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Molecular Cloning of a *recA*-Like Gene from the Cyanobacterium *Anabaena variabilis*

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A *recA*-like gene isolated from the cyanobacterium *Anabaena variabilis* was cloned and partially characterized. When introduced into *Escherichia coli recA* mutants, the 7.5-kilobase-pair plasmid-borne DNA insert restored resistance to methyl methanesulfonate and UV irradiation, as well as recombination proficiency when measured by Hfr-mediated conjugation. The cyanobacterial *recA* gene restored spontaneous but not mitomycin C-induced prophage production. Restriction analysis and subcloning yielded a 1.5-kilobase-pair *Sau*3A fragment which also restored methylmethane sulfonate resistance and coded for a 38- to 40-kilodalton polypeptide when expressed in an in vitro transcription-translation system.

Genetic and biochemical studies of homologous recombination and DNA repair in *Escherichia coli* have clearly defined the central role of the *recA* protein in these processes (13, 17). The invasion of duplex DNA by single-stranded homologous DNA is stimulated in vitro by the addition of purified *recA* protein (3). Other studies support the conclusion that with the addition of ATP, this protein facilitates reciprocal strand transfer between two double helices (18).

Agents that damage DNA or interfere with DNA replication in *E. coli* result in the induction of a DNA repair process described as the SOS response. The SOS network regulates the expression of genes whose products are required for excision repair, daughter-strand gap repair, and double-strand break repair, as well as SOS processing (17). Activation of the *recA* protein by an as yet uncharacterized signal results in the induction of the SOS network. The activated *recA* protein either directly or indirectly proteolytically cleaves the *lexA* repressor, which represses the expression of genes in the SOS network, thus allowing expression of the SOS genes (17). The activated *recA* protein is also capable of proteolytic cleavage of the bacteriophage lambda repressor, resulting in the lytic growth of endogenous lambda prophage (15).

Complementation of well-defined *E. coli recA* mutations is a technique that has been recently used to obtain the *recA* gene from a number of bacterial species (7, 9, 11). Complementation has also been used to isolate genes from cyanobacteria, a unique group of oxygen-evolving, photosynthetic procaryotes (10). We have used this process to obtain a *recA*-like gene from the cyanobacterium *Anabaena variabilis*.

MATERIALS AND METHODS

Cyanobacteria, bacteria, bacteriophages, and plasmids. The cyanobacterial and bacterial strains and phages used in this study are listed in Table 1. In the text, the plasmid content of the strains is given in parentheses. Plasmid pDPL13 (6), a derivative of pBR322 that confers ampicillin resistance, was used as a vector for the construction of chromosomal libraries of *A. variabilis* DNA. These libraries were used in *recA* complementation studies.

Media. *E. coli* strains were grown in LB or in LB containing ampicillin (LBA medium) at a final concentration of 30 μg/ml (12). For conjugation experiments, bacteria were grown on M9 minimal medium supplemented with the appropriate amino and organic acids (12). Bacteria resistant to methylmethane sulfonate (MMS) were selected by growth on LBA agar (LBA medium containing 1.5% agar) supplemented with MMS at a final concentration of 0.01%. Phage titers were determined with *E. coli* C600 as the indicator strain, grown in tryptone (B) medium containing 0.4% maltose. B plates contained B medium with 1.5% agar, and B-top agar contained B medium with 0.6% agar. Phages were diluted in TM buffer (10 mM Tris hydrochloride [pH 8.0] containing 10 mM $MgCl_2$). Cyanobacteria were grown in liquid BG-11 medium (1) at 30°C with a light intensity of 200 microeinsteins $\cdot m^{-2} \cdot s^{-1}$ (400 to 700 nm) and maintained on BG-11 plates (BG-11 medium with 1.0% agar).

