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Nucleotide Sequence and Further Characterization of the *Synechococcus* sp. Strain PCC 7002 *recA* Gene: Complementation of a Cyanobacterial *recA* Mutation by the *Escherichia coli recA* Gene

RANDY C. MURPHY, GAIL E. GASPARICH, DONALD A. BRYANT,* AND RONALD D. PORTER
Department of Molecular and Cell Biology, The Pennsylvania State University, University Park, Pennsylvania 16802

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The nucleotide sequence and transcript initiation site of the *Synechococcus* sp. strain PCC 7002 *recA* gene have been determined. The deduced amino acid sequence of the RecA protein of this cyanobacterium is 56% identical and 73% similar to the *Escherichia coli* RecA protein. Northern (RNA) blot analysis indicates that the *Synechococcus* strain PCC 7002 *recA* gene is transcribed as a monocistronic transcript 1,200 bases in length. The 5′ endpoint of the *recA* mRNA was mapped by primer extension by using synthetic oligonucleotides of 17 and 27 nucleotides as primers. The nucleotide sequence 5′ to the mapped endpoint contained sequence motifs bearing a striking resemblance to the heat shock (σ^{32}-specific) promoters of *E. coli* but did not contain sequences similar to the *E. coli* SOS operator recognized by the LexA repressor. An insertion mutation introduced into the *recA* locus of *Synechococcus* strain PCC 7002 via homologous recombination resulted in the formation of diploids carrying both mutant and wild-type *recA* alleles. A variety of growth regimens and transformation procedures failed to produce a *recA Synechococcus* strain PCC 7002 mutant. However, introduction into these diploid cells of the *E. coli recA* gene in *trans* on a biphasic shuttle vector resulted in segregation of the cyanobacterial *recA* alleles, indicating that the *E. coli recA* gene was able to provide a function required for growth of *recA Synechococcus* strain PCC 7002 cells. This interpretation is supported by the observation that the *E. coli recA* gene is maintained in these cells when antibiotic selection for the shuttle vector is removed.

The cyanobacteria (blue-green algae) represent the largest and most diverse group of the photosynthetic bacteria. They share with plants the ability to use two photosystems to evolve molecular oxygen from water. Current evidence indicates that the photosynthetic apparatus of the cyanobacteria is highly homologous to that found in the chloroplasts of higher plants (6). These organisms also share with the higher plants and algae the manner in which they assimilate and metabolize inorganic nitrogen compounds; additionally, some species are able to fix atmospheric nitrogen (43). Because the cyanobacteria encompass many basic plant functions in a relatively simple procaryotic genetic background, there is interest in developing these organisms as model systems for the study of various plant functions at the molecular level.

Several characteristics make the unicellular marine cyanobacterium *Synechococcus* sp. strain PCC 7002 an attractive candidate for development as a genetic model system. One of the simplest cyanobacteria, its structure and genetic complexity are similar to those of other gram-negative bacteria (38). *Synechococcus* strain PCC 7002 cells demonstrate natural competence for exogenous DNA uptake and transformation, and both chromosomal and plasmid transformation studies have been carried out in this species (35). Biphasic shuttle vectors, based on the fusion of native *Synechococcus* strain 7002 plasmids and *Escherichia coli* cloning vectors (e.g., pBR322), have been constructed (7; J. S. Buzby, Ph.D. thesis, The Pennsylvania State University, University Park, 1984). Allowing for genetic manipulation in *E. coli*, these plasmids can be transformed into and propagated in *Synechococcus* strain PCC 7002. However, these plasmids cannot at present be usefully employed as bearers of cloned *Synechococcus* sp. fragments for merodiploid analysis. Homologous recombination between plasmid- and chromosome-borne alleles leads to gene conversion and integration events that render merodiploid analysis in this species problematic (36).

In *E. coli*, homologous recombination is largely governed by the *recA* gene product. Since the elimination of RecA protein activity results in mutant *E. coli* strains that are capable of maintaining stable merodiploids, we have been interested in determining whether a similar strategy could lead to the production of a Rec$^-$ cyanobacterium. We have previously reported the cloning and partial characterization of the *Synechococcus* strain PCC 7002 *recA* gene (32). The cyanobacterial *recA* gene product was found to be similar in size to the *E. coli* RecA protein and was able to complement homologous recombination function when expressed in *E. coli recA* mutants. Hybridization experiments also indicated that the gene is widespread among the cyanobacteria (32). We report here the complete nucleotide sequence of the *Synechococcus* strain PCC 7002 *recA* gene, the results of experiments designed to eliminate *recA* function in the cyanobacterium, and the finding that the *E. coli* RecA protein is able to provide a function required for growth of *recA Synechococcus* strain PCC 7002 cells.

MATERIALS AND METHODS

Culture conditions and strain. The unicellular marine cyanobacterium *Synechococcus* strain PCC 7002 (formerly *Agmenellum quadruplicatum* PR-6) was maintained in liquid culture and on 1.5% agar plates in medium A as described previously (44). Various transformants of *Synechococcus* strain PCC 7002 were selected and maintained on antibiotic concentrations of 200 μg of kanamycin per ml, 2.0 μg of

* Corresponding author.

ampicillin per ml, and 50 μg of streptomycin per ml for both liquid culture and plate-growth conditions.

DNA isolation. Small-scale *E. coli* plasmid preparations were performed by following the rapid boiling procedure of Holmes and Quigley (21). Small-scale plasmid extractions from *Synechococcus* strain PCC 7002 for analysis of transformants was carried out by following an alkaline extraction procedure (28), with two modifications: cells were frozen and subsequently thawed at 37°C prior to reagent I-lysozyme treatment, and cells were treated with proteinase K (50 μg/ml overnight) after RNase treatment. *Synechococcus* strain PCC 7002 chromosomal DNA was isolated from 10-ml liquid cultures by the following method. Cells were harvested by centrifugation and suspended in buffered lysis solution (25% sucrose, 50 mM Tris hydrochloride, pH 7.5, 10 mM EDTA). Following freezing and subsequent thawing at room temperature, cells were incubated first at 37°C in the presence of 10 mg of lysozyme per ml for 30 min and then overnight at 55°C, after the addition of sodium dodecyl sulfate and proteinase K (Sigma Chemical Co.) to 10 mg/ml and 100 μg/ml, respectively. Lysates were subsequently extracted at least twice with phenol-chloroform (1:1) and two final chloroform extractions before precipitation of the DNA with isopropanol.

Cloning, transformation, and hybridization procedures. Various restriction endonuclease enzymes were used to digest both plasmid and chromosomal DNA in accordance with instructions provided by the manufacturers (Bethesda Research Laboratories, Inc.; International Biotechnologies, Inc.). *E. coli* plasmid transformations were performed by using either the 24-h $CaCl_2$ procedure of Dagert and Ehrlich (11) or the method of Kushner (26); transformants were selected on LB agar plates containing 50 μg of ampicillin per ml or 100 μg of kanamycin per ml or both. Both plasmid and chromosomal transformations of *Synechococcus* strain PCC 7002 were carried out as previously described (7, 44) for chromosomal transformation of the cyanobacterium. DNA fragment isolation and purification were performed by agarose gel electrophoresis as previously described (32). DNA ligations, nick translations, and Southern and Northern (RNA) hybridizations were performed by using standard procedures (28). *Synechococcus* strain PCC 7002 RNA was isolated by following the mechanical breakage protocol of Golden et al. (14). Primer extension mapping of the 5′ endpoint of the *Synechococcus* strain PCC 7002 *recA* mRNA was performed by the method of Ausubel et al. (2) and used avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim Biochemicals) and the aqueous hybridization option recommended for oligonucleotide primers. The hybridization solution contained 0.3 M NaCl, 0.5 M HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), pH 7.5, and 1 mM EDTA. S1 nuclease mapping of the 5′ end of the *Synechococcus* strain PCC 7002 *recA* transcript was carried out by the procedure outlined by Maniatis et al. (28), with slight modifications. The overhanging 5′ termini of the 252-base-pair (bp) gel-purified *Eco*RI-*Bst*EII fragment from plasmid pAQPR75 were labeled by using [γ-^{32}P]ATP and T4 polynucleotide kinase (Bethesda Research Laboratories) after dephosphorylation with bacterial alkaline phosphatase (Bethesda Research Laboratories). A 1/2-μg portion of this fragment was allowed to hybridize overnight at 50°C with approximately 200 μg of *Synechococcus* strain PCC 7002 RNA. S1 nuclease treatment at 1,000 U/ml was allowed to proceed for 1 h at 37°C. After precipitation and suspension in TE (0.01 M Tris hydrochloride, 1 mM EDTA, pH 8.0), the sample was denatured and electrophoresed on a polyacrylamide gel alongside the sequence reactions of a DNA sample of known sequence.

