Molecular Cloning and Characterization of the *recA* Gene from the Cyanobacterium *Synechococcus* sp. Strain PCC 7002†

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The *recA* gene of *Synechococcus* sp. strain PCC 7002 was detected and cloned from a λ gtw10 genomic library by heterologous hybridization by using a gene-internal fragment of the *Escherichia coli* *recA* gene as the probe. The gene encodes a 38-kilodalton polypeptide which is antigenically related to the RecA protein of *E. coli*. The nucleotide sequence of a portion of the gene was determined. The translation of this region was 55% homologous to the *E. coli* protein; allowances for conservative amino acid replacements yield a homology value of about 74%. The cyanobacterial *recA* gene product was proficient in restoring homologous recombination and partial resistance to UV irradiation to *recA* mutants of *E. coli*. Heterologous hybridization experiments, in which the *Synechococcus* sp. strain PCC 7002 *recA* gene was used as the probe, indicate that a homologous gene is probably present in all cyanobacterial strains.

Although genetic studies of general recombination in a number of organisms have led to the enumeration of a variety of mechanistic possibilities, much of the biochemistry of that process and of the relevant proteins remains a mystery. The RecA protein of *Escherichia coli* is the most notable exception to this general lack of biochemical information about recombination proteins. The role of the RecA protein in general recombination has been carefully defined by genetic analysis, and the biochemistry of how it participates in the process of recombination has been characterized in detail. Its abilities to bind DNA, form synapses among a variety of combinations of DNA molecules, promote the assimilation of a single strand of DNA into a DNA duplex, and mediate polarized branch migration have all been demonstrated in vitro (38). Its regulatory and direct biochemical roles in DNA repair have also been examined in considerable detail (23, 31, 47).

The importance of this protein in both general recombination and DNA repair has made it a subject of general interest, and many investigators have looked for analogous genes or proteins in other systems. RecA-like proteins or RecA-like activities have been described in several other species of gram-negative bacteria, including *Salmonella typhimurium*, *Proteus vulgaris*, *Proteus mirabilis*, *Shigella flexneri*, *Neisseria gonorrhoeae*, *Rhizobium meliloti*, *Erwinia carotovora*, *Pseudomonas aeruginosa*, *Rhodobacter capsulatus*, *Vibrio cholerae*, *Methylophilus methylotrophus*, and *Agrobacterium tumefaciens* (2, 10-12, 15, 17, 19, 21, 22, 28). RecA-like proteins or activities have also been characterized in the gram-positive bacteria *Bacillus subtilis* (24) and *Streptococcus faecalis* (50). In cases which have been examined in detail, the RecA-like protein has been shown to be immunologically related to the *E. coli* prototype (17, 19, 24). Reports concerning DNA sequence homology to the *E. coli* *recA* gene have been much more varied. The results of hybridization analyses done with sequences isolated from diverse bacteria suggest that these sequences have diverged

considerably from that of the *E. coli* *recA* gene. Other studies have suggested that some DNA sequence homology exists (11, 17, 19, 28).

There are a number of reasons why the cyanobacteria have recently become an increasingly popular model system for the genetic dissection of structural and functional aspects of the photosynthetic apparatus. Of primary importance is the fact that the photosynthetic apparatus of the cyanobacteria is highly homologous to that found in the chloroplasts of higher plants (2a). Of essentially equal importance is the rapid rate at which workable genetic systems are being developed for several cyanobacteria. These systems take advantage of the ability of a number of cyanobacteria to undergo DNA-mediated transformation (35) or to serve as recipients in conjugation involving *E. coli* donor cells (49). Although the ability to use plasmid vectors in the production of merodiploids already exists in several of the cyanobacteria, meaningful genetic analyses with such merodiploids is severely limited by the high level of recombination events that occur. In one study with *Synechococcus* sp. strain PCC 7942 (also called *Anacystis nidulans* R2), it was observed that the genomic DNA carried by the plasmid vector had a pronounced tendency to recombine with the homologous region on the genome (45). High levels of apparent gene conversion have also been observed in plasmid-based heterozygous merodiploids of *Synechococcus* sp. strain PCC 7002 (36).

Although mutations in many of the *rec* genes of *E. coli* produce significant reductions in the observed levels of general recombination, null alleles of the *recA* gene have the distinctive phenotype of essentially eliminating this process (8, 26, 38). This ability of a *recA* mutation to prevent totally general recombination makes the development of similar mutations in other organisms highly desirable, if not essential, when genetic analysis involving heterozygous merodiploids is to be done in those organisms. We have therefore sought to identify a *recA*-like gene in *Synechococcus* sp. strain PCC 7002 by heterologous hybridization so that we might ultimately attempt the production of Rec⁻ cyanobacterial mutants in which heterozygous merodiploids would be stable.

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TABLE 1. *E. coli* strains used in this study

Strain	Relevant genotype	Reference or source
AB1157	F ⁻ <i>recA</i> ⁺ <i>rpsL31</i>	1
AB2463	F ⁻ <i>recA13 rpsL31 (recA13 AB1157)</i>	1
RDP145	F ⁻ <i>recA13</i>	6
RDP211	F128 <i>lacZΔM15 lacI^a (proA⁺ proB⁺) traD36 (lac-pro)XIII hsdR sbcB15 endA</i>	33
RDP236	F421 <i>lacZ118 lacI3/lacZ813 lacI3 Δ(srl-recA)306</i>	51
RDP256	F ⁻ <i>sfiA lexA3 recA13</i>	Lab stock
RDP257	F ⁻ <i>Δ(srl-recA)306 λ⁻</i>	Lab stock
RDP258	<i>recA</i> ⁺ derivative of RDP145	Lab stock
RDP259	Hfr Cavalli <i>zji::Tn10</i>	Lab stock

This report describes the cloning and characterization of a cyanobacterial gene which encodes a protein that is structurally homologous to the *E. coli* RecA protein. When the protein from this cyanobacterial gene is expressed in *recA* strains of *E. coli*, it functions in general recombination and also appears to contribute to cellular resistance to UV irradiation.

(A preliminary account of some of these results was presented at the VIIth International Congress on Photosynthesis [30].)

MATERIALS AND METHODS

Materials. Restriction endonucleases, T4 DNA ligase, DNA polymerase I, and the Klenow fragment of DNA polymerase were purchased from Bethesda Research Laboratories (Gaithersburg, Md.). [α -³²P]dATP and [³⁵S]methionine were purchased from New England Nuclear Corp. (Boston, Mass.). Purified *EcoRI*-digested arms of bacteriophage λ vector gt10 were purchased from Amersham Corp. (Arlington Heights, Ill.). Packaging extracts were purchased from Promega Biotec (Madison, Wis.). Antibiotics were purchased from Sigma Chemical Co. (St. Louis, Mo.). Nitrocellulose was purchased from Schleicher & Schuell, Inc. (Keene, N.H.).