Cloning and DNA techniques. Cyanobacterial chromosomal DNA was isolated from cultures grown to late log phase essentially by a modification of the Marmur procedure as described by Kallas et al. (8). For the construction of genomic libraries, DNA isolated from *A. variabilis* was partially digested with *Sau*3A, ethanol precipitated, suspended in TE buffer (10 mM Tris hydrochloride [pH 8.0], 1 mM EDTA), and layered onto a 5 to 20% sucrose gradient containing 1 M NaCl and 10 mM EDTA. The gradient was centrifuged in a Beckman SW 41 rotor at 26,000 rpm for 18 h at 5°C and fractionated, and the individual fractions were analyzed for DNA content and size by electrophoresis on a 0.7% agarose gel. Fractions containing DNA fragments of approximately 5 to 7 kilobase pairs (kbp) were pooled, dialyzed against TE buffer, ethanol precipitated, and suspended in sterile H_2O at a concentration of 1 μg/μl.

The plasmid pDPL13 was digested with *Bam*HI; dephosphorylated with calf intestinal alkaline phosphatase; extracted sequentially with phenol, phenol-chloroform, and chloroform; and then ethanol precipitated. Ligations with T4 DNA ligase (Pharmacia, Inc.) with a vector/insert ratio of 1:3 (total DNA concentration, 30 μg/ml) were performed at 15°C for 12 to 18 h. Transformation of competent *E. coli* HB101 cells resulted in the isolation of 1,000 to 3,000 ampicillin-resistant colonies per ligation. A number of these libraries were produced and screened for growth on LBA-MMS agar.

UV sensitivity. Cells grown in LB to approximately 10^8/ml were centrifuged at 3,000 × *g* for 10 min and suspended in an

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TABLE 1. Bacterial strains and phage

Strain or phage	Relevant genotype	Reference or source
Strains		
A. variabilis ATCC 29413	Wild type	ATTC[a]
Anacystis nidulans R2	Wild type	N. A. Straus
E. coli HB101	*leu hsdS20 proA2 supE44 recA13 rpsL20*	12
E. coli JC10289	*leuB6 thr-1 proA2 hisG4 argE3 thi-1 supE44 rpsL31 Δ(recA-srlR) 306*	B. Bachmann
E. coli JF1754	*leu*B *his*B *rec*$^+$	J. Friesen
E. coli C600	Lambda$^-$	12
E. coli KL16-99	Hfr *recA1*	B. Bachmann
Phage		
Lambda	Wild type (isolated from *E. coli* Y10)	C. R. Fuerst

[a] ATCC, American Type Culture Collection, Rockville, Md.

equal volume of 0.9% saline. A 1-ml portion was placed in the bottom of a petri dish (diameter, 86.5 mm), diluted 10-fold with saline, and exposed to various fluences of UV light generated from a G15T8-15W germicidal lamp (General Electric Co.). Survivors were determined by plating appropriate dilutions (in saline) on LB or LBA agar and incubating them overnight at 37°C. All procedures were carried out under red light or in total darkness to prevent photoreactivation.

MMS resistance. Bacterial strains to be tested were grown to a cell density of approximately 10^8/ml in LBA medium, diluted in LBA medium, and plated on LBA agar containing the stated concentrations of MMS. Survival was determined after overnight growth at 37°C.

Bacterial conjugation. Cells to be mated were grown at 37°C in LB or LBA medium to approximately 2×10^8/ml, harvested by centrifugation, washed, and suspended in LB at a donor/recipient ratio of 1:10. Conjugation was allowed to occur for 2 h at 37°C without shaking. After this period, the cell mixtures were vigorously vortexed, serially diluted, plated on M9 minimal agar, and incubated for 36 to 48 h at 37°C. Transconjugants were selected on the basis of their acquisition of an amino acid prototrophy, while counterselection against the parental cells was by either amino acid auxotrophy or antibiotic (streptomycin or ampicillin) sensitivity.

Prophage induction. Lysogens of various bacterial strains were produced by placing a drop of phage suspension (ca. 10^6 PFU/ml) on the surface of an agar overlay containing the bacteria to be lysogenized. Colonies growing in the turbid zone after overnight incubation at 37°C were selected and plated on B agar. We tested individual colonies growing on these plates for the presence of prophage, as indicated by plaque formation, by growing these colonies in B medium, removing the cells by centrifugation, and placing a drop of the supernatant on the surface of B agar overlaid with B-top agar containing *E. coli* C600 cells. Lysogens to be tested for prophage induction were grown in LB or LBA medium to a density of 2×10^8 cells per ml, washed, resuspended in LB or LBA medium with or without mitomycin C (final concentration, 5 μg/ml), and incubated in the dark for 150 min at 37°C with agitation. The cells were then lysed by the addition of chloroform, cell debris was removed by centrifugation, and the supernatants were titrated for phages on B agar with *E. coli* C600 as the host.

