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Regulation of Expression and Nucleotide Sequence of the *Anabaena variabilis recA* Gene

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The expression of the cyanobacterial *recA* gene, isolated from *Anabaena variabilis*, has been examined at the levels of transcript and protein abundance. Exposure of the cyanobacterium to a variety of DNA-damaging agents, including mitomycin C, methyl methanesulfonate, and UV irradiation, results in a rapid increase in the abundance of the *recA* transcript above basal levels as determined by Northern (RNA) blot analysis. A concomitant increase in the abundance of a 37- to 38-kilodalton polypeptide was also detected by Western (immuno-) blot analysis of soluble cyanobacterial polypeptides using polyclonal antiserum directed against the *Escherichia coli recA* protein. The cyanobacterial polypeptide is of the same molecular mass as that synthesized by an in vitro, DNA-directed procaryotic transcription-translation system primed with an *A. variabilis* genomic fragment containing the *recA* gene. Nucleotide sequence analysis of the cyanobacterial gene revealed a protein of 358 amino acids with a molecular weight of 38,403 daltons. The *A. variabilis* and *E. coli recA* genes share similarity at 58% of the amino acid residues; however, an *E. coli*-like *lexA* repressor-binding site is not present in the *A. variabilis* promoter region. The similarities of *A. variabilis* and *E. coli recA* expression and gene sequence are discussed.

The cyanobacteria are a diverse group of procaryotic, obligate photoautotrophs with growth habitats including marine, freshwater, and terrestrial environments (20). Within these habitats, prolonged exposure to a number of DNA-damaging agents, including UV irradiation as well as soil- or waterborne chemical agents, can be expected. This suggests that the cyanobacteria possess efficient recombinational and DNA repair systems. It has been shown that cyanobacteria are capable of photoreactivation and excision repair of DNA damaged by exposure to UV irradiation, and it was suggested that *Synechocystis* sp. strain PCC 6308 may also have an inducible repair mechanism (15). However, a detailed biochemical and genetic study of an inducible, cyanobacterial DNA repair system has not been performed.

In *Escherichia coli*, a postreplication error-prone repair system, termed the SOS response, is induced after exposure of the cells to agents which either cause DNA damage or inhibit DNA replication (21). Induction of the SOS response results in the transient, coordinated expression of several unlinked genes. These gene products are responsible for DNA repair as well as for other processes required for cell viability. SOS gene expression is controlled at the level of transcription by a complex interaction between the *recA* and *lexA* gene products (21). Under noninducing conditions, the *lexA* protein represses the expression of at least 20 SOS genes, including itself and *recA*, by binding to a conserved sequence, the SOS box, present in the promoter region of each gene (22). A low, basal level of *recA* expression also occurs and functions as a DNA-dependent ATPase essential for general homologous DNA recombination (22). DNA damage produces a metabolic signal which activates the *recA* protein, facilitating its ability to recognize and enhance autodigestion of the *lexA* repressor polypeptide. The subsequent derepression of SOS gene transcription results in significant increases in *recA* transcription and translation, with the consequent enhancement of both recombinational and error-prone *recA*-mediated DNA repair. Increased levels of mutagenesis, inhibition of cell division, and prophage induction as a result of prophage repressor cleavage are also associated with SOS induction (22).

The essential role that the *E. coli recA* protein performs in important processes such as recombination and DNA repair suggests that *recA*-like analogs could be conserved among other procaryotes. In fact, *recA*-like genes have recently been isolated from a number of organisms (6, 10, 11, 13). In an earlier publication we reported the isolation and characterization of a *recA* analog isolated from the cyanobacterium *Anabaena variabilis* ATCC 29413 (16). We were able to show that the cyanobacterial gene was able to functionally complement *E. coli recA* mutants with respect to repair of DNA damage and restoration of conjugal proficiency but not induction of temperate prophage (16). Other researchers have recently reported similar findings with *recA* analogs isolated from other cyanobacteria (5, 14). In this study we have used Northern (RNA) and Western (immuno-) blot analyses to determine the levels of cyanobacterial *recA* mRNA and protein abundance after exposure of *A. variabilis* to a variety of DNA-damaging agents. We have also sequenced the *A. variabilis* genomic DNA fragment which complements *E. coli recA* mutants and compared the cyanobacterial sequence with that of the bacterial *recA* gene.