Strains. The relevant genotypes of the *E. coli* strains used in this study are presented in Table 1. *E. coli* strains were routinely grown in liquid culture or on plates consisting of LB medium (29) at 37°C unless otherwise stated. Ampicillin (Na⁺ salt) concentrations were 50 μ g/ml for solid medium and 100 μ g/ml for liquid medium. Streptomycin and tetracycline concentrations for solid media were 100 and 10 μ g/ml, respectively. Cyanobacterial strain *Synechococcus* sp. strain PCC 7002 was grown in medium A as described previously (44). All other cyanobacterial strains were obtained from the Pasteur Culture Collection and were grown in medium BG-11 as described previously (39).

DNA isolation and genomic library construction. Total genomic DNA *Synechococcus* sp. strain PCC 7002 was prepared as previously described (9). For the construction of the λ gt10 library of *Synechococcus* sp. strain PCC 7002, total genomic DNA was digested with endonuclease *EcoRI*, mixed at a ratio of 4:1 (wt/wt) with purified *EcoRI*-digested arms of λ gt10, ligated overnight at 14°C with T4 DNA ligase, and packaged in vitro. The titer of the recombinant library thus generated was approximately 10⁶ PFU/ μ g of DNA packaged.

Plasmid DNA preparation cloning, hybridization, and other manipulations. Large-scale plasmid extraction and purification

were done as previously described (42). DNA fragment isolation and purification were done by agarose gel electrophoresis in a Tris-borate buffer (90 mM Tris, 2.7 mM disodium EDTA, 86 mM boric acid, pH 8.2), followed by electroelution of the DNA into hydroxylapatite. DNA ligations, plasmid transformations, nick translations, and Southern (43) and plaque hybridizations were done by using standard procedures (27). All hybridizations included at least a 6-h prehybridization in 6 $\times$ SET (1 $\times$ SET is 150 mM NaCl, 30 mM Tris, 1 mM EDTA, pH 8.0)–5 $\times$ Denhardt solution (27)–0.5% sodium dodecyl sulfate (18). No carrier DNA or RNA was used in the prehybridization or hybridization solutions. Hybridized nitrocellulose filters were washed four times for at least 30 min per wash at room temperature in 6 $\times$ SET. Fluorography was done at –70°C with Kodak X-Omat AR film (Eastman Kodak Co., Rochester, N.Y.).

DNA sequence analysis. A 1,200-base-pair (bp) *AluI* fragment from pAQPR75 was subcloned into the *HincII* site of the multiple cloning site of the plasmid vector pIBI21 (International Biotechnologies, Inc., New Haven, Conn.) to generate plasmid pAQPR77. Single-stranded template DNA was produced by superinfection with phage M13K07 as described in a protocol provided by International Biotechnologies. Nucleotide sequence analysis was done by the chain termination (dideoxy) method by using the M13 reverse sequencing primer as previously described (4, 9).

Merodiploid recombination assay. Lac⁻ colonies of *E. coli* RDP236, with or without the various recombination plasmids used, were selected from lactose MacConkey agar plates and grown overnight in a rotating incubator in 5.0 ml of LB medium. From these cultures, 0.1 ml was transferred to 5.0 ml of a glycerol-based, supplemented minimal medium (34, 37). This culture was allowed to grow overnight and was used to inoculate a second glycerol-based minimal medium overnight culture. Platings from both minimal medium cultures were made on LB agar for determination of CFU and on minimal lactose agar medium to determine Lac⁺ recombinants. Similar experiments, in which LB liquid medium replaced the glycerol-based minimal medium, produced results identical to those reported in Table 2.

Hfr conjugation assays. The donor strain RDP259 was grown to approximately 10⁸ cells per ml in LB medium, and 0.5 ml of this culture was immediately added to 2.0 ml of an LB overnight culture of the appropriate recipient strain.

TABLE 2. Homologous recombination assay for two *lacZ* ochre alleles in *E. coli*^a

Plasmid	Day	10 ⁻⁵ Lac ⁺ CFU/ml	10 ⁻⁸ CFU/ml	Lac ⁺ CFU/total CFU
None	1	1.3	8.1	0.00016
	2	1.3	3.6	0.00036
pAQPR75	1	507	6.2	0.082
	2	5,400	6.0	0.90
pAQPR76	1	36	6.8	0.0053
	2	680	9.1	0.075
pAQPR82 ^b	1	0.78	4.4	0.00018
	2	2.3	4.8	0.00048

^a Data represent the averages of three independent determinations for all values. *E. coli* RDP236 (Table 1) harboring the plasmids indicated was assayed as described in Materials and Methods.

^b Plasmid pAQPR82 is a pUC9 derivative which carries a 2.8-kilobase-pair insert encoding the *Synechococcus* sp. strain PCC 7002 *psaA* gene (A. Cantrell and D. A. Bryant, unpublished results).

Conjugation was allowed to proceed for 2 h at 37°C with gentle shaking. Platings were made on LB-streptomycin plates to determine recipient CFU and on LB-tetracycline-streptomycin plates to select for transconjugants.

UV sensitivity. Exponentially growing cells at approximately 10^8 cells per ml in LB medium were placed on ice for 10 min, harvested by centrifugation, and suspended in 10.0 ml of modified 56/2 minimal medium (25). UV irradiation of 9.0 ml of cells was done in an otherwise dark room at $0.5 \text{ J m}^{-2} \text{ s}^{-1}$ in 100-mm-diameter petri dishes with gentle agitation. Cells were then diluted and plated on LB plates to determine CFU.

Maxicell and crude extract preparations. Whole-cell extracts were prepared and polyacrylamide gel electrophoresis was done as previously described (5). Plasmid-encoded polypeptides were identified by the maxicell procedure of Sancar et al. (41). A polyclonal antiserum directed against the *E. coli* RecA protein was kindly provided by J. Roberts, Cornell University, Ithaca, N.Y. Radiolabeled polypeptides were immunoprecipitated by using this antiserum as previously described (5, 3).

RESULTS

Isolation and molecular cloning of the *recA* gene of *Synechococcus* sp. strain PCC 7002. Figure 1 shows the results obtained when a Southern (43) blot carrying restriction endonuclease digests of *Synechococcus* sp. strain PCC 7002 genomic DNA was probed with the 550-bp gene-internal *EcoRI*-*PstI* fragment of the *E. coli* *recA* gene (40). This DNA fragment corresponds to approximately one-half of the *E. coli* *recA* gene and comprises the middle portion of the gene, which is known to include the ATP-binding site (20). At 61°C under the hybridization and wash conditions used (see Materials and Methods), sequences with approximately 40% mismatched bases can be detected (D. A. Bryant and J. M. Dubbs, unpublished results). Strong unique hybridization signals were obtained for all restriction endonuclease digests tested (Fig. 1). Although weaker, minor hybridization signals were detectable when the autoradiograms were heavily overexposed, the DNA sequences corresponding to these weaker hybridization signals have not been studied further. Data obtained from the hybridization experiments (Fig. 1) were used to develop the physical map of the genomic region that hybridized to the probe (Fig. 2).