Southern hybridizations. Genomic DNA was digested with various restriction endonucleases as specified by the suppliers, and the fragments were separated by overnight electrophoresis on 0.7% agarose gels. Southern transfer to nitrocellulose was performed as described previously (12). The 1.5-kbp fragment produced by partial *Sau*3A digestion of the 3.4-kbp *Xba*I fragment, present in precAVX12, was recovered from the plasmid precAVS1 as an *Eco*RI-*Hin*dIII fragment and labeled in vitro with [^{32}P]dCTP (Amersham Corp.) by using a nick translation kit (Bethesda Research Laboratories). After prehybridization, the labeled probe was allowed to hybridize to Southern blots for 18 h at 55°C as described previously (12). The blots were then washed three times in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate for 15 min at room temperature. Autoradiographic analysis was used to determine the hybridization pattern.

In vitro transcription-translation. Partial digestion of the 3.4-kbp *Xba*I fragment from precAVX12 with *Sau*3A yielded a 1.5-kbp fragment which, when cloned into the expression vector pUC18, also restored MMS resistance in *E. coli recA* mutants. This plasmid, precAVS1, was cleaved with *Eco*RI and *Hin*dIII, and the resulting insert was cloned into pUC19 in the opposite orientation, yielding precAVS2. The plasmids precAVS1, precAVS2, and pUC19 were then subjected to transcription-translation in an in vitro procaryotic DNA-directed transcription-translation system as specified by the supplier (Amersham Corp.). After the mixture had been incubated at 37°C for 60 min with [^{35}S]-methionine and then for 5 min with excess unlabeled methionine, the reaction was terminated by the addition of sodium dodecyl sulfate and glycerol to final concentrations of 1.7 and 10%, respectively. A portion was boiled for 1 min and electrophoresed on a 7.5 to 15% linear-gradient sodium dodecyl sulfate-polyacrylamide gel (14). The gel was stained with Coomassie brilliant blue G-250, and the newly synthesized polypeptides were visualized by autoradiography.

RESULTS

Isolation of the *A. variabilis recA* gene. A library of *A. variabilis* chromosomal DNA was constructed by cloning size-fractionated, partially digested *Sau*3A restriction fragments into the *Bam*HI site of the plasmid pDPL13 and then transforming them into *E. coli* HB101. The library consists of approximately 2,000 independent clones. Plasmids containing the *A. variabilis recA* gene were obtained by screening the amplified library for clones that restored MMS resistance to *E. coli* HB101. *E. coli* strains with mutations in *recA*, such as HB101, are incapable of growth in media containing MMS (2). After an 18-h incubation at 37°C, ampicillin- and MMS-resistant colonies appeared at a frequency of approximately 4.4×10^{-3}. Colonies were not observed on plates containing ampicillin and MMS when cells were transformed with the vector alone. Analysis of the plasmid DNA isolated from 20 MMS-resistant colonies indicated that with the exception of one clone, all contained the same *A. variabilis* DNA insert. This result was not unexpected, as the clone bank was amplified before MMS resistance selection. Plasmid DNA isolated from one of these 19 identical clones was used to retransform *E. coli* HB101 and yielded ampicillin- and MMS-resistant colonies. This plasmid, termed precAV2, also restored to MMS resistance the