MATERIALS AND METHODS

Culture conditions. *A. variabilis* (ATCC 29413) and *Anacystis nidulans* R2 (PCC 7942) were cultured axenically in BG-11 medium at 30°C and at a light intensity of 150 μE/m^2 per s (400 to 700 nm) as described previously (16). The cells were aerated vigorously and harvested at early- to mid-log phase of growth. Culture stocks were maintained under similar conditions on BG-11 medium solidified with 1.0% agar.

Exposure to DNA-damaging agents. Cyanobacteria were harvested by centrifugation (10,000 × *g*, 7 min), suspended in fresh BG-11 medium at a concentration of 5 to 10 μg of

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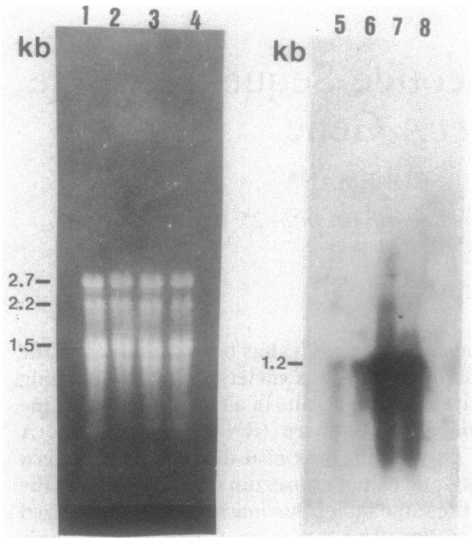

FIG. 1. Effect of mitomycin C on abundance of the *A. variabilis recA* transcript. Total RNA was isolated from cells before and during exposure to mitomycin C at a concentration of 5 μg/ml. After electrophoretic separation on a 1.0% agarose gel containing 0.66 M formaldehyde, the RNA was transferred to a nylon membrane and probed with a ^{32}P-labeled 1.5-kb *Sau*3A fragment containing the entire *A. variabilis recA* coding region. The ethidium bromide-stained gel (lanes 1 to 4) and the resulting hybridization profiles (lanes 5 to 8) are shown for RNA isolated from cells before (lanes 1 and 5) and after mitomycin C exposure of 1 h (lanes 2 and 6), 3 h (lanes 3 and 7) and 6 h (lanes 4 and 8). The sizes of the transcript and the stained ribosomal bands were determined by using a synthetic RNA ladder.

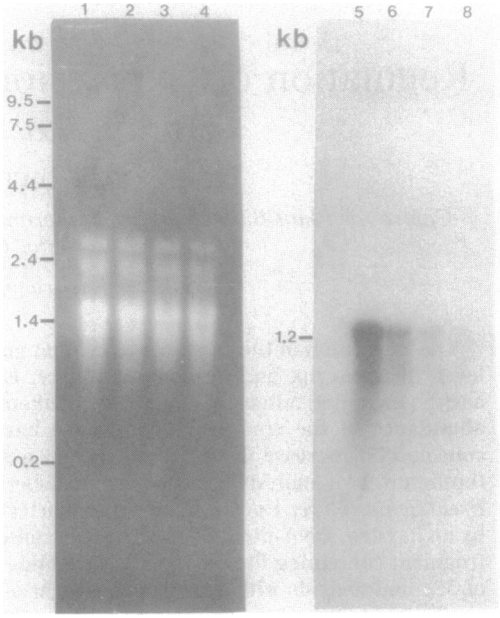

FIG. 2. Reduction of the *recA* transcript abundance after removal of mitomycin C. *A. variabilis* was exposed to mitomycin C (5 μg/ml) for 3 h in the light at 30°C. The cells were then harvested, washed, suspended in fresh medium, and incubated under growth conditions. Total RNA (lanes 1 to 4) was isolated at various periods of time after exposure to mitomycin C and treated as described for Fig. 1. The autoradiograph (lanes 5 to 8) shows the hybridization pattern of the *recA* gene to RNA isolated from cells immediately after exposure to mitomycin C (lanes 1 and 5) and 1 h (lanes 2 and 6), 3 h (lanes 3 and 7), and 6 h (lanes 4 and 8) postexposure. The positions of the RNA standards are indicated.

chlorophyll per ml, and incubated under normal growth conditions for various periods of time with the DNA-damaging chemicals. Final concentrations were as follows: mitomycin C, 5 μg/ml; methyl methanesulfonate (MMS), 0.015% (wt/vol); and nalidixic acid, 80 μg/ml. UV irradiation of the cyanobacteria was performed in the dark in a shaking, sterile Pyrex (Corning Glass Works) dish. A prewarmed G15T8-15W germicidal lamp (General Electric) was used to deliver an irradiance level of 1 J/m^2 per s. Immediately after UV treatment, the cells were transferred to a darkened, sterile growth flask and incubated under normal growth conditions.