The *E. coli* *recA* probe hybridized to a unique 7.5-kilobase-pair *EcoRI* fragment (Fig. 1). A recombinant phage (λ RCM1) carrying this *EcoRI* fragment was isolated from a λ gtw10 genomic library of *Synechococcus* sp. strain PCC 7002 *EcoRI* fragments. Additional restriction endonuclease mapping of the insert DNA in λ RCM1 confirmed the genomic map shown in Fig. 2. The 2.45-kilobase-pair *BglII*-*EcoRI* fragment, which carried the *Synechococcus* sp. strain PCC 7002 sequences that hybridized to the *recA* probe, was subcloned from λ RCM1 into the *Bam*HI-*EcoRI* sites of plasmid vector pUC8 to generate plasmid pAQPR75. A detailed restriction map of the insert DNA in plasmid pAQPR75 is shown in Fig. 2. Plasmid pAQPR76 carries an insert fragment in the *Bam*HI-*EcoRI* sites of plasmid vector pUC9 which is approximately 100 bp larger than that in plasmid pAQPR75. The detailed restriction map of the insert DNA in plasmid pAQPR76 differs from that of the insert in pAQPR75 only in that it carries a 100-bp extension from the *BglII* site which is shown in Fig. 2. Plasmid pAQPR76 may have arisen from the ligation of an *EcoRI*-*BglII* partial digestion product of the λ RCM1 phage DNA used in the construction of plasmids pAQPR75 and pAQPR76.

FIG. 1. Fluorogram showing results of hybridization experiments with the 550-bp gene-internal *PstI*-*EcoRI* fragment of the *E. coli* *recA* gene as the probe. Restriction endonuclease digests of *Synechococcus* sp. strain PCC 7002 genomic DNA were electrophoresed, transferred to nitrocellulose, and probed with the nick-translated probe DNA at 61°C. Restriction endonuclease digest (lane): 1, *Bam*HI; 2, *Hind*III; 3, *Pst*I; 4, *Bgl*II; 5, *Eco*RI; 6, *Eco*RI-*Pst*I; 7, *Eco*RI-*Bgl*II; 8, *Hind*III-*Pst*I; 9, *Hind*III-*Bgl*II; 10, *Pst*I-*Bgl*II. The bars at the left show the positions of size markers (the *Hind*III fragments of phage λ), whose sizes are indicated in kilobase pairs.

Partial nucleotide sequence analysis of the *recA* gene of *Synechococcus* sp. strain PCC 7002. At present, only a portion of the nucleotide sequence of the *Synechococcus* sp. strain PCC 7002 *recA* gene has been determined. The plasmid pAQPR77, which carries a 1,200-bp *Alu*I fragment derived from pAQPR75 (Fig. 2), was partially sequenced on one strand by the chain termination method. The region sequenced is compared with the nucleotide sequence of the homologous region of the *E. coli* *recA* gene in Fig. 3. The two nucleotide sequences are 57% homologous in the region sequenced. This homology value is in good agreement with the value estimated from the heterologous hybridization conditions which were required to obtain hybridization between the two sequences (see above). The region sequenced encodes an open reading frame which is 55% homologous to the corresponding region of the *E. coli* RecA protein (residues 195 to 292) (Fig. 3). This region includes the binding site for ATP (20). Allowing for some conservative amino acid replacements, the amino acid sequence homology in this region is approximately 74%. Although stretches of considerable, even complete, homology exist between the two polypeptides, regions in which the sequences have diverged considerably also occur (Fig. 3). However, the comparisons in Fig. 3 unambiguously demonstrate the homology of the two genes and their products (also see below).

FIG. 2. Physical maps of the genomic region surrounding the *recA* gene of *Synechococcus* sp. strain PCC 7002 (top) and of the insert fragment cloned in plasmid pAQPR75 (bottom). The orientation of the gene is shown by the arrow below. Abbreviations of restriction endonucleases: H, *Hind*III; E, *Eco*RI; G, *Bgl*II; P, *Pst*I; B, *Bam*HI; V, *Pvu*II; F, *Hin*fI; A, *Alu*I. Regions exhibiting hybridization to the 550-bp gene-internal fragment of the *E. coli* *recA* gene are indicated by the thick line.

The nucleotide sequence results, in combination with the restriction map comparisons of plasmids pAQPR75 and pAQPR77, allowed us to orient the *recA* gene on the cloned fragments as shown in Fig. 2. In plasmid pAQPR75, the gene is properly juxtaposed for transcription from the *lacZ* promoter borne on plasmid pUC8; in plasmid pAQPR76, the gene is not properly juxtaposed for transcription from the

lacZ promoter borne on plasmid pUC9. The orientation of the *recA* gene relative to the *lacZ* promoter is possibly significant in considering the complementation studies done in *E. coli* as described below.

Functional assays of the cyanobacterial *recA* gene in recombination-deficient *E. coli* strains. One characteristic property of the *E. coli* *recA* gene product is its essential role in

COMPARISON OF THE *SYNECHOCOCCUS* PCC 7002 AND *E. COLI* *recA* NUCLEOTIDE SEQUENCES.

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7002:  ATTCGCCAAAAAATTGGCATTAGTTACGGCAATCCAGAGGTAACCAACCGGGGAACCGCCCTCAAATTTTATGCCTCT
E. coli:  C TATG      TG G TG T  T C G AACC T  T T A G G C C

7002:  GTGCGCTAGATATCCGCCGATCCAAACCTCAAGAAAGGAGCGAAGGGAATTTGCATCCGCGCCAAGGTTAAAGTG
E. coli:  T T C C    T T  GGCG GG G AG G C AA C TGG GG T G GAAA CGC G

7002:  GCGAAAAATAAGTTGCTCCACCGTTCGCCATTGCCGAATTTGACATTATTTTGGCAAAGGAATCTCCGTGTGGGC
E. coli:  T G C A C G G  TAAACAG T T CC G CC C AC T G  T AA TTCTAC

7002:  TGCATGCTAGACTCGCGGAACAAACAGTGTCTTACGCCGCAAGGCGCTTGGTACAGC
E. coli:  GAAC G T  CT G CGTA  G AAG TGAT  AGAAAGC  G

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COMPARISON OF THE *SYNECHOCOCCUS* PCC 7002 AND *E. COLI* *RecA* AMINO ACID SEQUENCES.

