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Organization of the Genes for Protein Synthesis Elongation Factors Tu and G in the Cyanobacterium *Anacystis nidulans*

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The genes for protein synthesis elongation factors Tu and G were cloned from the cyanobacterium *Anacystis nidulans*. The locations of these genes were mapped within the cloned DNA fragment by hybridization with *Escherichia coli* probes. The organization of the cloned fragment and the DNA flanking it in the *A. nidulans* chromosome was also determined. The elongation factor Tu and G genes are adjacent to one another and in the same 5′- to- 3′ orientation. In contrast to other gram-negative bacteria, *A. nidulans* contains only one gene for elongation factor Tu.

Cyanobacteria (blue-green algae) are gram-negative bacteria (18). Although all cyanobacteria are capable of photosynthetic CO_2 fixation, these organisms display diverse morphological and biochemical properties. At present, little is known about the relationships among various species of cyanobacteria and between cyanobacteria and other bacteria. Examination of the organization of genetic information in these organisms may provide some insight into these relationships. Since the bacterial genes coding for elongation factor Tu (EF-Tu) are well-conserved across species lines (6, 7), they may be used as probes to study the relatedness among procaryotic species.

In *Escherichia coli*, EF-Tu is encoded by two unlinked genes, designated *tufA* and *tufB* (3, 11). The *tufA* gene is linked to the genes for EF-G (*fus*) and ribosomal proteins S7 and S12 and is transcribed with them as part of the *str* operon (10). All of the gram-negative bacteria that have been screened with *E. coli tuf* probes have been shown to contain two genes for EF-Tu (7, 8). In contrast, the two gram-positive bacteria that have been tested have only one gene for EF-Tu (7).

In this paper we describe the cloning of a fragment of DNA which carries the genes for EF-Tu and EF-G in the cyanobacterium *Anacystis nidulans*. An analysis by hybridization with cloned *E. coli* EF-Tu and EF-G gene sequences showed that the two elongation factor genes are in the same relative orientation as the *tufA* and *fus* genes of *E. coli*. Hybridization of the cloned *Anacystis nidulans* elongation factor genes to genomic blots indicated that *Anacystis nidulans*, unlike other gram-negative bacteria previously examined, has only one gene for EF-Tu.

MATERIALS AND METHODS

Bacterial strains and plasmids. A culture of *Anacystis nidulans* R_2 was obtained from L. Sherman, University of Missouri. A culture of *E. coli* NO1379, which carries λ *fus-2* was obtained from M. Nomura, University of Wisconsin-Madison. *E. coli* strains carrying plasmids pG1 and pT1 were obtained from A. Furano, National Institute of Arthritis, Metabolism, and Degenerative Diseases.

Hybridization conditions. Hybridization was carried out as described by Wahl et al. (20), except that we changed the hybridization and wash buffers to decrease the stringency (F. S. Mickel, Ph.D. thesis, University of North Carolina, Chapel Hill, 1985).

Isolation of *Anacystis nidulans* DNA. *Anacystis nidulans* cells were harvested by centrifugation, and the resulting cell pellet was suspended in one-half the original culture volume of 50 mM Tris hydrochloride (pH 8)–0.1 M EDTA–0.15 M NaCl. The cells were treated with lysozyme at 37°C for 1 h. Lysis was completed by adding 1% sodium dodecyl sulfate and incubating the preparation at 37°C for 20 min. The nucleic acids were precipitated with ammonium acetate and isopropanol and then suspended in one-tenth the original culture volume of 10 mM Tris hydrochloride (pH 8)–10 mM EDTA–0.15 M NaCl–1% Sarkosyl. The solution was incubated overnight at 4°C in the presence of 1 mg of pronase per ml. Following treatment with 1 mg of RNase A per ml for 1 h at 37°C, the solution was extracted three times with phenol and chloroform. The DNA was ethanol precipitated and then dialyzed extensively.

Isolation of plasmid DNAs. Plasmid DNAs were isolated by the alkaline lysis method (1), which was scaled up or down as required.

Construction of pAnG2 and pAnG21. *Pst*I-digested *Anacystis nidulans* DNA in the desired size range (2.5 to 3.5 kilobase pairs [kbp]) was electroeluted from an agarose gel (14). A portion of this DNA was ligated to *Pst*I-cleaved pUC9 (19) and used to transform (5) *E. coli* JM83 cells (19). Clones were screened by hybridizing DNAs from lysates to the insertion of pG1 (Fig. 1). A positive clone, pAnG2, was selected for further study. The insertion of *Anacystis nidulans* DNA was cloned into the *Pst*I site of pBR322 (2) to yield pAnG21.