DNA sequencing. Double-strand nucleotide sequencing was carried out by allowing specific dideoxynucleotides to terminate ^{32}P-labeled primer extension reactions randomly by using base-denatured plasmid DNAs as templates (16, 40). The radiolabeled fragments were subsequently separated by electrophoresis at a constant power of 60 watts through a 40 by 33 by 0.4 cm 5% acrylamide gel (acrylamide: bisacrylamide = 19:1); in TEB buffer (TEB = 275 mM sodium EDTA, 89 mM boric acid, 89 mM Tris hydrochloride, pH 8.3) containing 8.5 M urea. Following electrophoresis, the gel was exposed to Kodak X-Omat AR film at −70°C for about 15 h prior to film development. Initial sequencing at the extreme ends of the insertion fragment used M13 universal and reverse primers (48). As nucleotide sequence data were obtained, appropriate primers were prepared as synthetic oligonucleotides on a model 380A automated DNA synthesizer (Applied Biosystems, Inc.). The entire nucleotide sequence was determined on both DNA strands. The synthetic 27-mer used in the primer extension mapping experiments was purified by reverse-phase high-pressure liquid chromatography prior to detritylation.

RESULTS

Nucleotide and amino acid sequence analysis. Plasmid pAQPR75 was previously shown to encode the *Synechococcus* strain PCC 7002 *recA* gene on a 2.2-kilobase-pair *Eco*RI-*Bgl*II fragment that had been cloned into the *Eco*RI-*Bam*HI sites of *E. coli* plasmid vector pUC8 (R. C. Murphy, Ph.D. dissertation, The Pennsylvania State University, University Park, 1989). The entire cyanobacterial DNA fragment (2,218 bp) was subjected to double-strand nucleotide sequence analysis (Fig. 1). The cloned *Synechococcus* sp. fragment was found to contain both the complete coding sequence of the *recA* gene (nucleotides 1 to 1047 in Fig. 1) as well as the 5′ portion of a second open reading frame (nucleotides 1192 to 1960 in Fig. 1) which begins 144 bp downstream from the *recA* termination codon. Although the deduced amino acid sequence of this open reading frame is shown in Fig. 1, the significance of this potential coding sequence is unknown. No homology to sequences in GenBank could be found.

The cyanobacterial *recA* gene (1,047 bp) and the *E. coli recA* gene (1,062 bp) are similar in size. A comparison of the two coding sequences for the region in which the two sequences overlap demonstrates 56% sequence identity at the nucleotide level. Similarly, the deduced amino acid sequence of the *Synechococcus* strain PCC 7002 *recA* gene shows 56% sequence identity with the *E. coli* RecA protein (Fig. 2 and Table 1). This homology increases to about 73% when allowances for conservative amino acid substitutions are made (30). The *Synechococcus* strain PCC 7002 RecA protein is approximately equally homologous to those of *E. coli*, *Proteus mirabilis*, *Thiobacillus ferooxidans*, *Pseudomonas aeruginosa* PAO, *Rhizobium meliloti*, and *Agrobacterium tumefaciens* (see Table 1). Several regions of complete or nearly complete amino acid identity among the various *recA* gene products are observed (Fig. 2). However, the amino acid sequence homology of all RecA proteins is considerably reduced at their carboxyl termini.

An acceptable ribosome-binding (Shine-Dalgarno-type) sequence, 5′ GAGG 3′, is found 5 bp upstream from the ATG initiation codon of the cyanobacterial *recA* gene (Fig. 1). The downstream open reading frame is preceded by the

NUCLEOTIDE SEQUENCE OF SYNECHOCOCCUS SP. PCC 7002 RECA GENE AND ITS DEDUCED AMINO ACID SEQUENCE

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  -250      -240      -230      -220      -210      -200      -190      -180      -170      -160      -150      -140
GAATTCTTGGCCTGGCTCCAGTTACCAACCTTTATCCAAGCGGCCCCCGTCAAAACCCAGACCATCTATCACTTCCAGCAGCCCAAATAATCATCCCGGTGAGGGTTGTTTGCCGCCGGA

  -130      -120      -110      -100      -90       -80       -70       -60       -50       -40       -30       -20
ATATCACCTGGATCTGCCTTTCGTCAGATTGTAAAAATCCTGCGACAGCTTTCCGGGAAAATTGCGATACCATAAGTACGATTGAACTCAGAAATCCAATCACTTTTGACCCATTACGCC

  -10      -1 1         10          20          30          40          50          60          70
ACGGCGATCGAGGTAACC ATG TCA GCC ATC AGC AAT AAT CCC GAC AAA GAA AAG GCC CTA AAC CTA GTG CTC AAC CAA ATT GAG CGT AAC TTT
                    M   S   A   I   S   N   N   P   D   K   E   K   A   L   N   L   V   L   N   Q   I   E   R   N   F

 80          90          100         110         120         130         140         150         160
GGT AAG GGG GCG ATT ATG CGG TTG GGG GAT GCC GCC CAG ATG AAG GTC GCA ACC ATT CCC AGT GGG GCT TTG ACC CTA GAT CAG GCG ATG
 G   K   G   A   I   M   R   L   G   D   A   A   Q   M   K   V   A   T   I   P   S   G   A   L   T   L   D   Q   A   M

 170         180         190         200         210         220         230         240         250
GGC GGT GGG TTT CCG CGT GGG AGA ATT GTG GAA ATC TAT GGC CCG GAG AGT TCT GGG AAA ACG ACG GTT GCT CTC CAT GCG ATC GCC GAA
 G   G   G   F   P   R   G   R   I   V   E   I   Y   G   P   E   S   S   G   K   T   T   V   A   L   H   A   I   A   E

 260         270         280         290         300         310         320         330         340
GTC CAA AAA GCG GGT GGC GTC GCT GCT TTT ATT GAC GCT GAA CAT GCC CTG GAT CCC ACC TAT TCC GCG GCC TTG GGA GTT GAT ATT GAA
 V   Q   K   A   G   G   V   A   A   F   I   D   A   E   H   A   L   D   P   T   Y   S   A   A   L   G   V   D   I   E

 350         360         370         380         390         400         410         420         430
AAC CTG CTG GTG GCC CAA CCC GAT AAC GGC GAA TCT GCT TTA GAA ATT GCC GAC CAA TTG GTT CGC TCG GCG GCT GTG GAT CTA ATC GTG
 N   L   L   V   A   Q   P   D   N   G   E   S   A   L   E   I   A   D   Q   L   V   R   S   A   A   V   D   L   I   V