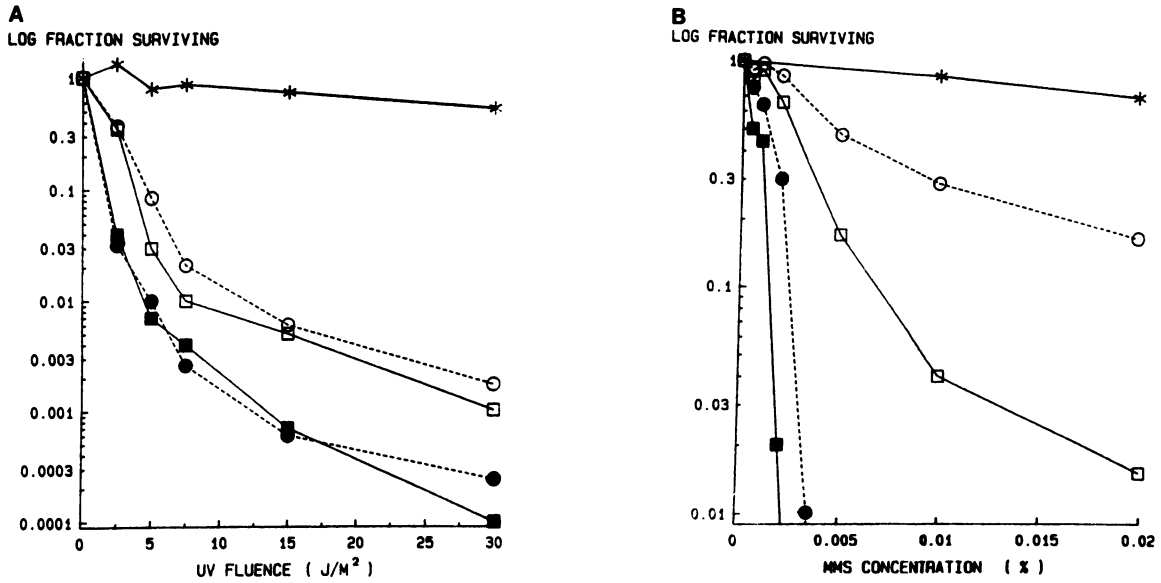

FIG. 1. (A) UV survival of various *recA*$^+$ and *recA E. coli* strains containing either pDPL13 or precAV2. Cells were grown to approximately 10^8/ml, pelleted, suspended at 1/10 concentration in 0.9% saline, and exposed to various fluences of UV irradiation. After dilution in saline, the irradiated cells were plated on LBA agar, and survival was determined after overnight incubation at 37°C in the dark. (B) MMS resistance of various *recA*$^+$ and *recA E. coli* strains containing either pDPL13 or precAV2. Cells were grown to approximately 10^8/ml, diluted in LBA, and plated on LBA agar containing various concentrations of MMS. Survival was determined after overnight incubation at 37°C. Symbols: *, JF1754(pDPL13); ●, HB101(pDPL13); ○, HB101(precAV2); ■, JC10289(pDPL13); □, JC10289(precAV2).

mutant *E. coli* strain, JC10289, in which the *recA* region is deleted [Δ(*recA-srlR*)*306*] (4). Attempts to isolate the *A. variabilis recA* gene from the genomic library by using the restoration of UV resistance in *E. coli* HB101 as the selection pressure were not successful. Although Amp^r UV^r colonies were obtained, none of the isolates contained autonomous plasmid.

UV sensitivity. The ability of precAV2 to complement the UV sensitivity of a number of *E. coli recA* mutants was determined (Fig. 1A). The plasmid increased the UV resistance of *E. coli* strains containing either a point mutation or a deletion of the *recA* gene by approximately 10- to 15-fold.

MMS resistance. The ability of the *A. variabilis recA* gene to impart MMS resistance to *E. coli recA* mutants was tested over a range of concentrations (Fig. 1B). The *recA* mutant HB101 containing the plasmid precAV2 was found to be significantly more resistant to MMS than was HB101 containing the vector alone. It was, however, less resistant than that observed with the *E. coli recA*$^+$ strain, JF1754. The *recA*-deleted strain JC10289 containing the *A. variabilis recA* gene exhibited lower levels of MMS resistance but considerably higher levels than that exhibited by this strain containing the vector alone.

