaplasmid of *Anabaena* sp. strain PCC 7120. Conjugation between cyanobacteria was sought by using this marker.

We sought to transfer *nucA*::C.K3 from strain AMP2 to NW18, an Sm^r derivative of *Anabaena* sp. strain PCC 7118. Like its parent strain PCC 7118, strain NW18 is unable to

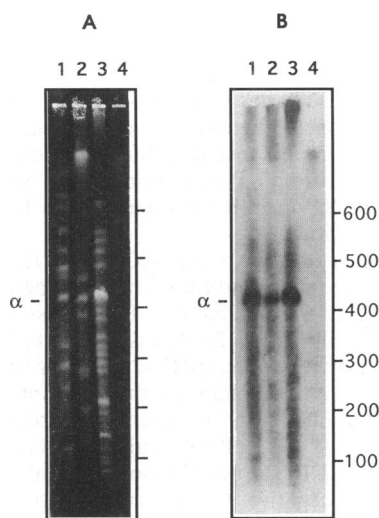


FIG. 2. Localization of the *nucA* gene in the α megaplasmid. (A) High-molecular-weight DNA from *Anabaena* sp. strain PCC 7120 was digested with *BlnI* (lane 1), *PstI* (lane 2), or *SalI* (lane 3), and the fragments were resolved by PHOGE. Lane 4 contains lambda DNA oligomers. (B) Hybridization of the PHOGE-resolved fragments with ^{32}P -labeled *nucA*-containing plasmid pRLA1 is shown. The position of the linearized α megaplasmid is indicated. The positions of several size markers (in kilobases), as deduced from the 48.5-kb lambda ladder, are shown to the right of the figure.

develop heterocysts or to fix dinitrogen under aerobic conditions, whereas AMP2, like its parent PCC 7120, makes heterocysts and can grow on dinitrogen under aerobic conditions. *Anabaena* sp. strain PCC 7120 and its derivative AMP2 have the 410-kb megaplasmid mentioned above, whereas derivative NW18 of *Anabaena* sp. strain PCC 7118 bears a 510-kb plasmid, detected by pulsed-field gel electrophoresis of unrestricted DNA (data not shown). Cells of strains AMP2 and NW18 were mixed and spread on filters set atop plates of solid cyanobacterial medium (see Materials and Methods for details). From a total amount of ca. 6.6×10^8 cells of strain NW18 and ca. 3.3×10^8 cells of strain AMP2, in several experiments, 117 $\text{Nm}^r \text{Sm}^r$ colonies were obtained. Seventy-six of these colonies were tested for the ability to grow on dinitrogen under aerobic conditions (an unselected marker), and all were found to be unable to do so, implying that they were Nm^r derivatives of strain NW18 rather than Sm^r derivatives of strain AMP2. Because we have found that spontaneous Nm^r mutants appear at a frequency of less than 10^{-9} per cell, Nm^r derivatives of strain NW18 were expected to have received the C.K3 cassette from strain AMP2 rather than to have arisen from spontaneous mutation. Southern blot analysis of total DNA isolated from several $\text{Nm}^r \text{Sm}^r$ derivatives that was probed with a ^{32}P -labeled, C.K3-containing DNA fragment showed that those derivatives contained the C.K3 cassette (Fig. 3A). In addition, a Southern blot of total DNA isolated from strains AMP2 and NW18 and five $\text{Nm}^r \text{Sm}^r$ derivatives was probed with a ^{32}P -labeled internal fragment of the *nucA* gene (Fig. 3B). The results obtained (see lower band in each lane) indicate that the *nucA*::C.K3 construct originally present in strain AMP2 (that results in a *ClaI* band of ca. 5.7 kb) had replaced the wild-type *nucA* gene of strain NW18 (that results in a *ClaI* band of ca. 4.4 kb) in the five $\text{Nm}^r \text{Sm}^r$ derivatives that were examined. Moreover, unlike NW18, these strains exhibited no activity of the 29-kDa nuclease in SDS-PAGE assays (Fig. 4). The upper *nucA*-hybridizing bands observed in AMP2