RNA isolation and Northern analysis. RNA was purified from *A. variabilis* by sedimentation through cesium chloride after mechanical lysis of the cells and extensive phenol-chloroform extraction as described by Golden et al. (7). RNA was separated electrophoretically in 1.0% agarose gels containing 0.66 M formaldehyde and transferred to Biotrace nylon membrane (ICN Pharmaceuticals Inc.) as previously described (4). Northern blots were prehybridized overnight and hybridized for 36 h at 42°C in a solution containing 50% (vol/vol) deionized formamide, 5× SSC (1× SSC is 0.15 M NaCl–0.015 M sodium citrate), 50 mM sodium phosphate buffer (pH 6.5), 1× Denhardt solution, and 250 μg of denatured calf thymus DNA per ml. A 1.5-kilobase (kb) *Sau*3A I fragment containing the entire *A. variabilis recA* gene (16) was labeled with [α-^{32}P]dCTP by the random primer labeling procedure and was added at a final concentration of 10^6 cpm/ml to the hybridization solution (3). The hybridized blots were washed four times for 5 min each in 2× SSC containing 0.1% sodium dodecyl sulfate at room temperature; this was followed by two 15-min washes in 0.1× SSC containing 0.1% sodium dodecyl sulfate at 50°C. The hybridization pattern was determined by autoradiography, and the sizes of the stained ribosomal bands and transcripts were estimated by comparison with a synthetic RNA ladder (Bethesda Research Laboratories, Inc.).

Protein isolation and Western analysis. Exponentially growing *A. variabilis* and *Anacystis nidulans* cells were harvested by centrifugation, washed once with buffer (20 mM Tris hydrochloride, pH 8.0), and then suspended in ice-cold extraction buffer (20 mM Tris hydrochloride, pH 8.0, containing 100 mM NaCl, 5 mM dithiothreitol, 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride). The cells were lysed by passage through a French pressure cell (16,000 lb/in^2) and then centrifuged (17,000 × *g*) for 30 min at 4°C to remove unbroken cells and large membrane fragments. Soluble polypeptides were precipitated from the supernatant by the addition of trichloroacetic acid (final concentration, 10%) and centrifuged, and the pelleted protein was washed with ice-cold acetone. The proteins were suspended in 0.1 M Na_2CO_3–0.1 M dithiothreitol, and a 0.5 volume of 5% sodium dodecyl sulfate–30% sucrose–0.1% bromophenol blue was added to each sample. The proteins were boiled and electrophoresed at 4°C on a denaturing polyacrylamide gel (7 to 15% linear gradient) by using Laemmli buffers (17). Replicate halves of the gels were either stained with Coomassie brilliant blue R250 or used for electrotransfer of the polypeptides to nitrocellulose (BA-85; Schleicher & Schuell, Inc.) in a Trans-Blot cell (Bio-Rad Laboratories) at 30 V for 16 h in 25 mM Tris–192 mM glycine–20% methanol. The nitrocellulose was preincubated for 0.5 h in 1× Tris-buffered saline (TBS; 10 mM Tris hydrochloride, pH 8.0,