 440         450         460         470         480         490         500         510         520
ATT GAC TCG GTG GCG GCC CTT GTG CCC AGG GCA GAG ATC GAA GGA GAA ATG GGC GAT GTG CAG GTG GGT CTC CAG GCC CGT CTC ATG AGT
 I   D   S   V   A   A   L   V   P   R   A   E   I   E   G   E   M   G   D   V   Q   V   G   L   Q   A   R   L   M   S

 530         540         550         560         570         580         590         600         610
AAA GCC CTC CGT AAA ATT GCC GGG AAT ATG GGG CGT TCG GGT TGT ACG GTG ATT TTT CTC AAT CAA TTG CGC CAA AAA ATT GGC ATT AGT
 K   A   L   R   K   I   A   G   N   M   G   R   S   G   C   T   V   I   F   L   N   Q   L   R   Q   K   I   G   I   S

 620         630         640         650         660         670         680         690         700
TAC GGC AAT CCA GAG GTA ACC ACC GGG GGA ACC GCC CTC AAA TTT TAT GCC TCT GTG CGC CTA GAT ATC CGC CGC ATC CAA ACC CTC AAG
 Y   G   N   P   E   V   T   T   G   G   T   A   L   K   F   Y   A   S   V   R   L   D   I   R   R   I   Q   T   L   K

 710         720         730         740         750         760         770         780         790
AAA GGG AGC GAA GGG GAA TTT GGC ATC CGC GCC AAG GTT AAA GTG GCG AAA AAT AAA GTT GCT CCA CCG TTC CGC ATT GCC GAA TTT GAC
 K   G   S   E   G   E   F   G   I   R   A   K   V   K   V   A   K   N   K   V   A   P   P   F   R   I   A   E   F   D

 800         810         820         830         840         850         860         870         880
ATT ATT TTT GGC AAA GGA ATC TCC CGT GTG GGC TGC ATG CTA GAC CTC GCG GAA CAA ACA GGT GTC ATT ACC CGC AAA GGC GCT TGG TAC
 I   I   F   G   K   G   I   S   R   V   G   C   M   L   D   L   A   E   Q   T   G   V   I   T   R   K   G   A   W   Y

 890         900         910         920         930         940         950         960         970
AGC TAT GAA GGG GAT AAC ATT GCC CAG GGT CGC GAC AAT GCA GTG AAG TAT CTA GAG GAA AAT CCG GAC GTT GCG GCG ATC GTC ACC CAA
 S   Y   E   G   D   N   I   A   Q   G   R   D   N   A   V   K   Y   L   E   E   N   P   D   V   A   A   I   V   T   Q

 980         990         1000        1010        1020        1030        1040        1050      1060      1070
AAA GTC CGG GAA AAT CTG GAT ATG AGT TCC ATG GGT TTC GGG GAT GAG CAC CAC ACC ACC GAA GAA GAA TAA GCATTTTTGTGGCAGCAAATATT
 K   V   R   E   N   L   D   M   S   S   M   G   F   G   D   E   H   H   T   T   E   E   E  END

     1080      1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190
CCTGATCCAATGCAAGATGGTAAGACAAGGGGCGAATTTAAGCCTCTTTTTTCTTGAGTTTTTTGAAAATTAGGGAAACAGGCACCAGACAACAGCATAATTTGCGGCGTGCTAGAATCG

         1200        1210        1220        1230        1240        1250        1260        1270
G   ATG CGT CAT TTT GAA AAA AAA CGT TAC GTT CAC TAT GGG TAT TCG TTT CTC TCG TTG GTT AGT GGG TTG CGG TGT GGC GAT CGC TTC
     M   R   H   F   E   K   K   R   Y   V   H   Y   G   Y   S   F   L   S   L   V   S   G   L   R   C   G   D   R   F

1280     1290        1300        1310        1320        1330        1340        1350        1360
 GGT GTA TTG TCC CCT TCA GTC ACT TGG GCT AAT CCC CAA CTC AAT GCC CTC CTC GAA CAG GGA AAT GAA CAG CTG ACC AAT CGG AAT TTT
  G   V   L   S   P   S   V   T   W   A   N   P   Q   L   N   A   L   L   E   Q   G   N   E   Q   L   T   N   R   N   F

1370     1380        1390        1400        1410        1420        1430        1440        1450
 GCC CAA GCA GTG CAG CAT TAC CGC CAG GCT CTC ACC CTA GAA GCC AAC AAC GCC CGG ATC CAT GGT GCC CTC GGT TAC GCC CTT TCC CAA
  A   Q   A   V   Q   H   Y   R   Q   A   L   T   L   E   A   N   N   A   R   I   H   G   A   L   G   Y   A   L   S   Q

1460     1470        1480        1490        1500        1510        1520        1530        1540
 CTG GGC AAT TAT TCA GAA GCG GTG ACG GCC TAC CGC CGG GCT ACG GAA TTA GAA GAT GAT AAT GCA GAG TTT TTC AAT GCC CTG GGT TTC
  L   G   N   Y   S   E   A   V   T   A   Y   R   R   A   T   E   L   E   D   D   N   A   E   F   F   N   A   L   G   F

1550     1560        1570        1580        1590        1600        1610        1620        1630
 AAT TTG GCC CAA AGT GGC GAT AAC CGC AGT GCC ATC AAC GCC TAC CAG CGA GCC ACC CAA CTG CAG CCG AAT AAC CTG GCC TAC AGT CTG
  N   L   A   Q   S   G   D   N   R   S   A   I   N   A   Y   Q   R   A   T   Q   L   Q   P   N   N   L   A   Y   S   L

1640     1650        1660        1670        1680        1690        1700        1710        1720
 GGT TTA GCG ACC GTA CAA TTT CGG GCC GGG GAC TAC GAT CAA GCT CTG GTC GCG TAC CGT AAA GTT TTG GCT AAA GAC AGC AAC AAC ACC
  G   L   A   T   V   Q   F   R   A   G   D   Y   D   Q   A   L   V   A   Y   R   K   V   L   A   K   D   S   N   N   T

1730     1740        1750        1760        1770        1780        1790        1800        1810
 ATG GCG CTG CAA AAT AGT TTA ACC AGT TTG TTG CAA TTG GGA CGT AAC CAA GAG GCG GCA GTG CTG TTT CCT GAT TTG CTG CGC CAA CGA
  M   A   L   Q   N   S   L   T   S   L   L   Q   L   G   R   N   Q   E   A   A   V   L   F   P   D   L   L   R   Q   R

1820     1830        1840        1850        1860        1870        1880        1890        1900
 CCC AAT GAC GCG GAA CTC CGC ATT AAG GCA GCG GTG ACG TGG TTT GGC CTC AAT GAT CGT GAC CAG GCG ATC GCC TTC CTG GAA GAA GCC
  P   N   D   A   E   L   R   I   K   A   A   V   T   W   F   G   L   N   D   R   D   Q   A   I   A   F   L   E   E   A

1910     1920        1930        1940        1950        1960
 CGC CGC CTC AGC ACC CGC GAC AGT GCG ATG CAA ATT CGG GTC GGC AAG ATC T
  R   R   L   S   T   R   D   S   A   M   Q   I   R   V   G   K   I
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FIG. 1. Nucleotide sequence of the *Synechococcus* strain PCC 7002 *recA* gene and flanking sequences (GenBank accession no. M29495). Nucleotide numbers 1 through 1047 encompass the *recA* coding sequence. The coding region of the downstream open reading frame begins with nucleotide number 1192. Nucleotide −26 is the transcription initiation site as determined by primer extension and S1 nuclease mapping. An interrupted inverted repeat which could play a role in transcription termination and/or mRNA stabilization occurs at nucleotides 1046 to 1073.

sequence 5′ AGAA 3′ 4 bp prior to the translation start codon. The nucleotide sequence coding for the structural portion of the RecA protein contains two short regions of dyad symmetry (nucleotides 398 to 407 and 458 to 468 in Fig. 1), but the "pseudo-ISs" observed in the *E. coli recA* gene sequence (39) are not found in the cyanobacterial homolog. Finally, the A+T-rich interrupted inverted repeat that is observed immediately 3′ to the coding sequence (nucleotides 1046 to 1073) may play a role in transcription termination or mRNA stabilization.