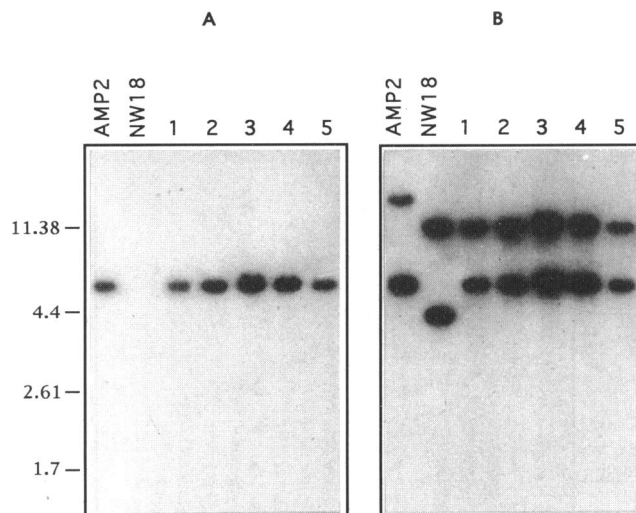


FIG. 3. Southern analysis of $\text{Nm}^r \text{Sm}^r$ derivatives. Total DNA from donor strain AMP2, recipient strain NW18, and five $\text{Nm}^r \text{Sm}^r$ derivatives (lanes 1 to 5) was digested with *ClaI* and hybridized with a 1.1-kb *BamHI* DNA fragment containing the Tn5 *npt* gene in cassette C.K3 (A) or a 473-bp *HindIII-BstNI* DNA fragment internal to the *nucA* gene of *Anabaena* sp. strain PCC 7120 (B). Hybridization was carried out under conditions of high stringency (see Materials and Methods). The positions of several size standards (in kilobases) are shown to the left of the figure.

and NW18 in Fig. 3B do not correspond to a gene that encodes an active 29-kDa nuclease (16) (Fig. 4).

Figure 5 presents a Southern blot of PHOGE-resolved high-molecular-weight DNA from strains PCC 7120, AMP2, and NW18, and two $\text{Nm}^r \text{Sm}^r$ derivatives, probed with pRLA1 (*nucA*). The α megaplasmid of *Anabaena* sp. strain PCC 7120 has single restriction sites for *SphI* and *SalI* that are about 105 kb distant from one another (4). Introduction of C.K3 into *nucA* added a second *SalI* site ca. 19 kb distant from the first (sizing data not shown), a *PstI* site at which the plasmid can be linearized, and a second *SphI* site ca. 126 kb distant from the first (Fig. 5, lanes 4 and 5; Fig. 1). The 510-kb megaplasmid of strain NW18 also appears to have single sites for *SphI* and *SalI* (not shown) as well as a locus that hybridizes with pRLA1 (Fig.

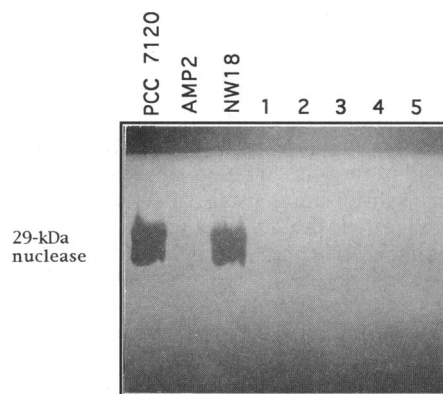


FIG. 4. Nuclease activity of cell suspensions from wild-type *Anabaena* sp. strain PCC 7120, donor strain AMP2, recipient strain NW18, and several $\text{Nm}^r \text{Sm}^r$ derivatives (lanes 1 to 5). Culture samples containing 5 to 10 μg of protein were used. The location of the 29-kDa nuclease (NucA) is indicated.