Conjugational proficiency. The ability of precAV2 to restore homologous recombination capacity to various *E. coli recA* mutants was determined by measuring genetic recombination after conjugation with the Hfr strain KL16-99. Recombinational proficiency was restored in *E. coli* strains containing either point mutations or deletions of the *recA* gene. The recombinational proficiency (expressed as the number of *leuB*$^+$ recombinants per 100 donors) was 4.0 for the *recA*$^+$ strain JF1754. For the *recA* strains HB101 and JC10289, the recombinational proficiency was zero, but HB101(precAV2) and JC10289(precAV2) had recombinational proficiencies of 2.3 and 1.15, respectively. In the absence of precAV2, transconjugants were not observed when *recA* parental strains were used as recipients.

Prophage induction. *E. coli recA* mutants are defective in the ability to induce temperate prophage either spontaneously or after mitomycin C-induced DNA damage. Wild-type lambda lysogens of various *E. coli* strains with or without precAV2 were prepared from individually isolated clones, and the level of spontaneous and mitomycin C-induced phage production was determined. The presence of precAV2 allowed the spontaneous production of phage in the two *E. coli recA* mutants tested, although at a lower level than in the control *recA*$^+$ strain, JF1754 (Table 2). Mitomycin C did not significantly enhance phage production in *recA E. coli* strains containing precAV2. Exposure of the *recA*$^+$ strain, JF1754, to mitomycin C resulted in a 10^4-fold increase in phage production (Table 2).

Restriction mapping. A physical map of precAV2 was generated after digestion of the plasmid with nine restriction enzymes (Fig. 2). Subcloning of various restriction fragments and screening for their ability to recover MMS resistance in *E. coli recA* mutants indicates that the *A. variabilis recA* gene is located within a 3.4-kbp *Xba*I fragment (Fig. 2). Restoration of MMS resistance was accomplished when the *Xba*I fragment was inserted in either orientation in the plasmid pDPL13 (precAVX12).

In vitro transcription-translation. Subcloning of *Sau*3A

TABLE 2. Spontaneous and mitomycin C-stimulated induction of lambda prophage[a]

Lysogen	Phage produced (PFU/ml)	
	Spontaneous	Induced
JF1754	1.9×10^5	2×10^9
HB101	50	50
HB101(precAV2)	4×10^4	1.7×10^5
JC10289	50	50
JC10289(precAV2)	1.4×10^4	2×10^4

[a] Mitomycin C was present at a final concentration of 5 μg/ml.

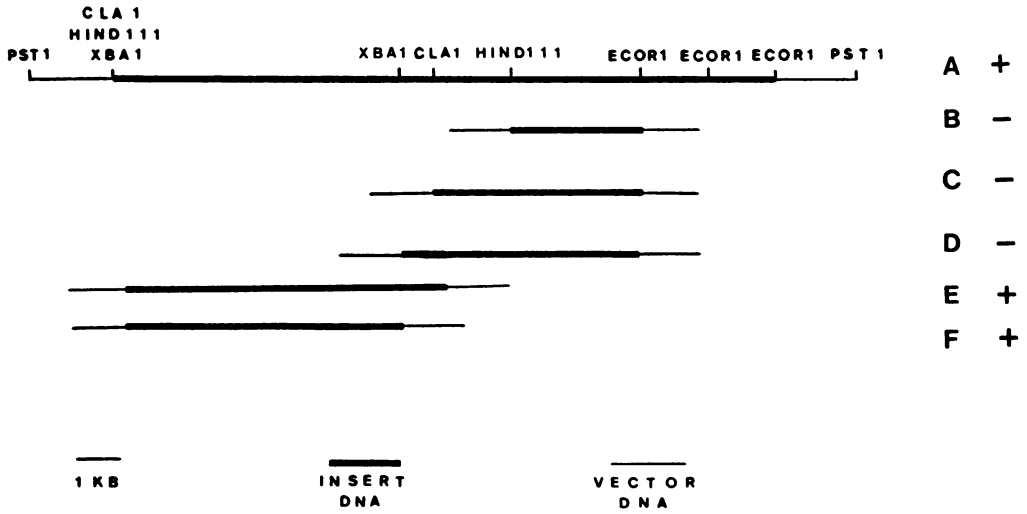

FIG. 2. Restriction endonuclease map of precAV2. precAV2 was digested with nine restriction enzymes, and all cleavage sites were determined. Subcloning of various fragments produced the following subclones: A, precAV2; B, precAVEH; C, precAVEC; D, precAVEX; E, precAVC1; and F, precAVX12. The ability or inability of these subclones to confer MMS resistance on *recA E. coli* mutants is indicated by + or −, respectively. Cleavage sites for *Bam*HI, *Bgl*II, *Pst*I, *Sal*I, and *Xho*I were not observed.