Northern blot and primer extension analyses. Northern blots of RNA prepared from exponentially growing *Synechococcus* strain PCC 7002 cells or from cells which had been starved for a combined nitrogen source for 5 h were hybridized with two different radiolabeled probes. The first probe was prepared from the gel-purified 980-bp *AluI* fragment of

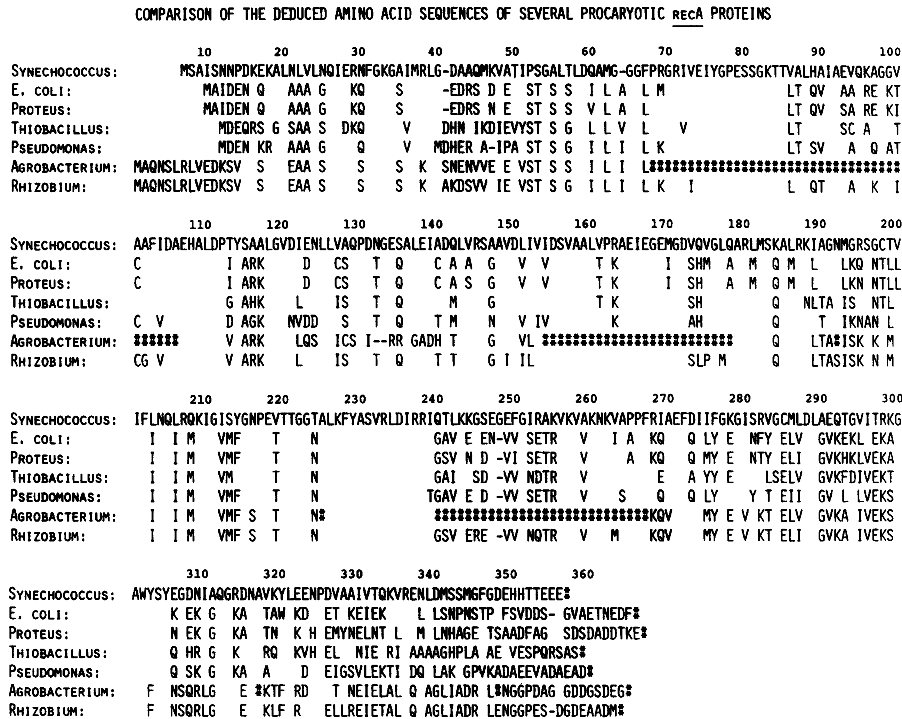

FIG. 2. Comparison of the deduced amino acid sequences of the RecA proteins of *Synechococcus* strain PCC 7002, *E. coli* (39), *P. mirabilis* (1), *T. ferrooxidans* (37), *P. aeruginosa* PAO (41), *A. tumefaciens* (Buikama, Ph.D. thesis), and *R. meliloti* (Buikama, Ph.D. thesis). Blank spaces indicate amino acids identical to those of the *Synechococcus* strain PCC 7002 RecA protein; hyphens indicate deletions inserted to improve the alignment, and asterisks in the *A. tumefaciens* sequence indicate regions which have not yet been sequenced.

plasmid pAQPR75 (nucleotides −92 to 886); this probe is highly specific for the *Synechococcus* strain PCC 7002 *recA* gene. Hybridization with total *Synechococcus* strain PCC 7002 RNA revealed a transcript of 1,200 nucleotides (Fig. 3A), suggesting that the *Synechococcus* strain PCC 7002 *recA* gene is transcribed as a monocistronic transcript approximately 1,200 nucleotides in length. From the continuous smear of smaller hybridizing species, it appears that the *recA* transcript may be rather unstable. The pattern and strength of the hybridization obtained with RNA from cells which had been starved of combined nitrogen for 5 h were indistinguishable from those obtained with exponentially growing cells from nitrogen-replete media.

A second radiolabeled probe was prepared from the gel-purified 780-bp *Hin*dIII-*Hin*fI fragment of plasmid pAQPR75 (the *Hin*dIII site is in the pUC8 multiple-cloning site). This probe contains DNA sequence exclusively from the open reading frame that begins immediately downstream from the *Synechococcus* strain PCC 7002 *recA* gene (nucleotides 1185 to 1960; Fig. 1). Hybridization of this probe with *Synechococcus* strain PCC 7002 RNA yielded an apparent transcript size of approximately 7,200 nucleotides (Fig. 3B). The Northern blot data suggest that the *recA* gene and the downstream open reading frame are not cotranscribed.

The 5′ endpoint of the *recA* mRNA was mapped by primer extension by using synthetic oligonucleotides of 17 bases (5′ CCAAAGTTACGCTCAAT 3′; nucleotides 77 to 61; data not shown) or 27 bases (5′ CGCCCCCTTACCAAAGTTA CGCTCAAT 3′; nucleotides 87 to 61; Fig. 4). The two oligonucleotide primers produced identical extension patterns. The major 5′ endpoint of the *recA* mRNA determined with either primer occurred 26 nucleotides upstream from the translation start codon of the *recA* gene (nucleotide −26 in Fig. 1). Consistent with the results of the primer extension reactions, protected fragments of 24 to 28 nucleotides were also observed when the 252-bp *Eco*RI-*Bst*EII fragment of

TABLE 1. % Amino acid sequence identity of RecA proteins from various bacterial species[a]

Bacterial species	% Identity						
	E. coli	*P. mirabilis*	*P. aeruginosa*	*T. ferrooxidans*	*R. meliloti*	*A. tumefaciens*[b]	*Synechococcus* sp.
E. coli	100	84	70	68	65	59	56
P. mirabilis		100	71	68	65	58	55
P. aeruginosa			100	68	64	55	58
T. ferrooxidans				100	66	59	59
R. meliloti					100	86	55
A. tumefaciens[b]						100	46
Synechococcus sp.							100

[a] References for sequences: *E. coli* (39), *P. mirabilis* (1), *P. aeruginosa* PAO (41), *T. ferrooxidans* (37), and *R. meliloti* and *A. tumefaciens* (Buikama, Ph.D. thesis).
[b] Based on incomplete sequence data.

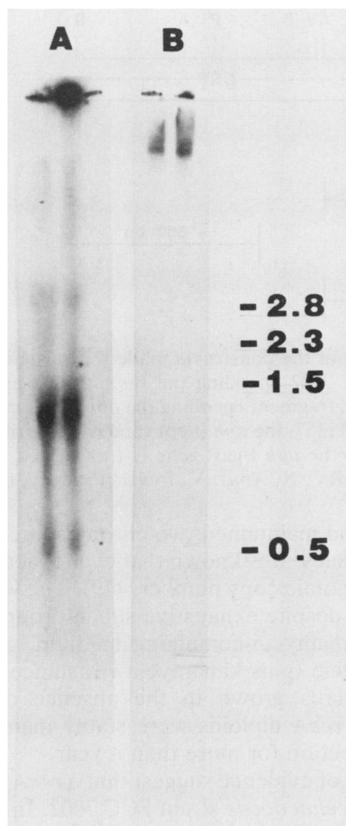

FIG. 3. Fluorogram resulting from Northern blot hybridization of *Synechococcus* strain PCC 7002 RNA. (A) Hybridization with a radiolabeled *Synechococcus* strain PCC 7002 *recA* gene internal probe (nucleotides −92 to 860; see Fig. 1). (B) Hybridization with a radiolabeled *Synechococcus* strain PCC 7002 fragment comprising sequences derived exclusively from the open reading frame located downstream from the *recA* gene (nucleotides 1185 to 1960; see Fig. 1). The left and right lanes of both panels A and B represent equal portions (20 μg) of RNA from *Synechococcus* strain PCC 7002 grown in medium with and without (for 5 h) combined nitrogen, respectively. The bars at the right show the position of the *Synechococcus* strain PCC 7002 ribosomal RNA molecules, whose approximate sizes are expressed in kilobases.