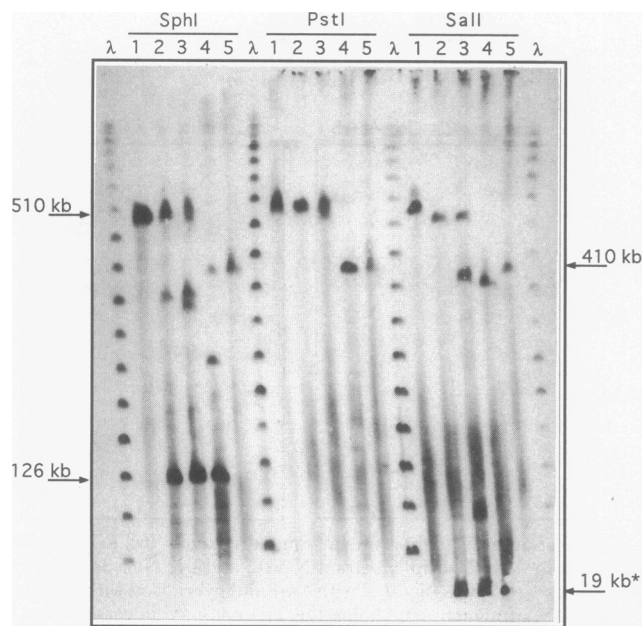


FIG. 5. Southern analysis of PHOGE gel of $Nm^r Sm^r$ derivatives. Agarose beads containing DNA derived from NW18 (lanes 1) $Nm^r Sm^r$ derivative 2 (lanes 2), $Nm^r Sm^r$ derivative 1 (lanes 3), AMP2 (lanes 4), and wild-type PCC 7120 (lanes 5) were subjected to PHOGE analysis as described previously (11) after digestion with *SphI* (partial digest [11]), *PstI*, or *SalI*. A Southern blot of the gel was probed with pRLA1. λ , beads containing concatemers of DNA from coliphage lambda; *, size of this fragment measured separately.

5, lanes 1). We shall refer to this locus as the *nucA* gene of NW18. The $Nm^r Sm^r$ derivatives contain plasmids that are approximately equal in size to the ca. 510-kb megaplasmid of NW18 (Fig. 5, lanes 1 to 3; *PstI* digestion). Upon partial digestion with *SphI*, these plasmids as well as the smaller α megaplasmid of AMP2 give rise to a fragment of about 126 kb (Fig. 5, lanes 2 to 4; *SphI* digestion) that measures the distance from *SphI* in C.K3 (in *nucA*) to the unique *SphI* of the megaplasmid. *SalI* cuts the same megaplasmids and gives rise to fragments with a size close to 19 kb (sizing data not shown). (Bands present in lanes 3 that are absent from lanes 2 are of unclear origin.) According to these results, the *SphI* and *SalI* sites are as far from *nucA* in the largest megaplasmid of the $Nm^r Sm^r$ derivatives of strain NW18 as they are in the α megaplasmid of PCC 7120. These results suggested that, in the two $Nm^r Sm^r$ derivatives investigated, the *nucA*::C.K3 marker had been incorporated by recombination in the megaplasmid of strain NW18. The presence of the C.K3 marker in the 510-kb plasmid of the $Nm^r Sm^r$ derivatives of strain NW18 was confirmed by hybridization using cassette C.K3 as a probe. When DNA digested with *SalI* was tested, C.K3 was observed to hybridize (under the conditions of hybridization used for the PHOGE gels) only to the 19-kb band (data not shown).

Transfer of the C.K3 cassette from strain AMP2 to strain NW18 was not affected by the presence of bovine pancreatic DNase (grade II; Boehringer Mannheim), which was added at 7.5 $\mu\text{g/ml}$ both to the mixture of cells before they were spread in the solid medium and to the medium in the plates. Therefore, the transfer presumably did not involve DNA free in the medium. Transfer by conjugation is possible, although other mechanisms are not excluded (21).

Because the donor strain used in the matings, AMP2, is itself

an exconjugant, derived from *Anabaena* sp. strain PCC 7120, which originated in an RP-4-promoted conjugation with *E. coli*, it was possible that strain AMP2 bore the *tra* genes from RP-4 and that these genes were responsible for the phenomenon of DNA transfer between cyanobacteria that we have observed. Therefore, we tested by hybridization the presence of DNA sequences from RP-4 in strain AMP2. No evidence for the presence of RP-4 or RP-4 fragments in this strain was found (data not shown).

To test whether transfer occurs specifically for genes in the α megaplasmid, we sought to transfer the *npt* gene from strain EF116(pRL52) to strain NW18. EF116 is a mutant of *Anabaena* sp. strain PCC 7120 that is unable to fix dinitrogen under aerobic conditions, and pRL52 is an *npt*-bearing shuttle plasmid whose insert complements the mutation in EF116 (9, 28). In matings involving, in several experiments, a total amount of ca. 2.3×10^8 cells of strain NW18 and ca. 1.15×10^8 cells of strain EF116(pRL52), no $Nm^r Sm^r$ colonies were obtained. These negative results provide no evidence that genes located outside the α megaplasmid can be transferred.