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7002:  Ile Arg Gln Lys Ile Gly Ile Ser Tyr Gly Asn Pro Glu Val Thr Thr Gly Gly Thr
E. coli:  Met      Val Met Phe      Thr

7002:  Ala Leu Lys Phe Tyr Ala Ser Val Arg Leu Asp Ile Arg Arg Ile Gln Thr Leu Lys
E. coli:  Gly Ala Val

7002:  Lys Gly Ala Lys Gly Ile Cys Ile Arg Ala Lys Val Lys Val Ala Lys Asn Lys Val
E. coli:  Glu  Glu Asn Val Val Gly Ser Glu Thr Arg      Val

7002:  Ala Pro Pro Phe Arg Ile Ala Glu Phe Asp Ile Ile Phe Gly Lys Gly Ile Ser Arg
E. coli:  Ala      Lys Gln      Leu Tyr      Glu      Asn Phe

7002:  Val Gly Cys Met Leu Asp Ser Arg Asn Lys Gln Cys His Tyr Ala Arg Lys Gly Ala
E. coli:  Tyr  Glu Leu Val  Leu Gly Val  Glu Lys Leu Ile Glu Lys Ala

7002:  Trp Tyr Ser
E. coli:

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FIG. 3. Comparison of portions of the nucleotide sequences and the deduced amino acid sequences of the *E. coli* and *Synechococcus* sp. strain PCC 7002 *recA* genes. The partial nucleotide sequence shown for the *Synechococcus* sp. strain PCC 7002 *recA* gene was determined for a region of the 1,200-bp *Alu*I insert fragment in plasmid pAQPR77 (Fig. 2). The two sequences were aligned by using a computer program written by one of us (R.D.P.). The regions of the *E. coli* sequences shown correspond to nucleotides 634 to 927 and amino acids 195 to 292 in the sequences determined by Sancar et al. (40). This region includes the ATP-binding domain of the *E. coli* RecA protein (20). Omission of a base or amino acid in the *E. coli* sequences indicates that the identical base or amino acid occurs at that position in the *Synechococcus* sp. strain PCC 7002 sequences.

recombination between homologous DNA sequences. To test the cyanobacterial nucleotide sequences carried by pAQPR75 and pAQPR76 for the ability to produce a functional RecA-like protein, various *recA* *E. coli* mutants were transformed with these plasmids, and the resulting strains were tested for evidence of recombination proficiency by two different assays.

E. coli RDP236 (Table 1) carries a deletion of the *recA* gene as well as two different *lacZ* alleles. The *lacZ*813(Oc) allele is located on the chromosome, and the *lacZ*118(Oc) allele is located on the F42 *lac* plasmid. The nonsense polypeptides produced by these two ochre alleles fail to produce a functional β -galactosidase by intracistronic complementation (37), so this strain exhibits a Lac⁻ phenotype. To examine the ability of the cyanobacterial *recA* gene to complement homologous recombination activity, Lac⁻ colonies of RDP236 harboring either plasmid pAQPR75 or pAQPR76 were selected and serially subcultured twice overnight under nonselective conditions in liquid medium. After each subculturing, platings were done to determine Lac⁺ CFU and total CFU. The results of these experiments are shown in Table 2. The results shown indicate clearly that the presence of plasmid pAQPR75 or pAQPR76 is accompanied by greatly increased levels of homologous recombination as reflected by the greatly increased ratio of Lac⁺ CFU to total CFU.

RDP236 cells harboring either no recombinant plasmid or the control plasmid pAQPR82 (a pUC9 derivative carrying an insert fragment encoding the *psaA* gene of *Synechococcus* sp. strain PCC 7002; A. Cantrell and D. A. Bryant, unpublished results) exhibit background levels of Lac⁺ cells. This background of Lac⁺ cells in a *recA* deletion strain presumably arises from either *lacZ* mutation reversion or from *recA*-independent recombination. In striking contrast, 8.2% of the cells harboring plasmid pAQPR75 demonstrated a Lac⁺ phenotype after one subculturing, and 90% of the cells harboring this plasmid demonstrated a Lac⁺ phenotype after two subculturings. The results obtained with plasmid pAQPR75 represent an approximately 2,000-fold increase above the control levels. Cells harboring plasmid pAQPR76 were less effective at recombining the two alleles. Nonetheless, values 170-fold above control levels were obtained after the second day of subculturing.

A second complementation assay tested the ability of the cyanobacterial *recA* gene to complement the recombination function of a *recA*-deficient recipient cell in an Hfr conjugation. In these experiments, quantitative liquid matings were done between the streptomycin-sensitive Hfr Cavalli strain RDP259 and the streptomycin-resistant, *recA* recipient mutant AB2463 harboring either plasmid pAQPR75 or pAQPR76 (Table 1). Strain RDP259 carries a Tn10 insertion (conferring tetracycline resistance) near the *thr* operon; transconjugants were therefore selectable as Str^r Tc^r colonies. The results of these experiments are shown in Table 3.

Transconjugant levels were, on average, 400-fold higher for recipient cells harboring plasmid pAQPR76 than for cells harboring no plasmid at all or a pUC9 control plasmid. The apparently high level of Tc^r transconjugants observed in the control conjugations is most likely the result of *recA*-independent Tn10 transposition in the zygotes. Introduction of the pAQPR75 plasmid into AB2463 resulted in a decrease in the viability of the cells, and repeated attempts to assay the effects of this plasmid in the conjugation assay failed. In this context, it should be noted that the cyanobacterial *recA* gene causes all strains of *E. coli* examined thus far to filament to some extent. Filamentation is more severe in

TABLE 3. Homologous recombination assay in *E. coli* after Hfr conjugation^a

Strain	Plasmid	Tc ^r transconjugants/ 10 ⁷ recipient CFU
AB1157	None	15,000
AB2463	None	7.9
AB2463	pAQPR82 ^b	1.1
AB2463	pAQPR76	320

^a Results are the averages of four independent experiments. *E. coli* RDP259 (Table 1) was the donor to the recipient strains listed.

^b Plasmid pAQPR82 is a pUC9 derivative which carries a 2.8-kilobase-pair insert encoding the *Synechococcus* sp. strain PCC 7002 *psaA* gene (A. Cantrell and D. A. Bryant, unpublished results).

strains harboring pAQPR75 than in strains harboring pAQPR76. Filamentation is still observed in an *sfiA* strain (RDP256), an observation which suggests that the filamentation phenotype is at least not entirely caused by the effects of the cyanobacterial *recA* gene product on the SOS system of *E. coli* (47).