partial fragments of the *Xba*I insert from precAVX12 generated a 1.5-kbp fragment which, when cloned into pUC18 and pUC19 in opposite orientations, also restored MMS resistance in *E. coli recA* mutants. These plasmids, designated precAVS1 and precAVS2, respectively, were then used to prime an in vitro transcription-translation system, and the newly synthesized polypeptides were detected by autoradiographic analysis (Fig. 3).

Transcription-translation of pUC19 produced a polypeptide corresponding to the truncated β-galactosidase gene (Fig. 3, lanes 1 and 5). This polypeptide was not synthesized from either precAVS1 or precAVS2 but was replaced by a 38- to 40-kilodalton polypeptide (Fig. 3, lanes 2 and 6; lanes 3 and 7). Therefore, in both orientations, the 1.5-kbp fragment directed the expression of a single 38- to 40-kilodalton polypeptide (Fig. 3).

Southern hybridizations. Hybridization of the 1.5-kbp partial *Sau*3A fragment containing the *A. variabilis recA* gene to the cyanobacterial genomic DNA restricted with *Xba*I or *Eco*RI is shown in Fig. 4. The probe hybridized to single 3.4-kbp *Xba*I and 6.6-kbp *Eco*RI genomic fragments. A similar 6.6-kbp hybridizing fragment was observed with

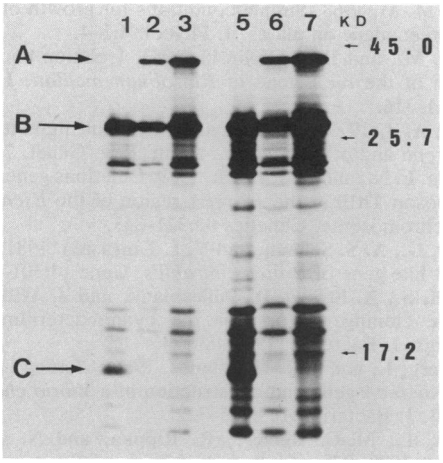

FIG. 3. In vitro transcription-translation. Autoradiogram of a 7.5 to 15% linear-gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel loaded with the polypeptide products synthesized from various plasmids in an in vitro DNA-directed transcription-translation system. Lanes: 1 and 5, pUC19; 2 and 6, precAVS1; 3 and 7, precAVS2. Lanes 1 to 3 and 5 to 9 were loaded with 2- and 5-μl samples, respectively. The molecular mass is shown in kilodaltons (KD). The letters indicate the positions of the *A. variabilis recA* (A), the β-lactase (B), and the truncated β-galactosidase (C) gene products.

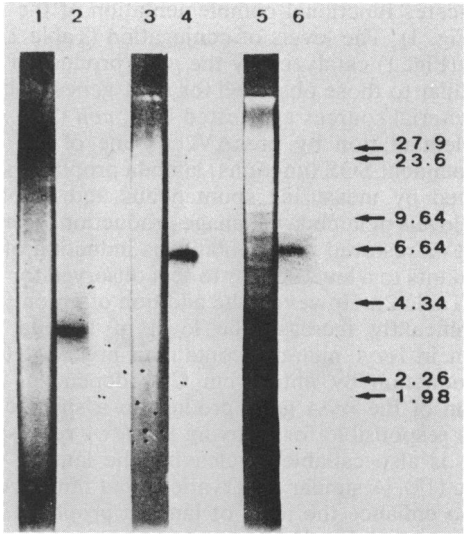

FIG. 4. Southern hybridization. Autoradiogram of a Southern blot of *A. variabilis* and *A. nidulans* R2 genomic DNA probed with a 1.5-kbp *Sau*3A fragment containing the *A. variabilis recA* gene. Lanes 1, 3, and 5 show ethidium bromide-stained *A. variabilis* genomic DNA digested with *Xba*I and *Eco*RI and *A. nidulans* R2 genomic DNA digested with *Eco*RI, respectively. Lanes 2, 4, and 6 show the corresponding hybridization pattern. Molecular size markers (*Hin*dIII-digested lambda DNA) are indicated.