Widespread occurrence of *nucA* in heterocyst-forming cyanobacteria. It was of interest to test how widespread the *nucA* gene is in cyanobacteria other than strain PCC 7120. Judging by DNA-DNA reassociation experiments, *Anabaena* sp. strains

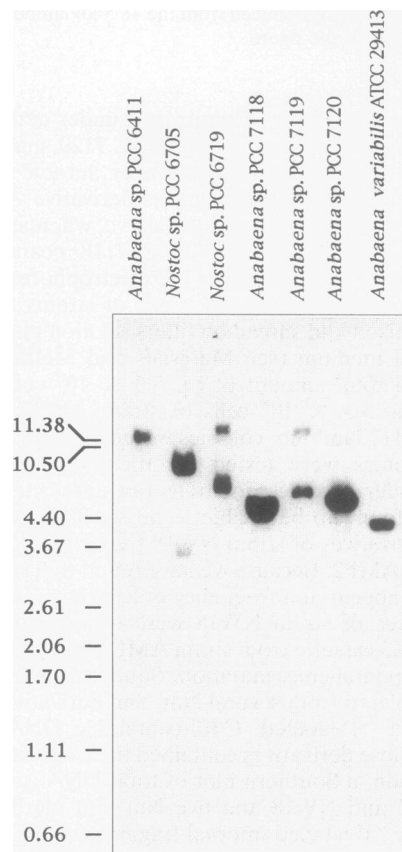


FIG. 6. Hybridization of DNA from several heterocyst-forming cyanobacteria with the *nucA* gene from *Anabaena* sp. strain PCC 7120. Total DNA from each of the cyanobacteria was digested with *EcoRV* and hybridized to a 473-bp *HindIII-BstNI* fragment internal to the *nucA* gene of *Anabaena* sp. strain PCC 7120. Hybridization was carried out under conditions of high stringency (see Materials and Methods). The positions of size standards (in kilobases) are indicated to the left of the figure.

TABLE 1. Presence or absence of *nucA* gene-hybridizing DNA and a nuclease of about 29 kDa in some unicellular and filamentous, heterocyst-forming cyanobacteria

Strain ^a	Hybridization ^b	29-kDa nuclease ^c
Group A		
<i>Anabaena variabilis</i> ATCC 29413-FD	+	+
<i>Anabaena</i> sp. strain PCC 6411 (ATCC 27898)	+	+
<i>Anabaena</i> sp. strain PCC 7118 (ATCC 27892)	+	+
<i>Anabaena</i> sp. strain PCC 7119 (ATCC 29151)	+	+
<i>Nostoc</i> sp. strain PCC 6705 (ATCC 29131)	+	+
<i>Nostoc</i> sp. strain PCC 6719 (ATCC 29105)	+	+
Group B		
<i>Calothrix</i> sp. strain PCC 7601	+	+
<i>Fischerella muscicola</i> UTEX 1829	+	-
<i>Nostoc ellipsosporum</i> B 1453-7 ^d	+	+
<i>Nostoc</i> sp. strain ATCC 43237	+	ND ^e
<i>Nostoc</i> sp. strain PCC 7107 (ATCC 29150)	+	-
<i>Nostoc</i> sp. strain PCC 7422 (ATCC 29132)	+	+
<i>Nostoc</i> sp. strain PCC 7413 (ATCC 29106)	+	+
* Group C		
<i>Gloeobacter</i> sp. strain PCC 7421 (ATCC 29082)	-	ND
<i>Synechococcus</i> sp. strain PCC 7202 (ATCC 29140)	-	-
<i>Synechococcus</i> sp. strain PCC 7425 (ATCC 29141)	-	-
<i>Synechococcus</i> sp. strain PCC 7942	-	-
<i>Synechocystis</i> sp. strain PCC 6308 (ATCC 27150)	-	-
<i>Synechocystis</i> sp. strain PCC 6803 (ATCC 27184)	-	-

^a Group A includes derivative FD of *A. variabilis* (6a) and cyanobacteria that, as described by Lachance (12), are closely related to *Anabaena* sp. strain PCC 7120. a Group B includes heterocyst-forming cyanobacteria that are not (see footnote d below) as closely related to *Anabaena* sp. strain PCC 7120. Group C includes unicellular strains. ATCC, American Type Culture Collection; PCC, Pasteur Culture Collection; UTEX, University of Texas Culture Collection. *N. ellipsosporum* B 1453-7 was obtained from the University of Göttingen.