Another recognized activity of the *E. coli* *recA* gene product is its ability to confer resistance to the lethal effects of UV irradiation. *E. coli* RDP145, which carries the *recA*13 allele, was transformed with plasmids pAQPR75 and pAQPR76. These cells and appropriate control cells were irradiated with UV light for various periods and assayed for survival by plating for CFU. The results of these experiments are shown in Fig. 4. The introduction of either plasmid into strain RDP145 greatly increases the ability of these cells to survive the effects of UV irradiation. Again, the results are most striking for the cells harboring plasmid pAQPR75, as these cells demonstrate UV-survival characteristics comparable to those of *recA*⁺ *E. coli* control cells. Cells harboring plasmid pAQPR76, although somewhat less UV resistant than the *recA*⁺ control cells, were nonetheless more than 1,000-fold more UV resistant than the *recA* mutant control cells.

Expression and immunological studies on the RecA protein of *Synechococcus* sp. strain PCC 7002. The complementation assays described above indicated that the *Synechococcus* sp. strain PCC 7002 *recA* gene was being expressed in *E. coli*; additionally, the complementation assays suggested that the expression was greater in cells harboring plasmid pAQPR75 than in cells harboring plasmid pAQPR76. When whole-cell extracts of *E. coli* harboring plasmid pAQPR75 were subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, a prominent 38-kilodalton (kDa) polypeptide was observed which did not occur in control cells (Fig. 5A, compare lanes 1 and 2 with lane 3). This 38-kDa polypeptide was likewise not detectable in extracts of cells harboring plasmid pAQPR76 (Fig. 5A, compare lanes 3 and 4). These results are consistent with the idea that the cells harboring plasmid pAQPR75 are expressing the *Synechococcus* sp. strain PCC 7002 gene at a higher level than are cells harboring plasmid pAQPR76.

Specific labeling of plasmid-encoded proteins from *E. coli* cells harboring plasmids pUC9, pAQPR75, and pAQPR76 was obtained by the maxicell procedure of Sancar et al. (41). Although this procedure normally requires UV-sensitive, *recA* mutant cells, specific labeling of plasmid-encoded proteins (see Fig. 5B) in cells harboring plasmids pAQPR75 and pAQPR76 was apparently achieved by increasing the UV dose used on these cells to 15 J/m² (normal treatment for strain RDP145 requires 5 J/m²). Identical results were obtained with plasmids pAQPR75 and pAQPR76. Each plasmid

FIG. 4. UV survival of *E. coli* strains RDP258, RDP145 (pAQPR75), RDP145(pAQPR76), and RDP145(pUC9). The relevant genetic background information for the *E. coli* strains is shown in Table 1. Plasmids pAQPR75 and pAQPR76 are pUC8 and pUC9 derivatives, respectively, encoding the *Synechococcus* sp. strain PCC 7002 *recA* gene (see the text). The UV fluence was $0.5 \text{ J m}^{-2} \text{ s}^{-1}$.

directed the synthesis of three polypeptides, with apparent molecular masses of 38, 21, and 20 kDa, in addition to the β -lactamase polypeptides detectable in the control cells harboring plasmid pUC9 (Fig. 5B, lanes 1 to 3). Also shown in Fig. 5B are the results obtained by immunoprecipitating the specifically labeled, plasmid-encoded proteins with a polyclonal antiserum directed against the *E. coli* RecA protein (Fig. 5B, lanes 4 to 6). The 38-kDa polypeptide produced in cells harboring plasmids encoding the cyanobacteria *recA* gene is immunoprecipitated by the antiserum to the *E. coli* RecA protein. In addition, a 31-kDa polypeptide, originally masked by its comigration with the vector-encoded β -lactamase polypeptide, was also immunoprecipitated. The 31-kDa polypeptide is probably a proteolytic product of the 38-kDa polypeptide, as it is known that RecA proteins from several species are unstable and sensitive to proteolytic degradation (15, 19, 24). The 20- and 21-kDa polypeptides were not precipitated by the *E. coli* RecA antiserum, however. These polypeptides could originate from other sequences borne on the cyanobacterial DNA

fragment cloned. The results of these experiments indicate that the cyanobacterial *recA* gene product is antigenically related to the *recA* gene product of *E. coli*, a result that is consistent with the results shown in Fig. 3.

Distribution of sequences homologous to the *Synechococcus* sp. strain PCC 7002 *recA* gene in cyanobacteria. Hybridization analyses of nitrocellulose filters carrying restriction endonuclease digestion products of genomic DNAs from a variety of phylogenetically diverse cyanobacteria revealed that all strains tested harbor sequences homologous to the *recA* gene of *Synechococcus* sp. strain PCC 7002. For these studies, the 1,200-bp *AluI* fragment, which by sequence analysis and analogy to the *E. coli* *recA* gene should be largely coding sequence (Fig. 2 and 3), was used as the hybridization probe. Strong hybridization signals were observed under moderately stringent conditions for all cyanobacterial strains tested, which included many strains frequently used in physiological and genetic analyses. Unique hybridization signals resulted for most strains and restriction enzymes employed: *Synechococcus* sp. strains PCC 6301

FIG. 5. Expression studies with *E. coli* cells harboring plasmids pAQPR75 and pAQPR76, which encode the *Synechococcus* sp. strain PCC 7002 *recA* gene. (A) Coomassie blue-stained polypeptides of whole-cell extracts separated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Whole-cell extracts were prepared from *E. coli* AB2463 (lane 1); strain AB2463 (pAQPR82) (lane 2; pAQPR82 is a pUC9 derivative carrying an insert encoding the *psaA* gene of *Synechococcus* sp. strain PCC 7002; A. Cantrell and D. A. Bryant, unpublished results); strain AB2463(pAQPR75) (lane 3); and strain AB2463(pAQPR76) (lane 4). The arrow indicates the 38-kDa polypeptide, whose production is apparently directed by plasmid pAQPR75. The bars to the right indicate the relative mobilities of standard proteins, whose molecular masses are indicated in kilodaltons. (B) Fluorogram of [^{35}S]methionine-labeled, plasmid-encoded proteins of *E. coli* cells subjected to the maxicell procedure of Sancar et al. (41). Plasmid-encoded proteins from whole-cell extracts of *E. coli* RDP145 harboring plasmids pUC9 (lane 1), pAQPR75 (lane 2), and pAQPR76 (lane 3). Whole-cell extracts were prepared as described previously (5) and subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. For the samples in lanes 4 to 6, the whole-cell extracts for each plasmid-harboring strain subjected to the maxicell procedure (the extracts electrophoresed in lanes 1 to 3, respectively) were mixed with a polyclonal antiserum directed against the *E. coli* RecA protein. The resulting immunoprecipitates were collected on formalinized *Staphylococcus aureus* cells, solubilized, and electrophoresed. Immunoprecipitated proteins from cell extracts of *E. coli* RDP145 harboring plasmids pUC9 (lane 4), pAQPR75 (lane 5), and pAQPR76 (lane 6) are shown.