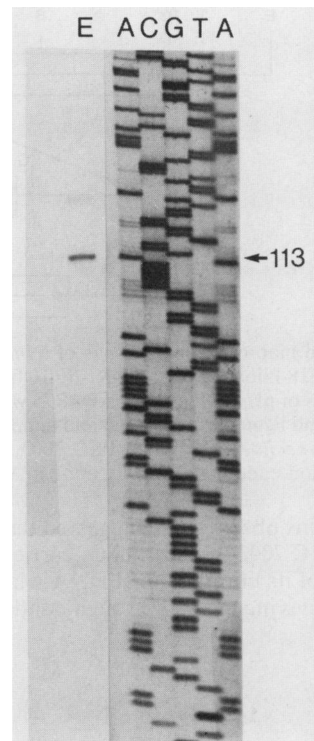

FIG. 4. Primer extension analysis of the *Synechococcus* strain PCC 7002 *recA* transcript. Lane E contains the primer extension product. The nucleotide sequence shown is of known sequence and is derived from a subclone of a flanking sequence of the *Synechococcus* strain PCC 7002 *apcE* gene. The extension primer used in this experiment was the synthetic 27-mer 5′ CGCCCCCTTACCA AAGTTACGCTCAAT 3′ and corresponds to nucleotides 87 to 61 (complement) in Fig. 1. The extension product was 113 nucleotides long and thus corresponds to a transcription initiation site at nucleotide −26 (see Fig. 1). This length determination was confirmed by repeating the extension experiment with a sizing ladder generated by using the synthetic 27-mer as the sequencing primer and with plasmid pAQPR75 as template (results not shown).

plasmid pAQPR75 was used in S1 protection experiments (results not shown). Although the sequence 5′ to the mapped mRNA does not resemble the *E. coli* consensus (σ^{70}) promoter sequence (15, 17), the sequence does closely resemble the *E. coli* consensus heat shock (σ^{32}) promoter (10, 33). The sequence 5′ CGATTGAA 3′ occurs at 27 to 34 bp upstream from the transcription initiation point and is separated by 13 nucleotides from the sequence 5′ TCACTTTT 3′ which occurs 5 bases upstream from the transcription initiation point. These sequence motifs and their spacing are similar to the heat shock promoter sequence 5′ CCCTTGAA—13 to 15 bp—CCCCATTTA 3′ which occurs 7 to 8 bp upstream from the transcription initiation points for genes whose transcription is dependent upon the σ^{32} form of RNA polymerase in *E. coli* (10, 33). Finally, no sequence motif homologous to the "SOS box" (i.e., the operator sequence recognized by the LexA repressor [34]) was found in the sequence upstream from the *recA* translational start codon.

Introduction of an insertion mutation at the *recA* locus of *Synechococcus* strain PCC 7002. Plasmid pAQPR75 carries a single *Eco*RV restriction site located in the central portion of the *Synechococcus* strain PCC 7002 *recA* gene (nucleotides 679 to 684; Fig. 1; see Fig. 5). A 1,350-bp fragment carrying the *aph* gene of Tn*5* was inserted at this site to form plasmids pRM1357 and pRM6455, in which the *aph* gene is transcribed, respectively, in the same direction or in the opposite direction relative to the *recA* gene in plasmid pAQPR75 (Fig. 5). The *aph* gene encodes aminoglycoside 3′-phosphotransferase and confers resistance to the antibiotic kanamycin. Since equivalent results were obtained with both constructions, only the results obtained with pRM1357 will be described here. *Synechococcus* strain PCC 7002 cells were transformed with the gel-purified *Hin*dIII-*Eco*RI fragment of plasmid pRM1357 bearing the insertionally inactivated *Synechococcus* sp. *recA* allele, and chromosomal DNAs from 12 kanamycin-resistant transformants were subjected to endonuclease restriction, electrophoresis, and Southern blotting. Hybridization of the blotted DNA with radiolabeled plasmid pAQPR75 demonstrated that in all 12 transformants examined, the insertionally inactivated *recA* allele had recombined into the chromosome to produce the expected product. However, although all of the transformants yielded the predicted fragment sizes for displacement of the *recA* gene, the hybridization pattern also indicated that an uninterrupted wild-type *recA* gene remained in each of the transformants (data for only one transformant are shown in Fig. 6; compare

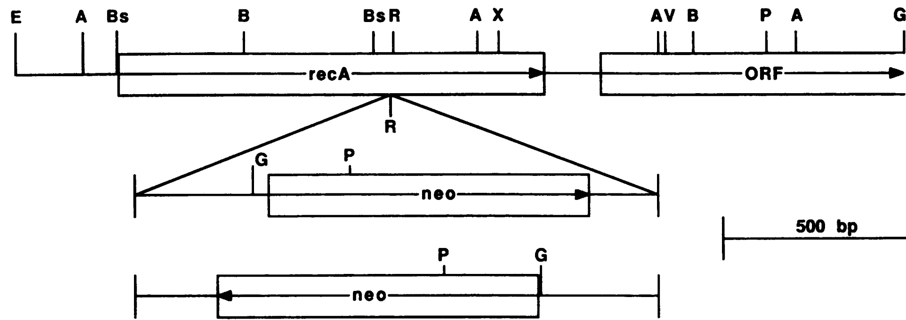

FIG. 5. Physical map of the *recA* locus of *Synechococcus* strain PCC 7002 and maps of the constructs made for plasmids RM1357 and pRM6455. The 2.218-kilobase-pair *Eco*RI-*Bgl*II fragment of *Synechococcus* strain PCC 7002 encoding the *recA* gene was cloned in the *Eco*RI-*Bam*HI sites of pUC8. Plasmid pAQPR75 was digested with *Eco*RV, and a 1,350-bp fragment encoding the *aph* gene, indicated as neo, of Tn*5* was blunt-end ligated into this plasmid in both directions as shown. In plasmid pRM1357, the *aph* (neo) gene is transcribed in the same direction as the *Synechococcus* strain PCC 7002 *recA* gene, while in plasmid pRM6455 the *aph* (neo) gene is transcribed in the opposite direction. Restriction endonucleases: E, *Eco*RI; A, *Alu*I; Bs, *Bst*EII; B, *Bam*HI; R, *Eco*RV; X, *Xba*I; V, *Pvu*II; P, *Pst*I; and G, *Bgl*II.

lanes 3 and 4). This observation suggested that the *Synechococcus* strain PCC 7002 transformants carried (at least) two genomic copies of its chromosomal DNA when transformed and that cells surviving growth through continuous kanamycin selection had maintained two chromosomes carrying the diploid *recA* alleles. It is known that cyanobacteria routinely exhibit high genomic copy numbers (19). The wild-type *recA* gene remained despite exhaustive subculturing of the transformants in kanamycin-containing medium, and the interrupted *recA* allele (plus kanamycin resistance) was quickly lost from cultures grown in the absence of kanamycin selection. The *recA* diploids were stably maintained under kanamycin selection for more than a year.