^b Hybridization was carried out at high stringency for strains in group A and at low stringency for strains in groups B and C (see Materials and Methods).

^c Presence of a nuclease of about 29 kDa as shown by the SDS-PAGE nuclease assay (see Materials and Methods) of cell extracts (30 µg of protein for strains in group A and 90 µg of protein for strains in groups B and C).

^d *N. ellipsosporum* gave a particularly strong hybridization signal and, as previously discussed (8), may be closely related to strains in group A.

^e ND, not determined.

PCC 6411, PCC 7118, and PCC 7119 and *Nostoc* sp. strains PCC 6719 and PCC 6705 are phylogenetically very closely related to strain PCC 7120 (12). We have investigated, by means of Southern analysis, whether the *nucA* gene is present in these cyanobacteria. A ³²P-labeled internal probe of the *nucA* gene (corresponding to amino acids 52 to 210 of the NucA protein) was used for hybridization with total DNA from the cyanobacteria mentioned above under conditions of high stringency. The results in Fig. 6 show that all of these strains, as well as *A. variabilis* ATCC 29413, carry DNA sequences that exhibit homology to *nucA*. DNA from other heterocyst-forming cyanobacteria that are not as closely related to strain PCC 7120 and from six diverse unicellular cyanobacteria was tested for hybridization with the *nucA* probe from strain PCC 7120 under conditions of low stringency. All of the filamentous, heterocyst-forming strains tested, but none of the unicellular strains, showed hybridization to the *nucA* probe (data not shown). The results obtained are summarized in Table 1. Table 1 also shows the results of testing, by means of a DNA-containing, SDS-PAGE assay (see Materials and Methods), whether some of the cyanobacterial strains contain a nuclease of about 29 kDa, which is the size of the protein encoded by the *nucA* gene of *Anabaena* sp. strain PCC 7120. With the excep-

tion of *Fischerella muscicola* and *Nostoc* sp. strain PCC 7107, in which no nuclease activity was detected, all of the strains tested whose DNA hybridized with the *nucA* probe also exhibited a nuclease of ca. 29 kDa. It is possible that the *nucA*-hybridizing sequences in *F. muscicola* and *Nostoc* sp. strain PCC 7107 do not encode an active nuclease or that assay by SDS-PAGE is not suitable for the nuclease from those particular cyanobacteria. We conclude that the *nucA* gene is commonly present in the heterocyst-forming cyanobacteria but not in some other cyanobacteria. If the gene transfer that we observed was due to conjugation, conjugation could account for lateral transfer of a megaplasmid, and thus of *nucA*, between strains.

Concluding remarks. The results presented in this work show that the *nucA* gene is located in the α megaplasmid of *Anabaena* sp. strain PCC 7120 and that a marked version of *nucA* (*nucA::npt*) can be transferred from strain PCC 7120 directly to another strain of *Anabaena* sp. Although the efficiency of transfer plus recombination appears low, it may be amenable to increase by mutation (27). The finding of a naturally occurring transfer system represents a novel aspect of the biology of cyanobacteria. Further research will be necessary if the process is to be made useful as a genetic tool.

The fact that the α megaplasmid from PCC 7120 recombined with the megaplasmid from PCC 7118 rather than replacing it may suggest that the α megaplasmid is not transferred entirely, although other interpretations are possible. Transfer by conjugation might be aborted before the plasmid is completely transferred so that (i) the frequency of transfer of a plasmid-borne genetic marker would depend on its distance from the origin of transfer, and (ii) acquisition of a marker would depend upon its incorporation into an endogenous replicon. If recombination is a low-frequency event, as it is upon conjugal transfer from *E. coli* (our unpublished observations), transfer of *nucA::npt* to NW18 may be a much more frequent event than we observed.

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