Construction of pEcT1. *Eco*RI-cleaved pACYC184 DNA (4) was ligated with *Eco*RI-cleaved λ *fus-2* DNA and used to transform *E. coli* HB101 cells. Colonies carrying plasmids with insertions were screened by colony hybridization (9) with the insertion of pT1 (Fig. 1).

RESULTS

Cloning of the *Anacystis nidulans* gene for EF-G. Screening of genomic blots of *Pst*I-cleaved *Anacystis nidulans* DNA with cloned *E. coli* fragments encoding the C-terminal portion of either EF-G or EF-Tu (see Fig. 1 for the hybridization probes used) revealed strong hybridization to a band at 2.7 kbp and weaker hybridization to a band at 3.9 kbp (data not shown). In some hybridizations, only the band at 2.7 kbp

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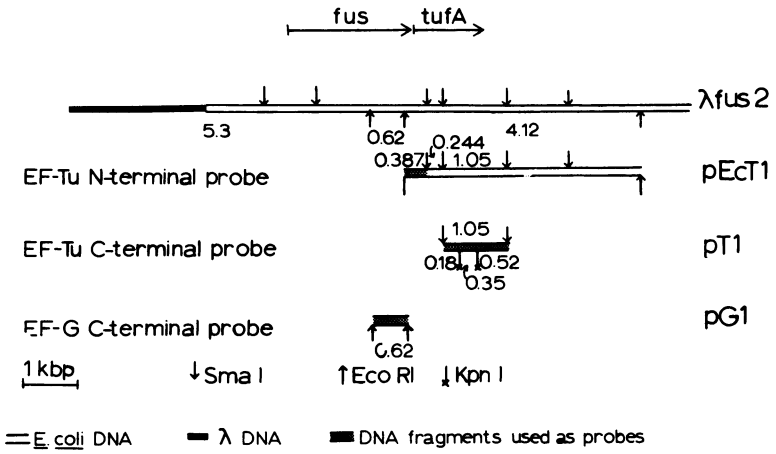

FIG. 1. Map of a portion of λ *fus-2* (13) carrying the *str* operon. The fragments of λ *fus-2* which are present in pT1, (21), pG1 (21), and pEcT1 are indicated. The sizes (in kilobase pairs) of fragments mentioned in the text are indicated. The fragments used as hybridization probes are indicated by stippling. The locations of the genes for EF-G (*fus*) and EF-Tu (*tufA*) and the direction in which they are transcribed are indicated above the restriction map of λ *fus-2*.

was observed, suggesting that the 3.9-kbp band represented a partial digestion product. Since *tuf* and *fus* genes are known to be linked in some bacterial species (10, 16), we examined the possibility that these two genes might also be linked in *Anacystis nidulans*. The 2.7-kbp *Pst*I fragment of *Anacystis nidulans* DNA which hybridized to the *E. coli* probe (pG1) was cloned (Fig. 1). A restriction map of the cloned DNA carried on plasmid pAnG21 is shown in Fig. 2.

When the insertion of pAnG21 was used as a probe against *Eco*RI-cleaved λ *fus-2* DNA (see Fig. 1 for a map), we observed strong hybridization to the 4.1-kbp fragment which carries the *tufA* gene, as well as a small portion of the *fus* gene (data not shown). There was also hybridization to fragments containing the other portions of the *fus* gene. These results indicated that the genes for both EF-Tu and EF-G are located on the insertion of pAnG21.

The locations of the elongation factor genes on the cloned fragment were determined by hybridizing *E. coli* gene sequences to restriction fragments of the insertion of pAnG21 (Fig. 3A). The hybridization probes which we used are shown in Fig. 1, and the results are summarized in Fig. 2. The EF-Tu C-terminal probe showed homology to the 0.65-kbp fragment (Fig. 3A, lanes a and b), while the EF-Tu N-terminal probe hybridized with the 0.94-kbp fragment