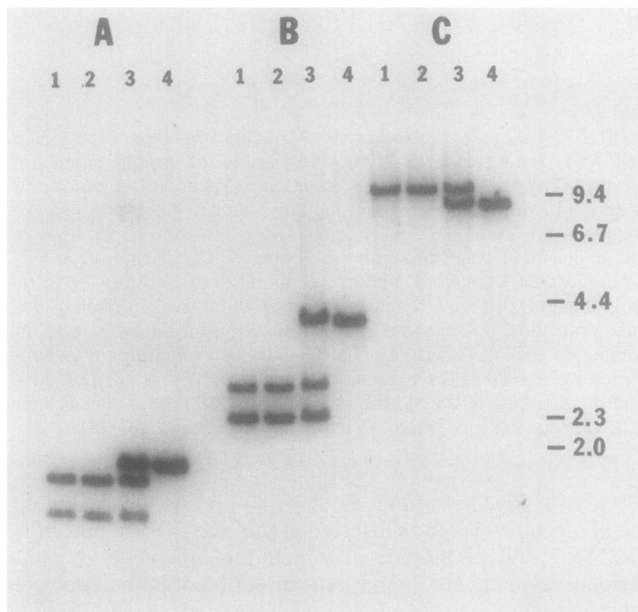

FIG. 6. Fluorogram resulting from Southern blot hybridization demonstrating the elimination of the wild-type *Synechococcus* strain PCC 7002 *recA* gene. The radiolabeled DNA insert of plasmid pAQPR75 (see Fig. 5) was used as the hybridization probe. (A) DNAs digested with *Eco*RI and *Pst*I. (B) DNAs digested with *Bgl*II. (C) DNAs digested with *Eco*RI. Lanes 1 and 2 contain chromosomal DNAs isolated from two *Synechococcus* strain PCC 7002 transformants harboring plasmid pAQE5/*recA* and the pRM1357-derived insertion mutation in the cyanobacterial *recA* locus. Lanes 3 contain chromosomal DNA isolated from *Synechococcus* strain PCC 7002 transformants carrying the pRM1357-derived insertion mutation at the chromosomal *recA* locus but not harboring plasmid pAQE5/*recA*. Lanes 4 contain chromosomal DNA isolated from the wild-type *Synechococcus* strain PCC 7002. A single fragment hybridizes to the probe for chromosomal DNA from the wild type. However, two fragments hybridize for DNAs isolated from transformants when these are digested with *Eco*RI and *Pst*I or *Bgl*II because of the *Pst*I and *Bgl*II sites within the *aph* gene fragment (see Fig. 5). The bars to the right show the position of size markers, the *Hin*dIII fragments of phage lambda DNA, whose sizes are indicated in kilobase pairs.

Several lines of evidence suggest that a *recA* null mutation is lethal in *Synechococcus* strain PCC 7002. In an attempt to enhance the probability of segregating the interrupted and wild-type alleles and identifying a *recA Synechococcus* strain PCC 7002 mutant, the transformed cyanobacterial cells were subjected to a number of growth regimens. Cells were grown and subcultured through liquid cultures containing up to 1,600 μg/ml (i.e., eight times the normal kanamycin concentration). Each liquid culture was allowed to grow into deep stationary phase prior to subculturing. Since the number of cyanobacterial chromosomes per cell is proportional to cell growth rate (19), it was anticipated that increasing the selection pressure for kanamycin resistance during exponential growth, followed by conditions leading to a reduced number of chromosome copies, might lead to an increase in the number of cells carrying only the mutant allele. Of surviving colonies examined, however, all maintained the wild-type and interrupted alleles. Similarly, the diploid condition was maintained in these cells following slow growth (in the presence of kanamycin) induced by CO_2 limitation, extremely reduced lighting, or filtered red lighting conditions. Interestingly, microscopic examination of plates streaked from these slow-growth liquid cultures demonstrated that a very high percentage (greater than 90%) of the cells did not yield colonies, even when plated on kanamycin-free media. Also evident on these nonselective plates were many microcolonies consisting approximately (by microscopic examination) of from 10 to 50 cells. These colonies did not grow larger and died relatively quickly. On the other hand, wild-type *Synechococcus* strain PCC 7002 cells grown under the same slow-growth regimens exhibited faster growth rates than the diploid cultures and yielded normal (nearly 100%) plating efficiencies.

In another experiment, an attempt was made to enrich the transformant cultures for cells carrying a stable kanamycin marker. The transformed cells were taken through three nonselective (i.e., no kanamycin) subculturings in liquid

TABLE 2. Stability of antibiotic markers in transformed *Synechococcus* strain PCC 7002

Chromosomal *recA* genotype(s)			Time of growth in liquid culture without antibiotic selection	Total colonies assayed	No. of colonies	
Wild type	Insertion mutation (Km^r)	Plasmid (Ap^r)			Ap^r	Km^r
+	−	None	6 days	50	0	0
+	−	pAQE5	6 days	200	15	ND[a]
+	+	None	6 days	500	ND	0
−	+	pAQE5/*recA*	5 mo	400	400	400

[a] ND, Not determined.

medium prior to streaking onto kanamycin plates. Although the vast majority of cells died upon reexposure to kanamycin, a very small number of kanamycin-resistant colonies did grow. However, Southern blotting and hybridization of DNA isolated from surviving kanamycin-resistant colonies demonstrated that these cells still carried both the interrupted and wild-type *recA* alleles. When wild-type *Synechococcus* strain PCC 7002 cells in deep stationary phase were transformed with the pRM1357-derived *recA* allele, transformation efficiency was reduced approximately 50-fold as compared with cells transformed while in exponential growth. Although kanamycin-resistant transformants were readily recovered, Southern blot analysis revealed that these cells also carried both mutant and wild-type *recA* alleles. Finally, it was noted that although plated wild-type *Synechococcus* strain PCC 7002 cells generally form smooth, round-edged colonies, microscopic observation of the transformant colonies plated on kanamycin medium displayed wedge-shaped indentations, possibly indicating lethal sectoring.

Complementation with *E. coli recA*. Plasmid pBEU14 carries the *E. coli recA* gene on a 3.0-kilobase-pair *Bam*HI fragment (46). This fragment was cloned into the single *Bam*HI site of the *E. coli*-*Synechococcus* strain PCC 7002 shuttle vector pAQE5 (7) to produce plasmid pAQE5/*recA*. Plasmids pAQE5 and pAQE5/*recA* both carry a functional *bla* gene and thus confer ampicillin resistance to both *E. coli* and *Synechococcus* strain PCC 7002 host cells. The diploid *Synechococcus* strain PCC 7002 carrying both wild-type and interrupted cyanobacterial *recA* alleles was transformed with plasmid pAQE5/*recA*. Selected transformants were serially subcultured three times through liquid media containing both ampicillin and kanamycin before streaking onto ampicillin-kanamycin plates. Isolated colonies of each transformant were chosen for inoculation of liquid cultures and subsequent DNA isolation. The presence of the intact plasmid pAQE5/*recA* in each transformant was confirmed by recovery of the original plasmid after transformation into *E. coli* (data not shown; see Murphy, Ph.D. dissertation). Southern blot hybridization using the DNA insert of plasmid pAQPR75 (see Fig. 5) as probe clearly demonstrated that the wild-type *Synechococcus* strain PCC 7002 *recA* gene had been eliminated from each of the two transformants analyzed (Fig. 6, compare lanes 1 and 2 to lane 3). Moreover, repeated subculturing (6 months) in the absence of both kanamycin and ampicillin did not result in the loss of either of these markers from the cells (Table 2). The stability of the kanamycin marker in these cells provided independent confirmation that the wild-type and interrupted *Synechococcus* strain PCC 7002 *recA* alleles had segregated. Maintenance of ampicillin resistance presumably indicates selection for the *E. coli recA* gene in these cells, since ampicillin resistance

TABLE 3. Chromosomal transformation efficiencies of *Synechococcus* strain PCC 7002

Chromosomal *recA* genotype(s)			Str^r transformants/ 10^6 CFU
Wild type	Insertion mutation	Plasmid	
+	−	None	1,400
+	+	None	560
−	+	pAQE5/*recA*	83

carried by the pAQE5 shuttle vector is normally lost from cell populations not kept under antibiotic selection (Table 2). Both the comparative ease with which introduction of the *E. coli recA* gene into these cells allowed segregation of the interrupted and wild-type cyanobacterial *recA* alleles and the continued selection of the *Synechococcus* strain PCC 7002 *recA* mutant for the *E. coli recA* gene argue strongly for the notion that the *E. coli recA* gene product is complementing in these cells a function normally provided by the *Synechococcus* strain PCC 7002 RecA protein.