(also called *A. nidulans*) and PCC 7942 (also called *A. nidulans* R2); *Synechocystis* sp. strains PCC 6701 and PCC 6803; *Pseudanabaena* sp. strains PCC 6901, PCC 7409, PCC 7429, and PCC 7955; *Anabaena* sp. strain PCC 7120; and *Calothrix* sp. strain PCC 7601 (also called *Fremyella diplosiphon*) (Fig. 6). For *Calothrix* sp. strain PCC 7601, two fragments exhibited hybridization after digestion of genomic DNA with either *Hind*III or *Eco*RI endonucleases (Fig. 6, lanes 17 and 18). Multiple hybridization signals were also detected for *Nostoc* sp. strain PCC 8009 (also called *Nostoc* sp. strain MAC; results not shown). It should be noted that no additional sequences in *Synechococcus* sp. strain PCC 7002 exhibited hybridization to the *recA* sequence used as a probe (Fig. 6, lane 10). This result suggests that the *recA* gene described in this report is a unique gene and that closely related sequences do not occur in this cyanobacterium.

DISCUSSION

We report here the isolation and characterization of the *recA* gene from *Synechococcus* sp. strain PCC 7002. This gene was initially identified and subsequently cloned by low-stringency heterologous hybridization that used a gene-internal fragment of the *E. coli recA* gene as a probe. Although conflicting reports exist in the literature concerning the detectability of diverse *recA*-like genes by heterologous hybridization (19), we had no trouble specifically detecting the gene under the hybridization conditions described. It is true, however, that the relatively low percentage of nucleotide sequence homology (57%) observed between the *recA* gene of *E. coli* and its analog from *Synechococcus* sp. strain PCC 7002 would not be detectable by the hybridization protocols that have been used in many published reports.

It is now well established that *recA* genes, similar to that of *E. coli* in both structure and function, are widely distributed among both gram-negative and gram-positive procaryotes (2, 10, 11, 15, 17, 19, 22, 24, 28). Our results extend these observations to the oxygenic photoautotrophic cyanobacteria. The results reported here suggest that perhaps all members of this large, diverse assemblage have homologs of the *Synechococcus* sp. strain PCC 7002 *recA* gene and, by extension, have homologs of the *E. coli* gene.

The *Synechococcus* sp. strain PCC 7002 *recA* gene apparently encodes a polypeptide of 38 kDa which shares both antigenic relatedness and considerable amino acid sequence homology with the *E. coli* RecA protein. The expression of the cyanobacterial protein was detected at relatively high levels in *E. coli*, and this expression occurred regardless of the orientation of the gene relative to the *lacZ* promoter carried by the vectors used. Although this does not unequivocally demonstrate that the promoter for this gene is functional in *E. coli*, these results are nonetheless consistent with this notion. The apparent ability of *Synechococcus* sp. strain PCC 7002 and *E. coli* to cross-utilize promoters has been suggested from results obtained in previous studies (5, 7, 36).

The protein product of the cyanobacteria *recA* gene was capable of at least partially replacing the *E. coli* RecA protein with regard to its role in general recombination as measured by two different assays. Although AB2463 containing pAQPR76 yielded Tc^r transconjugants at a level that was 50-fold lower than that observed with the Rec⁺ *E. coli* control strain AB1157, the levels are at least 40-fold higher than the background resulting from Tn10 transposition events in the Rec⁻ control recipient strains. The Hfr conjugation results are reported only for AB2463 containing

FIG. 6. Fluorogram of hybridization analyses demonstrating the presence of sequences homologous to the *Synechococcus* sp. strain PCC 7002 *recA* gene in phylogenetically diverse cyanobacterial strains. For the experiments shown, the 1,200-bp *Alu*I fragment (Fig. 2) of plasmid pAQPR77 was nick translated and used as the hybridization probe. From the sequence analysis of this fragment (Fig. 3), this fragment should encode most of the *recA* gene and some upstream sequence. Restriction endonuclease digest of genomic DNAs were electrophoresed on 0.5% agarose gels; DNA fragments were transferred to nitrocellulose filters and hybridized at 60°C with the probe DNA. Lanes: 1, *Pseudanabaena* sp. strain PCC 7409 digested with *Eco*RI; 2, *Synechocystis* sp. strain PCC 6701 digested with *Eco*RI; 3, *Pseudanabaena* sp. strain PCC 6901 digested with *Eco*RI; 4 and 5, *Pseudanabaena* sp. strain PCC 7429 digested with *Eco*RI and *Hind*III, respectively; 6 and 7, *Synechococcus* sp. strain PCC 7942 digested with *Eco*RI and *Hind*III, respectively; 8 and 9, *Pseudanabaena* sp. strain PCC 7955 digested with *Eco*RI and *Hind*III, respectively; 10, *Synechococcus* sp. strain PCC 7002 digested with *Eco*RI; 11 and 12, *Synechocystis* sp. strain PCC 6803 digested with *Eco*RI and *Hind*III, respectively; 13 and 14, *Synechococcus* sp. 6301 digested with *Eco*RI and *Hind*III, respectively; 15 and 16, *Anabaena* sp. PCC 7120 digested with *Eco*RI and *Hind*III, respectively; 17 and 18, *Calothrix* sp. PCC 7601 digested with *Eco*RI and *Hind*III, respectively. The bars indicate the positions of the *Hind*III fragments of phage λ which served as size markers, shown in kilobase pairs. The arrows (lanes 17 and 18) indicate the two pairs of fragments exhibiting hybridization of *Calothrix* sp. strain PCC 7601

pAQPR76, as the pAQPR75 derivative strain demonstrated significant viability problems in this experimental protocol.

Standard auxotrophic markers were not used in the Hfr conjugation assays because AB2463 containing either pAQPR75 or pAQPR76 gave dramatically reduced plating efficiencies on both glucose- and glycerol-based minimal media. It was possible, however, to assay the recombination of the two *lac* ochre alleles in the *lacZ* merodiploid system on minimal media, as Lac⁺ colonies of these strains exhibited high plating efficiencies on lactose-based minimal media. Very high levels of recombination were observed in these merodiploid assays in which recombination was permitted to occur during an extended period. It is also noteworthy that the merodiploid recombination assays were done in strains from which the *E. coli recA* gene had been deleted. This precaution ensures that no normally inactive polypeptide product of the *E. coli recA* gene is participating in the general

recombination via an interaction with the protein produced by the cyanobacterial *recA* gene.

The basis of the viability and minimal medium effects described above is unknown. The basis, however, may become clear as we investigate the possible interaction of the cyanobacterial RecA protein with the *E. coli* SOS system. The ability of pAQPR75 and pAQPR76 to enhance UV irradiation survival of RDP145 is quite dramatic (Fig. 4). The overexpression of the *E. coli* RecA protein in *lexA* mutants, in which a generalized induction of the SOS system cannot occur, suppresses much of the UV sensitivity of the cells (14, 46) as a result of increased recombinational repair or protection of gapped DNA by RecA protein binding. A parallel effect may be occurring when the cyanobacterial RecA protein is being expressed. It is also possible, however, that the cyanobacterial RecA protein is capable of participating in SOS induction; this possibility is under investigation.