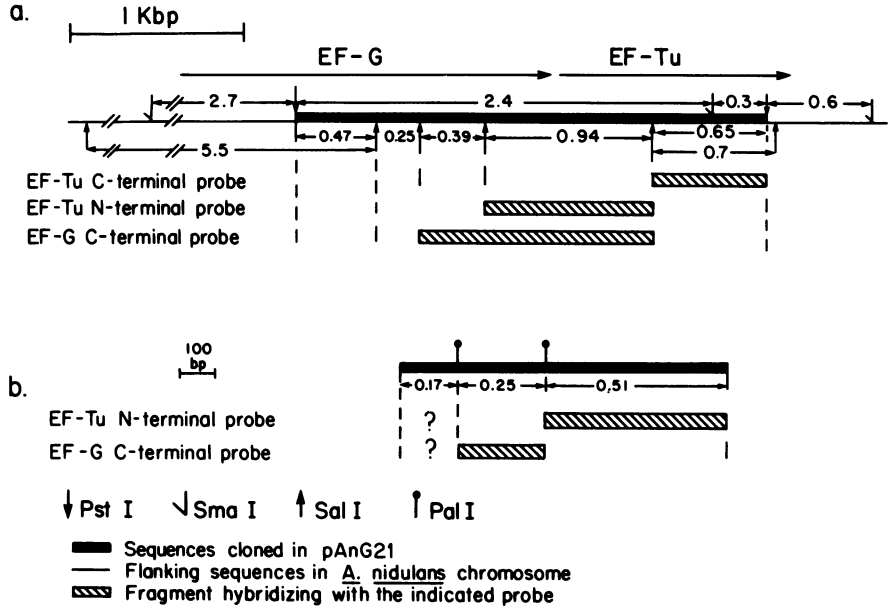

FIG. 2. Restriction map of the sequences contained in pAnG21 and flanking sequences within the *Anacystis nidulans* chromosome. (a) The genes for EF-Tu and EF-G are indicated above the restriction map. The exact boundaries of the genes have not been determined, and their sizes and spacing are based on the sizes and spacing observed for these genes in *E. coli*. The horizontal arrows indicate the direction of transcription, as inferred from the location of the C- and N-terminal sequences. (b) *Pal*I restriction sites within the 0.94-kbp *Sal*I fragment. The question marks indicate that the 0.17-kbp fragment was too small to transfer. The fragment sizes are indicated (in kilobase pairs). The fragments which hybridize with specific *E. coli* probes are indicated by cross-hatched boxes below the map. bp, Base pairs.

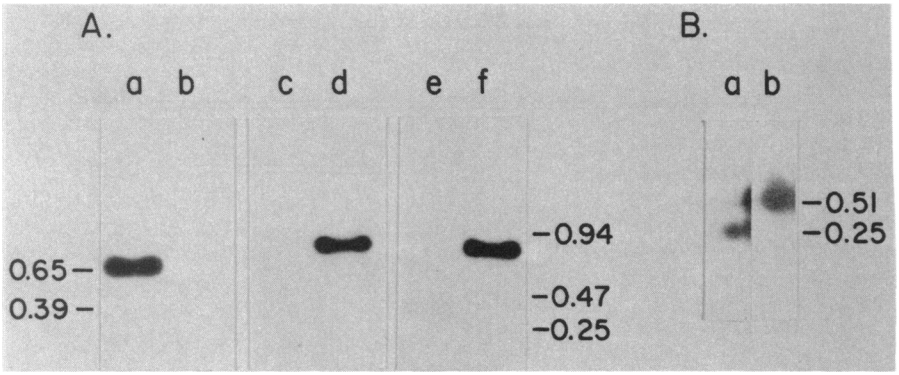

FIG. 3. Detection of sequences of pAnG21 which hybridize with the *E. coli* genes for ET-Tu and EF-G. (A) Blots of *SalI* subfragments of the insertion of pAnG21 hybridized with *E. coli* EF-Tu and EF-G probes. Lanes a, c, and e contained the 0.65- and 0.39-kbp fragments. Lanes b, d, and f contained the 0.94-, 0.47-, and 0.25-kbp fragments. The following probes were used: lanes a and b, EF-Tu C-terminal probe; lanes c and d, EF-Tu N-terminal probe; lanes e and f, EF-G C-terminal probe. (B) *PalI* subfragments of the 0.94-kbp *SalI* fragment of pAnG21 hybridized with *E. coli* DNA probes. The following probes were used: lane a, *E. coli* EF-G C-terminal probe; lane b, *E. coli* EF-Tu N-terminal probe.