Transformation efficiencies. The diploid *Synechococcus* strain PCC 7002 strain carrying the wild-type and interrupted *recA* alleles and the cyanobacterial strain carrying pAQE5/*recA* but no wild-type *Synechococcus* strain PCC 7002 *recA* gene were compared with wild-type *Synechococcus* strain PCC 7002 cells for their ability to undergo chromosomal transformation. All three strains were transformed with chromosomal DNA isolated from a streptomycin-resistant mutant of *Synechococcus* strain PCC 7002. Streptomycin-resistant colonies were scored following plating onto streptomycin medium. The results of these experiments (Table 3) indicate that the diploid strain carrying both the wild-type and interrupted *recA* alleles is approximately half as effective as wild-type *Synechococcus* strain PCC 7002 cells in undergoing chromosomal transformation. In contrast, the cyanobacterial strain carrying the *E. coli recA* gene but no wild-type *Synechococcus* strain PCC 7002 *recA* gene shows a roughly 17-fold decrease compared with wild-type cells in its ability to undergo chromosomal transformation.

DISCUSSION

Although *recA* analogs have reportedly been cloned from diverse bacterial species, *recA* nucleotide sequence data have been reported only *E. coli* (39), *P. mirabilis* (1), *T. ferrooxidans* (37), *P. aeruginosa* (41), *A. tumefaciens* (29; W. Buikama, Ph.D. thesis, Harvard University, Cambridge, Mass., 1985), and *R. meliloti* (Buikama, Ph.D. thesis). We report here the nucleotide sequence of the *recA* gene of the cyanobacterium *Synechococcus* strain PCC 7002.

Overall deduced amino acid homology between the *E. coli* and *Synechococcus* strain PCC 7002 RecA proteins is 56%. This is somewhat higher than the level of amino acid homology observed for the *phr* repair enzymes of *E. coli* and the cyanobacterium *Anacystis nidulans* (50). The *phr* gene product is responsible for photoreactivation of DNA lesions produced by UV irradiation. The observed amino acid homology between the *E. coli* and *Synechococcus* strain PCC 7002 RecA proteins is, however, somewhat lower than those observed between the *E. coli* protein and the deduced amino acid sequences of *P. aeroginosa*, *T. ferrooxidans*, *R. meliloti*, and *A. tumefaciens recA* proteins (Table 1). It is interesting to note, however, that although amino acid homologies among the *recA* genes varies from roughly 55 to 86%, the more divergent *Synechococcus* strain PCC 7002

gene product maintains a rather constant level of homology (about 55%) with each of the other RecA proteins. It is also clear that regions of strong homology between the *E. coli* and *Synechococcus* strain PCC 7002 proteins are for the most part maintained in the other homologs.

Sequence analysis supports the idea that the *E. coli* and *Synechococcus* strain PCC 7002 RecA proteins maintain a structural and functional relationship at the amino acid level. *E. coli recA1* mutants, for example, are deficient in all known RecA protein functions. The RecA1 protein contains a single point mutation resulting in the replacement of Gly-160 by an aspartic acid residue (22). Gly-160 is conserved in each of the sequenced *recA* homologs. Between the *E. coli* and cyanobacterial proteins there are several extensive regions of homology, one of which includes the 16 amino acids from Gly-59 to Thr-74. This sequence has been identified as the nucleotide-binding fold and is common to a number of ATP-ADP-binding proteins (20, 49). In contrast, the use of ATP-analog affinity labels has suggested that the region about Tyr-264 of the *E. coli* RecA protein is also involved in the binding of ATP (23, 24). However, this region is not especially conserved between the *E. coli* and *Synechococcus* strain PCC 7002 RecA proteins. Other regions exhibiting strong homology between the two proteins have been tentatively identified, via analysis of *E. coli recA* mutants, as the site for single-stranded DNA binding (Lys-6 to Gly-30), as the active site for ATP-hydrolysis (Ala-91 to Ile-128), and as the region for bacteriophage repressor recognition (Gly-204 to Gly-229) (34). Single point mutations in the latter region reportedly allow the *E. coli* protein to distinguish between the repressors of phage λ and Φ80 (34). Although cyanobacterial species have been found to harbor phage (42), one could expect that phage systems and host cell recognition proteins would be allowed some measure of freedom for divergence. The extreme degree of homology between the cyanobacterial and *E. coli* protein sequences may suggest that this region is also involved in a more fundamental RecA protein function.

The *E. coli* and cyanobacterial RecA proteins demonstrate considerable diversity in the carboxy-terminal region. No known *E. coli recA* mutants have been mapped in this region. In turn, evidence has been provided suggesting that this region of the *E. coli* RecA protein is required for binding of ATP to RecA protein (3), regulating binding of double-stranded DNA to *recA* protein (4), and regulating RecA protein protease activity (34). Ogawa and Ogawa found that a *recA* deletion mutant missing 25 amino acids at the carboxy terminus appeared to take an active protease form without DNA damage (34). It is perhaps interesting that although the deduced amino acid sequence of the *P. mirabilis recA* gene displays a much greater overall homology with the *E. coli* protein (Table 1), it also diverges considerably from the *E. coli* RecA sequence at the carboxy terminus. A similar divergence from the *E. coli* RecA protein at the carboxy terminus is observed for the *recA* homologs of *T. ferrooxidans*, *P. aeruginosa*, *A. tumefaciens*, and *R. meliloti*, although the RecA proteins of the latter two species appear to share more homology between themselves in this region than is found for any other RecA protein comparison.

The promoter regions of the *E. coli* and cyanobacterial *recA* genes demonstrate no real homology, and no sequence resembling the "SOS box," the binding site for the LexA repressor, was found. Although a sequence resembling the SOS box is present in the promoter region of the *P. aeruginosa* (41) and *P. mirabilis* (1) *recA* genes, no such sequence was found in *R. meliloti* (Buikama, Ph.D. thesis), *A. tumefaciens* (Buikama, Ph.D. thesis), or *T. ferrooxidans* (37). Attempts to identify a *lexA* homolog in *Synechococcus* strain PCC 7002 by low-stringency, heterologous hybridization with a radiolabeled gene internal fragment of the *E. coli lexA* gene were unsuccessful (Murphy, Ph.D. dissertation). These results suggest that regulation of expression of the *recA* gene in *Synechococcus* sp. might differ significantly from that in *E. coli*.