Anacystis nidulans R2 genomic DNA restricted with *Eco*RI (Fig. 4). These data indicate that a single copy of the *recA* gene resides in the *A. variabilis* genome.

DISCUSSION

We have cloned and partially characterized a chromosomal DNA fragment from the genome of the cyanobacterium *A. variabilis* which complements the recombinational deficiencies of *E. coli recA* mutants. Selection for this gene required that the transformed *recA* host could grow in the presence of the DNA-alkylating agent MMS. Growth under these conditions can occur only if DNA repair by the SOS system or if recombination deficiencies of the *recA* host, or both, have been complemented by the *A. variabilis* gene. Initial attempts to obtain the cyanobacterial *recA* gene by selecting for the acquisition of UV resistance resulted in the recovery of a number of Amp^r UV^r clones; however, plasmid could not be isolated from these cells. The reasons for this result are unclear, as this selection technique has been used for the isolation of the *recA* gene from *Proteus mirabilis* (5). These results suggest that the repair of UV irradiation damage has led to, or required, the integration of the *A. variabilis recA* gene.

The ability of the cloned fragment in precAV2 to functionally complement defects in homologous recombination and induction of the SOS repair system was tested with two *recA E. coli* mutants, HB101 and JC10289, which contain a point mutation and a deletion of the *recA* region, respectively. In all tests, both *E. coli recA* mutant strains gave similar results, indicating that complementation did not result from the cloning of a suppressor gene. Evidence for the functional complementation of the defect in homologous recombination was obtained by observing an increased number of transconjugants generated by Hfr-mediated conjugation when the *E. coli recA* mutants contained precAV2 (see Results). Restoration of the ability of the *E. coli recA* mutants to repair UV- or MMS-induced DNA damage when containing precAV2 also indicates functional complementation of the *recA* mutation (Fig. 1). The levels of conjugation (Table 2) and UV survival (Fig. 1) catalyzed by the gene product of precAV2 were similar to those observed for *recA* genes isolated from other bacterial sources and tested in *E. coli* (2, 7, 9, 11).

Complementation by precAV2 of one of the inducible *recA*-dependent SOS functions, lambda prophage induction, was tested by measuring spontaneous and mitomycin C-induced levels of lambda prophage production. The presence of precAV2 restored the spontaneous induction of phage in *recA* mutants to a level similar to that observed for wild-type *E. coli* (Table 2). However, the addition of mitomycin C did not significantly increase the level of lambda prophage induction in *recA* mutants containing precAV2 (Table 2). Phage induction by mitomycin C is dependent upon the activation of the *recA* gene product to a specific protease which is responsible for cleaving the *lexA* repressor molecule but is also capable of cleaving the lambda repressor molecule (17). A similar observation, that mitomycin C was unable to enhance the level of lambda prophage induction but that normal levels of spontaneous lambda prophage induction are restored in the *E. coli recA* host, has been reported for *recA* genes isolated from *Proteus vulgaris* and *Erwinia carotovora* (9). These data suggest that precAV2 is incapable of inducing the *E. coli* SOS system and therefore that the DNA damage resulting from UV illumination or MMS exposure could have been overcome by postreplication recombinational repair alone. It is possible that the inability of the cyanobacterial *recA* gene to induce lambda prophage induction also results from an inability of the *A. variabilis recA* gene product to recognize or cleave the lambda repressor. It is known that the *recA* protein-dependent proteolysis of the *lexA* repressor is more efficient than lambda repressor cleavage. Thus the absence of induced prophage induction may not indicate continued SOS repression. We are currently examining *recA* protein levels to determine whether *A. variabilis recA* gene expression is induced in *E. coli* by DNA damage.