(lanes c and d). This result suggests that the EF-Tu gene within the cloned *Anacystis nidulans* DNA fragment is oriented as indicated in Fig. 2. The EF-G C-terminal probe showed homology to the 0.94-kbp fragment and, to a small extent, to the 0.39-kbp fragment (Fig. 3A, lanes e and f). This observation indicated that the *fus* gene lies to the left of the *tuf* gene. In order to further clarify the organization, filters containing a *PalI* digest of the 0.94-kbp *SalI* fragment were probed with the *E. coli* EF-G C-terminal and EF-Tu N-terminal probes (Fig. 3B). The EF-G C-terminal probe hybridized to the 0.25-kbp fragment, and the EF-Tu N-terminal probe hybridized to the 0.51-kbp fragment (Fig. 3B, lanes a and b, respectively). These results established that the two genes are adjacent (Fig. 2) and in the same relative orientation as the *fus* and *tufA* genes of *E. coli* (Fig. 1 and 2).

Number of EF-Tu genes in *Anacystis nidulans*. In order to determine the number of genes for EF-Tu in *Anacystis nidulans*, subfragments of pAnG21 were hybridized to genomic blots of *Anacystis nidulans* DNA (Fig. 4). The fragments used as probes and the results obtained are summarized in Fig. 2. In addition, we were able to map *SalI* and *SmaI* sites in the *Anacystis nidulans* chromosome flanking the cloned region.

All of the probes examined hybridized strongly with a 2.7-kbp band and weakly with a 3.9-kbp band in blots of *PstI*-cleaved *Anacystis nidulans* DNA (Fig. 4A, lanes a, d, and g) (data not shown). This observation indicated that the sequences on the 2.7-kbp fragment are a subset of the sequences on the 3.9-kbp fragment. The 2.7-kbp fragment insertion of the cloned DNA hybridized strongly to bands at approximately 0.94, 0.6, and 0.4 kbp and weakly to a band at 1.5 kbp in the *PstI-SalI* digest of *Anacystis nidulans* DNA (Fig. 4A, lane b). The 0.65-kbp *SalI-PstI* fragment (EF-Tu C-terminal probe) hybridized strongly to the band at approximately 0.6 kbp and weakly to the 1.5-kbp band (lane e). The 0.51-kbp *PalI-SalI* fragment (EF-Tu N-terminal probe) hybridized strongly to the 0.94-kbp band and weakly to the 1.5-kbp band (lane h). The hybridization to the 1.5-kbp band by the two subfragments indicated that this band was a partial cleavage product and did not represent another

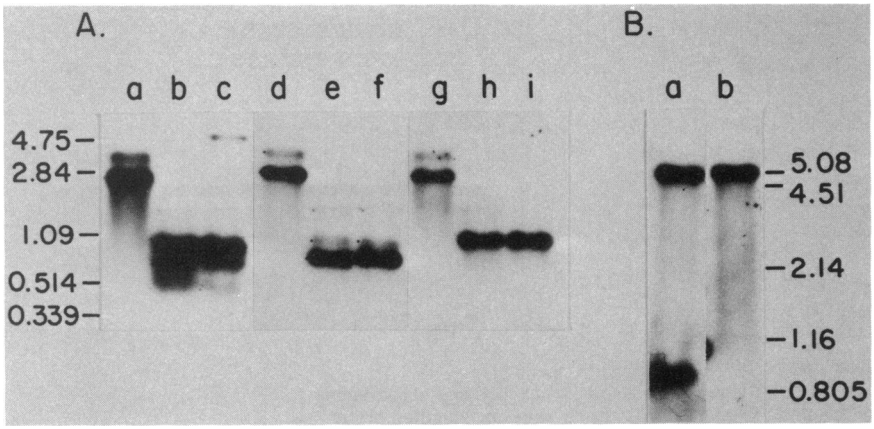

FIG. 4. *Anacystis nidulans* genomic blots probed with the insertion and subfragments of pAnG21. (A) *Anacystis nidulans* DNA was cleaved with *PstI* (lanes a, d, and g), *PstI* and *SalI* (lanes b, e, and h), or *SalI* (lanes c, f, and i). The following probes were used: lanes a through c, 2.7-kbp insertion of pAnG21; lanes d through f, *Anacystis nidulans* ET-Tu C-terminal sequences (0.65-kbp *SalI-PstI* fragment); lanes g through i, *Anacystis nidulans* EF-Tu N-terminal sequences (0.51-kbp *PalI-SalI* fragment). (B) *Anacystis nidulans* DNA cleaved with *SmaI*. The following probes were used: lane a, *Anacystis nidulans* EF-Tu C-terminal sequences; lane b, *Anacystis nidulans* EF-Tu N-terminal sequences. The sizes of molecular weight markers are indicated (in kilobase pairs).