Sequence motifs immediately 5′ to the mapped 5′ extremity of the *recA* transcript of *Synechococcus* strain PCC 7002 closely resemble, in both sequence and spacing, the consensus heat shock (σ^{32}) promoter sequence of *E. coli* (10, 33). The *Synechococcus* strain PCC 7002 *recA* promoter does not resemble the promoters for a wide variety of genes, including *cpcB*, *apcA*, *psaA*, *psaC*, *psaE*, *apcE*, *cpcG*, and *psbA1*, which encode components of the photosynthetic apparatus in this cyanobacterium (G. E. Gasparich and D. A. Bryant, unpublished results). We presently have no evidence to suggest that transcription of the *Synechococcus* strain PCC 7002 *recA* gene is enhanced following heat shock, but such experiments can now be attempted. It is important to note that heat shock proteins are constitutively expressed at low levels in *E. coli* but are hyperinduced in response to several stress signals, including heat shock (33). A heat shock response has been well documented in the cyanobacterium *Synechococcus* strain PCC 6301 (5), but the identities of proteins whose expression is enhanced by heat shock remains unknown. The more important aspect of these results is that the *recA* promoter appears to belong to a potentially minor and distinctive class in *Synechococcus* strain PCC 7002. Although multiple RNA polymerase sigma factors have not yet been suggested to occur in nonheterocystous cyanobacteria, the likelihood that multiple sigma factors should occur is quite high, based upon analogies with other procaryotes (17, 18, 45). Consistent with this possibility, preliminary hybridization experiments with the *rpoD* (σ^{43}) gene of *Bacillus subtillis* produced multiple (2 to 4) hybridization signals with DNAs from a wide variety of cyanobacteria, including *Synechococcus* strain PCC 7002 (D. A. Bryant, unpublished results).

The *recA* gene product is required for homologous recombination in *E. coli*. The ability of Rec^- *E. coli* strains to maintain stable merodiploids has rendered these mutants especially useful for genetic analysis and general cloning purposes. It is considered that elimination of homologous recombination function in the cyanobacterial species should be similarly advantageous for use of these organisms as model systems for genetic study. However, our attempts to produce a *recA Synechococcus* strain PCC 7002 mutant by introducing as insertionally inactivated *recA* allele into the cells did not prove successful. Mutagenesis was performed by transforming *Synechococcus* strain PCC 7002 with gel-purified recombinant DNA fragments in which the *aph* gene interrupted the cyanobacterial *recA* gene sequence. Subsequent Southern hybridization analysis demonstrated that in every kanamycin-resistant transformant examined, the mutant allele had recombined with the chromosome and replaced a wild-type *recA* gene. However, despite exhaustive subculturing in the presence of kanamycin, both Southern analysis and the instability of the kanamycin marker indicated that a wild-type *recA* gene also remained in the cells. Identical results were obtained for a deletion construction when the *Bam*HI fragment of plasmid pAQPR75 (see Fig. 5) was replaced in either orientation by the *aph* gene (data not shown; Murphy, Ph.D. dissertation).

Several investigators have found that attempts to muta-

genize certain cyanobacterial genes via homologous recombination results in displacement of the proper gene but that the cells also maintain a wild-type copy of the gene. *Synechococcus* and *Synechocystis* sp. strains carrying mutagenized genes encoding photosystem I (*psaA* and *psaB*; J. Zhou and D. A. Bryant, unpublished results) and II (*psbA*; 13) apoproteins, ferredoxin I (47), thioredoxin (31), and ribulose 1,5-bisphosphate carboxylase protein (John Pierce, E. I. Du Pont de Nemours, personal communication) have also been observed to maintain copies of the wild-type gene. Failure of these alleles to segregate has been interpreted as an indication that cells lacking the functional gene products are not viable.

A number of observations reported here lend support to the conclusion that *recA* is required for viability of *Synechococcus* strain PCC 7002 cells. All colonies formed from transformant cells harboring the diploid *recA* alleles appeared to demonstrate lethal sectoring, indicating that segregation of the alleles resulted in nonviable cells. The fact that segregation of the mutant and wild-type alleles readily occurred following introduction of the *E. coli recA* gene into the diploid cells demonstrates that the *E. coli recA* gene product was able to provide a function that allowed for growth of the cyanobacterial cells. Moreover, the observed maintenance of ampicillin resistance in these cells presumably reflects selection for the *E. coli recA* gene located on the shuttle plasmid pAQE5/*recA*. Although resistance to ampicillin is rather quickly lost from pAQE5-transformed *Synechococcus* strain PCC 7002 cells not maintained under ampicillin selection, ampicillin resistance was found to be completely stable in the pAQE5/*recA*-transformed cells not harboring a wild-type cyanobacterial *recA* gene (Table 2). Finally, despite the variety of growth regimens to which the diploid *recA* cells were subjected, segregation of the mutant and wild-type *Synechococcus* strain PCC 7002 *recA* alleles was never observed. Particularly interesting were the results obtained when liquid cultures were subjected to regimens inducing slow growth. Because the number of chromosomes carried by cyanobacteria is directly related to cell growth rate (19), segregation pressures should increase under slow-growth conditions. This should result in an increased percentage of cells carrying only one *recA* allele. Under normal exponential-growth conditions, the wild-type and transformed *Synechococcus* strain PCC 7002 cells demonstrated no obvious difference in growth rates. However, when both were placed under identical slow-growth liquid culture conditions, those cells harboring the diploid *recA* alleles experienced greatly reduced growth rates compared with wild-type cells. Plating of the former revealed that the majority of these cells did not form colonies either with or without kanamycin selection. This suggests that cells that had lost a functional *recA* gene were no longer viable. Many microcolonies resulting from approximately three to six cell divisions were also observed on the nonselective plates. The relatively large number of such colonies arising from slow-growing liquid cultures suggests they may be the product of recently segregated cells. Thus, the inability of these cells to maintain growth may be due to the dilution of intracellular RecA protein to levels below that necessary for cell viability.

The required function the *recA* gene product apparently provides in *Synechococcus* strain PCC 7002 is not known. It has been observed that growing cultures of *recA E. coli* strains contain a significant fraction of nondividing cells (8). The nonviable cells may result from the accumulated effects of routine genetic damage that is normally repaired by *recA* protein. It is possible that *Synechococcus* strain PCC 7002 cells rely more heavily upon the DNA repair activity of RecA protein than do other procaryotes for which *recA* mutants have been obtained. The cyanobacterium may lack secondary repair systems or more readily sustain genetic damage during growth due to its phototrophic growth mode. It is also possible that the cyanobacterium lacks the equivalent of the *rdgA*, *rdgB*, or *sdrT* genes that *recA E. coli* cells require for viability (9, 12, 27). The function of the *rdgB* gene product in *E. coli* is not understood. In *recA rdgA E. coli* cells, transcription appears to be blocked, while in *sdrT E. coli* strains, RecA function is required for initiation of DNA synthesis. Perhaps the cyanobacterial RecA protein is more intimately involved with DNA synthesis in *Synechococcus* strain PCC 7002. On the other hand, it is known that RecA is required for repair of double-strand breaks in *E. coli* (25). These lesions may occur more frequently in the cyanobacteria, either as a result of photodamage or as the result of an enzymatic system regulating chromosomal rearrangements.

Future investigations into the role played by the *Synechococcus* strain PCC 7002 *recA* gene product should be aided by the complementation ability demonstrated by the *E. coli* RecA protein. Although chromosomal transformation studies indicate that the level of recombination activity is somewhat reduced in cyanobacterial cells harboring pAQE5/*recA* but no wild-type *Synechococcus* sp. *recA* gene (Table 3), it seems doubtful that these cells would allow for meaningful merodiploid analysis except under the most transitory assay conditions. More promising, perhaps, is the possibility of introducing various *E. coli* mutant *recA* alleles into the cyanobacterium. In *E. coli*, the RecA protein does not act only as a recombinase but the protein also acts to effect the cleavage of certain repressor proteins. If the requirement of *Synechococcus* strain PCC 7002 for RecA is not due to the recombinase function per se, then a cyanobacterium deficient in recombination activity might be created by replacement of the host *recA* gene with a recombinase-deficient *E. coli recA* allele.

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