Restriction endonuclease mapping and subcloning of the 7.75-kbp insert of precAV2 revealed that the *A. variabilis recA* gene is located on a 3.4-kbp *Xba*I fragment (precAVX12). This fragment is capable of restoring MMS resistance when inserted in either orientation in pDPL13, which suggests that the *A. variabilis* gene is being expressed from its own promoter in *E. coli*. The in vitro transcription-translation of a 1.5-kbp *Sau*3A fragment, which is also capable of restoring MMS resistance, yielded a polypeptide of 38 to 40 kilodaltons, which is similar in size to other bacterial *recA* gene products (2, 9). Although a comparison of restriction maps from various *recA* genes did not reveal any similarities with the *A. variabilis* restriction map, preliminary DNA sequence analysis of the 1.5-kbp *Sau*3A fragment indicates that there is homology between this fragment and the nucleotide sequence of the *E. coli recA* gene (16) (manuscript in preparation).

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LITERATURE CITED

1. **Allen, M. M.** 1968. Simple conditions for growth of unicellular blue-green algae on plates. J. Phycol. **4**:1–4.
2. **Better, M., and D. R. Helinski.** 1983. Isolation and characterization of the *recA* gene of *Rhizobium meliloti*. J. Bacteriol. **155**:311–316.
3. **Clark, A. J.** 1973. Recombination deficient mutants of *Escherichia coli* and other bacteria. Annu. Rev. Genet. **7**:67–86.
4. **Csonka, L. N., and A. J. Clark.** 1979. Deletions generated by the transposon Tn10 in the *srl recA* region of the *Escherichia coli* K-12 chromosome. Genetics **93**:321–343.
5. **Eitner, G., A. S. Solonin, and V. I. Tanyashin.** 1981. Cloning of a *recA*-like gene of *Proteus mirabilis*. Gene **14**:301–308.
6. **Gendel, S., N. Straus, D. Pulleyblank, and J. Williams.** 1983. Shuttle cloning vectors for the cyanobacterium *Anacystis nidulans*. J. Bacteriol. **156**:148–154.
7. **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.
8. **Kallas, T., M.-C. Rebiere, R. Rippka, and N. Tandeau de Marsac.** 1983. The structural *nif* genes of the cyanobacterium *Gloeothece* sp. and *Calothrix* sp. share homology with those of *Anabaena* sp., but the *Gloeothece* genes have a different arrangement. J. Bacteriol. **155**:427–431.
9. **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*. J. Bacteriol. **160**:153–160.
10. **Kodaki, T., F. Katagiri, M. Asano, K. Izui, and H. Katsuki.** 1985. Cloning of the phosphoenolpyruvate carboxylase gene from a cyanobacterium, *Anacystis nidulans*, in *Escherichia coli*.

J. Biochem. **97**:533–539.
11. **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.
12. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **McEntee, K., G. M. Weinstock, and I. R. Lehman.** 1979. Initiation of general recombination catalyzed *in vitro* by the RecA protein of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **76**:2615–2619.
14. **Piccioni, R., G. Bellemare, and N.-H. Chua.** 1982. Methods of polyacrylamide gel electrophoresis in the analysis and preparation of plant polypeptides, p. 985–1014. *In* M. Edelman, R. B. Hallick, and N.-M. Chua (ed.), Methods in chloroplast molecular biology. Elsevier Biomedical Press, Amsterdam.
15. **Roberts, J. W., and C. W. Roberts.** 1975. Proteolytic cleavage of bacteriophage lambda repressor in induction. Proc. Natl. Acad. Sci. USA **72**:147–151.
16. **Sancar, A., C. Strachelek, W. Konigsbery, and W. D. Rupp.** 1980. Sequences of the *recA* gene and protein. Proc. Natl. Acad. Sci. USA **77**:2611–2615.
17. **Walker, G. C.** 1985. Inducible DNA repair sytems. Annu. Rev. Biochem. **54**:425–457.
18. **West, S. C., E. Cassuto, J. Mursalim, and P. Howard-Flanders.** 1980. Recognition of duplex DNA containing single-stranded regions by *recA* protein. Proc. Natl. Acad. Sci. USA **77**: 2569–2573.