EF-Tu gene. To obtain further information on the number of EF-Tu genes, the cloned fragment was hybridized against genomic blots of *Sal*I-cleaved DNA. In these blots, the 2.7-kbp fragment hybridized strongly with *Sal*I bands at 0.94 and about 0.6 kbp and weakly with bands at 5.5, 1.7, and approximately 0.4 kbp (Fig. 4A, lane c). The 0.65-kbp fragment from the C-terminal region of the EF-Tu gene hybridized strongly with the 0.6-kbp band and weakly with the 1.7-kbp band; the 0.51-kbp fragment (EF-Tu N-terminal probe) hybridized with the 0.94- and 1.7-kbp bands (lane i). Thus, the 1.7-kbp band represented a partial cleavage product. The 0.47-kbp *Pst*I-*Sal*I fragment (EF-G C-terminal probe) hybridized to the 5.5-kbp band (data not shown). The hybridization of the probes for *Anacystis nidulans* EF-Tu C-terminal and N-terminal sequences to one major band each in the *Sal*I and *Pst*I-*Sal*I digests of *Anacystis nidulans* DNA suggested that there was only one gene for EF-Tu. In addition, a comparison of the sizes of the bands in the single and double digests allowed us to map the two *Sal*I sites external to the cloned region (Fig. 2).

In order to further ensure that the EF-Tu gene was present in only one copy in the *Anacystis nidulans* genome, the 0.5- and 0.65-kbp fragments were hybridized to genomic blots of *Sma*I-cleaved *Anacystis nidulans* DNA. As indicated in Fig. 4B, lane b, the 0.51-kbp fragment hybridized to a band at 5.1 kbp. The 0.65-kbp fragment, which was cleaved by *Sma*I to yield 0.41- and 0.25-kbp fragments, hybridized to bands at 5.1 and about 0.9 kbp (Fig. 4B, lane a). The ratio of the levels of hybridization to the two bands, as determined by Cerenkov counts (data not shown), was about equal to the ratio of the sizes of the two fragments generated by cleavage of the 0.65-kbp fragment with *Sma*I. The correlation of hybridization and size ratios, when considered along with the presence of only one major band in *Sal*I and *Pst*I-*Sal*I digests of *Anacystis nidulans* DNA probed with either the C-terminal (0.65-kbp fragment) or N-terminal (0.51-kbp fragment) EF-Tu sequences, strongly indicated that a single gene for EF-Tu is present in *Anacystis nidulans*. Although it is possible that the entire region has been duplicated, so that *Anacystis nidulans* has two genes for both EF-Tu and EF-G, we consider this possibility unlikely. In *E. coli*, the duplicated *tuf* genes are in different sequence environments (10, 12).

DISCUSSION

Using heterologous hybridization with *E. coli* EF-Tu and EF-G gene sequences, we cloned a fragment of DNA from the cyanobacterium *Anacystis nidulans* containing sequences homologous to both *E. coli* genes. Our data show that the genes for EF-Tu and EF-G are adjacent and in the same relative orientation within the *Anacystis nidulans* genome. In contrast to other gram-negative bacteria (7, 8), including the cyanobacterium *Spirulina platensis* (17), there appears to be only one gene for EF-Tu present in *Anacystis nidulans*. In *E. coli*, one of two genes for EF-Tu, *tufA*, is linked to the gene for EF-G (3, 11), as is the sole gene for EF-Tu in *Bacillus subtilis* (16). The *tufA* and *fus* genes of *E. coli* are located next to one another in the *str* operon, with the *fus* promoter proximal (10). The relative 5′ -to- 3′ orientation of the genes for EF-G and EF-Tu in *Anacystis nidulans* is the same as that of *tufA* and *fus* in *E. coli*.

S. platensis has two genes for EF-Tu which are closely linked (17). A similar observation has been made for two other gram-negative bacteria, *Caulobacter crescentus* and *Pseudomonas putida* (7). Hybridization with probes for *tuf* genes from *E. coli* and *A. nidulans* to DNAs from other cyanobacteria has indicated that the arrangement of EF-Tu genes in *Plectonema boryanum* may be similar to that in *S. platensis* but that there is only one gene for EF-Tu in *Anabaena variabilis* (Mickel, Ph.D thesis). The arrangement of elongation factor genes in other cyanobacteria is not known.

The classification of cyanobacteria is based primarily on morphological and developmental criteria (15). The genetic relationships among species which have been grouped together are not well defined. The availability of cloned genes from cyanobacteria should allow workers to examine the genetic relatedness of strains of cyanobacteria by comparing the organization of genes and DNA sequence homologies.

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