It will be necessary to produce Rec⁻ cyanobacterial strains before biphasic plasmid transformation can be used as the basis for meaningful merodiploid analysis in these organisms. The high propensity of donor-plasmid-transforming DNA to recombine with the genome during transformation is clearly demonstrated by the high efficiency with which nonreplicating plasmids containing genomic DNA are added to the cyanobacterial genome in both *Synechococcus* sp. strain PCC 7942 (48) and *Synechococcus* sp. strain PCC 7002 (36). Even when a plasmid capable of replicating in the cyanobacterium carries the genomic DNA, recombination with the genome was shown to be preferred over plasmid establishment in one study with *Synechococcus* sp. strain PCC 7942 (45). The problem remains even after plasmid establishment, as established biphasic plasmids carrying genomic sequences continue to recombine frequently with the genome in *Synechococcus* sp. strain PCC 7002 (36).

The cloning of the *Synechococcus* sp. strain PCC 7002 *recA* gene should allow the generation of recombination-deficient strains by interposon mutagenesis, a technique in which a heterologous marker gene is inserted within the coding sequence of the gene and then transformed into the genome of the organism under study. This widely used technique has already been successfully used to produce other types of mutants in *Synechococcus* sp. strain PCC 7002 (3) and in other cyanobacteria (16, 32); experiments to produce a Rec⁻ strain by this method are under way.

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ADDENDUM

Since the initial submission of this manuscript for publication, two reports concerning *recA*-like genes in cyanobacteria have been published. C. M. Geoghegan and J. A. Houghton (J. Gen. Microbiol. 133:119–126, 1987) reported the cloning of a DNA fragment from *Gloeocapsa alpicola* which encodes a 39-kDa polypeptide and which confers increased survivability to UV irradiation and methyl methanesulfonate upon *recA* mutants of *E. coli*. G. W. Owttrim

and J. R. Coleman (p. 833–836, in J. Biggins, ed., *Progress in Photosynthesis Research*, vol. 4, 1987) reported the cloning of a DNA fragment from *Anabaena variabilis* which confers resistance to methyl methanesulfonate upon *recA* mutants of *E. coli*. In contrast to the results presented in this study, these authors report that the functional *Anabaena variabilis* DNA sequence did not hybridize to *E. coli* genomic DNA and did not exhibit sequence homology to the *E. coli recA* gene. In neither study was recombination assayed directly.

LITERATURE CITED

1. Bachmann, B. J. 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. *Bacteriol. Rev.* 36:525–557.
2. Better, M., and D. R. Helinski. 1983. Isolation and characterization of the *recA* gene of *Rhizobium meliloti*. *J. Bacteriol.* 155:311–316.
- 2a. Bryant, D. A. 1986. The cyanobacterial photosynthetic apparatus: comparisons to those of higher plants and photosynthetic bacteria. *Can. Bull. Fish. Aquat. Sci.* 214:423–500.
3. Bryant, D. A., R. de Lorimier, G. Guglielmi, V. L. Stirewalt, A. Cantrell, and S. E. Stevens, Jr. 1987. The cyanobacterial photosynthetic apparatus: a structural and functional analysis employing molecular genetics, p. 749–755. In J. Biggins (ed.), *Progress in photosynthesis research*, vol. 4. Martinus Nijhoff, Dordrecht, The Netherlands.
4. Bryant, D. A., R. de Lorimier, D. H. Lambert, J. M. Dubbs, V. L. Stirewalt, S. E. Stevens, Jr., R. D. Porter, J. Tam, and E. Jay. 1985. Molecular cloning and nucleotide sequence of the α and β subunits of allophycocyanin from the cyanobacterial genome of *Cyanophora paradoxa*. *Proc. Natl. Acad. Sci. USA* 82:3242–3246.
5. Bryant, D. A., J. M. Dubbs, P. I. Fields, R. D. Porter, and R. de Lorimier. 1985. Expression of phycobiliprotein genes in *Escherichia coli*. *FEMS Microbiol. Lett.* 29:343–349.
6. Buzby, J. S., R. D. Porter, and S. E. Stevens, Jr. 1983. Plasmid transformation in *Agmenellum quadruplicatum* PR-6: construction of biphasic plasmids and characterization of their transformation properties. *J. Bacteriol.* 154:1446–1450.
7. Buzby, J. S., R. D. Porter, and S. E. Stevens, Jr. 1985. Expression of the *Escherichia coli lacZ* gene on a plasmid vector in a cyanobacterium. *Science* 230:805–807.
8. Clark, A. J. 1973. Recombination deficient mutants of *E. coli* and other bacteria. *Annu. Rev. Genet.* 7:67–86.
9. de Lorimier, R., D. A. Bryant, R. D. Porter, W.-Y. Liu, E. Jay, and S. E. Stevens, Jr. 1984. Genes for the α and β subunits of phycocyanin. *Proc. Natl. Acad. Sci. USA* 81:7946–7950.
10. Eitner, G., B. Adler, V. A. Lanzov, and J. Hofmeister. 1982. Interspecies *recA* protein substitution in *Escherichia coli* and *Proteus mirabilis*. *Mol. Gen. Genet.* 185:481–486.
11. Finch, P. W., C. L. Brough, and P. T. Emmerson. 1986. Molecular cloning of a *recA*-like gene of *Methylophilus methylotrophus* and identification of its product. *Gene* 44:47–53.
12. Genthner, F. J., and J. D. Wall. 1984. Isolation of a recombination-deficient mutant of *Rhodopseudomonas capsulata*. *J. Bacteriol.* 160:971–975.
13. Gilman, S. C., J. J. Docherty, A. Clarke, and W. E. Rawls. 1980. Reaction patterns of herpes simplex virus type 1 and type 2 proteins with sera of patients with uterine cervical carcinoma and matched controls. *Cancer Res.* 40:4640–4647.
14. Ginsburg, H., S. H. Edmiston, J. Harper, and D. W. Mount. 1982. Isolation and characterization of an operator-constitutive mutation in the *recA* gene of *E. coli* K-12. *Mol. Gen. Genet.* 187:4–11.
15. Goldberg, I., and J. J. Mekalanos. 1986. Cloning of the *Vibrio cholerae recA* gene and construction of a *Vibrio cholerae recA* mutant. *J. Bacteriol.* 165:715–722.
16. Golden, S. S., J. Brusslan, and R. Haselkorn. 1986. Expression of a family of *psbA* genes encoding a photosystem II polypeptide in the cyanobacterium *Anacystis nidulans* R2. *EMBO J.* 5:2789–2798.
17. Hamood, A. N., G. S. Pettis, C. D. Parker, and M. A. McIntosh.

1986. Isolation and characterization of *Vibrio cholerae* *recA* gene. *J. Bacteriol.* **167**:375–378.
18. Hanahan, D., and M. Meselson. 1983. Plasmid screening at high colony density. *Methods Enzymol.* **100**:333–342.
19. Keener, S. L., K. P. McNamee, and K. McEntee. 1984. Cloning and characterization of *recA* genes from *Proteus vulgaris*, *Erwinia carotovora*, *Shigella flexneri*, and *Escherichia coli* B/r. *J. Bacteriol.* **160**:153–160.
20. Knight, K. L., and K. McEntee. 1986. Nucleotide binding by a 24-residue peptide from the RecA protein of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **83**:9289–9293.
21. Kokjohn, T. A., and R. V. Miller. 1985. Molecular cloning and characterization of the *recA* gene of *Pseudomonas aeruginosa* PAO. *J. Bacteriol.* **163**:568–572.
22. Koomey, J. M., and S. Falkow. 1987. Cloning of the *recA* gene of *Neisseria gonorrhoeae* and construction of gonococcal *recA* mutants. *J. Bacteriol.* **169**:790–795.
23. Little, J. W., and D. W. Mount. 1982. The SOS regulatory system of *Escherichia coli*. *Cell* **29**:11–22.
24. Lovett, C. M., Jr., and J. W. Roberts. 1985. Purification of a RecA protein analogue from *Bacillus subtilis*. *J. Biol. Chem.* **260**:3305–3313.
25. Low, B. 1973. Rapid mapping of conditional and auxotrophic mutations in *Escherichia coli* K-12. *J. Bacteriol.* **113**:798–812.
26. Low, K. B., and R. D. Porter. 1978. Modes of gene transfer and recombination in bacteria. *Annu. Rev. Genet.* **12**:249–287.
27. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. Miles, C. A., A. Mountain, and G. R. K. Sastry. 1986. Cloning and characterization of the *recA* gene of *Agrobacterium tumefaciens* C58. *Mol. Gen. Genet.* **204**:161–165.
29. Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. Murphy, R. C., D. A. Bryant, and R. D. Porter. 1987. Molecular cloning and preliminary characterization of a *recA* gene from the cyanobacterium *Synechococcus* PCC 7002, p. 769–772. In J. Biggins (ed.), Progress in photosynthesis research, vol. 4, Martinus Nijhoff, Dordrecht, The Netherlands.
31. Ossana, N., K. R. Peterson, and D. W. Mount. 1986. Genetics of DNA repair in bacteria. *Trends Genet.* **2**:55–58.
32. Pakrasi, H. B., J. G. K. Williams, and C. J. Arntzen. 1987. Genetically engineered cytochrome B559 mutants of the cyanobacterium *Synechocystis* 6803, p. 813–816. In J. Biggins (ed.), Progress in photosynthesis research, vol. 4, Martinus Nijhoff, Dordrecht, The Netherlands.
33. Person, S., S. C. Warner, D. J. Bzik, C. Debroy, and B. A. Fox. 1985. Expression in bacteria of gB-glycoprotein-coding sequence of herpes simplex virus type 2. *Gene* **35**:279–287.
34. Porter, R. D. 1981. Enhanced recombination between F42*lac* and λ *plac5*: dependence on F42*lac* fertility functions. *Mol. Gen. Genet.* **184**:355–358.
35. Porter, R. D. 1986. Transformation in cyanobacteria. *Crit. Rev. Microbiol.* **13**:111–132.
36. Porter, R. D., J. S. Buzby, A. Pilon, P. I. Fields, J. M. Dubbs, and S. E. Stevens, Jr. 1986. Genes from the cyanobacterium *Agmenellum quadruplicatum* isolated by complementation: characterization and production of merodiploids. *Gene* **41**:249–260.
37. Porter, R. D., T. McLaughlin, and B. Low. 1978. Transduction versus “conjugation”: evidence for multiple roles for exonuclease V in genetic recombination in *Escherichia coli*. *Cold Spring Harbor Symp. Quant. Biol.* **43**:1043–1047.
38. Radding, C. M. 1982. Homologous pairing and strand exchange in genetic recombination. *Annu. Rev. Genet.* **16**:405–437.
39. Rippka, R., J. Deruelles, J. B. Waterbury, M. Herdman, and R. Y. Stanier. 1979. Generic assignments, strain histories, and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* **111**:1–61.
40. Sancar, A., C. Stachelek, W. Konigsberg, and W. D. Rupp. 1980. Sequences of the *recA* gene and protein. *Proc. Natl. Acad. Sci. USA* **77**:2611–2615.
41. Sancar, A., R. P. Wharton, S. Saltzer, D. M. Kacinski, N. D. Clarke, and W. D. Rupp. 1981. Identification of the *uvrA* gene product. *J. Mol. Biol.* **148**:45–62.
42. Seifert, H. S., and R. D. Porter. 1984. Enhanced recombination between λ *plac5* and mini-F-*lac*: the *tra* regulon is required for recombination enhancement. *Mol. Gen. Genet.* **193**:269–274.
43. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**:503–517.
44. Stevens, S. E., Jr., and R. D. Porter. 1980. Transformation in *Agmenellum quadruplicatum*. *Proc. Natl. Acad. Sci. USA* **77**:6052–6056.
45. Tandeau de Marsac, N., W. E. Borrias, C. J. Kuhlemeier, A. M. Castets, G. A. van Arkel, and C. A. M. J. J. van den Hondel. 1982. A new approach for molecular cloning in cyanobacteria: cloning of an *Anacystis nidulans met* gene using a Tn901-induced mutant. *Gene* **20**:111–119.
46. Volkert, M. R., D. F. Spencer, and A. J. Clark. 1979. Indirect and intragenic suppression of the *lexA102* mutation in *E. coli* B/r. *Mol. Gen. Genet.* **177**:129–137.
47. Walker, G. C. 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. *Microbiol. Rev.* **48**:60–93.
48. Williams, J. G. K., and A. A. Szalay. 1983. Stable integration of foreign DNA into the chromosome of the cyanobacterium *Synechococcus* R2. *Gene* **24**:37–51.
49. Wolk, C. P., A. Vonshak, P. Kehoe, and J. Elhai. 1984. Construction of shuttle vectors capable of conjugative transfer from *Escherichia coli* to nitrogen-fixing filamentous cyanobacteria. *Proc. Natl. Acad. Sci. USA* **81**:1561–1565.
50. Yagi, Y., and D. B. Clewell. 1980. Recombination-deficient mutant of *Streptococcus faecalis*. *J. Bacteriol.* **143**:966–970.
51. Yancey, S. D., and R. D. Porter. 1985. General recombination in *Escherichia coli* K-12: in vivo role of RecBC enzyme. *J. Bacteriol.* **162**:29–34.