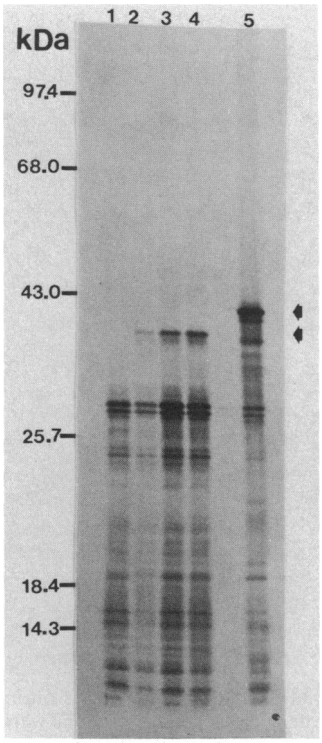

FIG. 3. In vitro transcription-translation of the *A. variabilis* and *E. coli recA* genes. The ^{35}S-labeled products of a DNA-directed transcription-translation system were separated on a sodium dodecyl sulfate-polyacrylamide gel (7 to 15% linear gradient) and subjected to autoradiography. The plasmids used to prime the system were pUC18 (lane 1), pUC18 containing the *A. variabilis recA* gene on a 1.5-kb *Sau*3A genomic fragment (lane 2), pUC18 containing the 1.5-kb *Sau*3A fragment in the alternate orientation (lane 3), pUC18 containing a 3.4-kb *A. variabilis Sau*3A fragment which spans the 1.5-kb fragment (lane 4), and pFK7 containing the *E. coli recA* gene (lane 5). The positions of the molecular mass protein standards (in kilodaltons) are indicated.

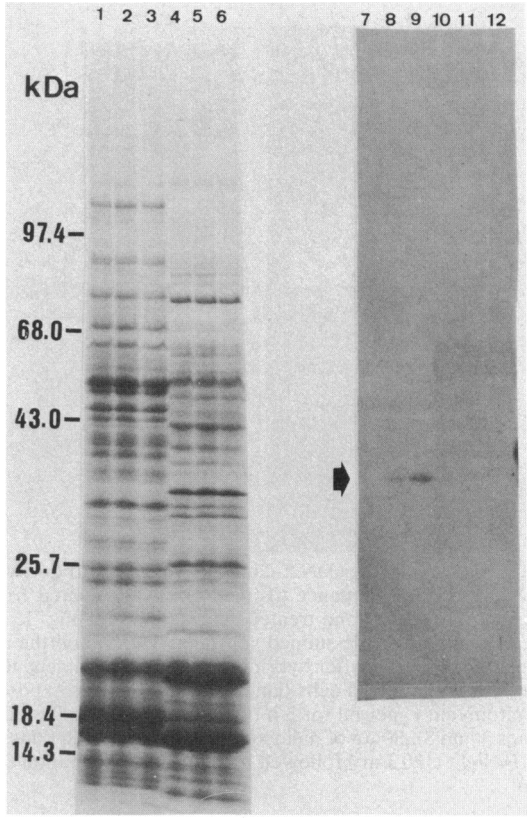

FIG. 4. Detection of the *A. variabilis recA* protein by Western blotting. Total soluble proteins were isolated from *A. variabilis* before (lanes 1 and 7) and after 3 h (lanes 2 and 8) and 9 h (lanes 3 and 9) of exposure to mitomycin C. Similarly, total soluble proteins were isolated from *Anacystis nidulans* R2 before (lanes 4 and 10) and after 3 h (lanes 5 and 11) and 9 h (lanes 6 and 12) of exposure to mitomycin C. After separation on a sodium dodecyl sulfate-polyacrylamide gel, the proteins were stained with Coomassie brilliant blue (lanes 1 to 6) or were electrotransferred to nitrocellulose and probed with polyclonal antiserum directed against the *E. coli recA* protein (lanes 7 to 12). The arrow indicates the position of the *A. variabilis recA* protein. The positions of the molecular mass protein standards (in kilodaltons) are also indicated.

0.15 M NaCl) containing 5% (wt/vol) nonfat dry milk powder (Carnation Inc.) and 0.02% sodium azide before the addition of *E. coli recA* antiserum (1:10,000 dilution) and a further incubation for 16 h at room temperature with gentle shaking. Nonspecifically bound primary antibody was removed by a single 2-min wash in 1× TBS followed by a 3-h incubation in 1× TBS containing the secondary antibody (peroxidase-conjugated goat anti-rabbit immunoglobulin G; Bio-Rad). After a 2-min wash in 1× TBS, specifically bound primary antibody was detected by using 4-chloro-napthol and H_2O_2.

In vitro transcription-translation. Plasmids containing the *A. variabilis* and *E. coli recA* coding regions (16) were subjected to transcription-translation in an in vitro, procaryotic DNA-directed system by following the instructions supplied by Amersham Corp. The proteins were separated by gel electrophoresis as described above, and the synthesized polypeptides were detected by autoradiography.

DNA sequencing and analysis. The 1.5-kb *Sau*3A I fragment was subcloned into M13mp18 and M13mp19, and a series of overlapping deletions of each of these clones was produced as previously described (1). DNA from both strands was sequenced completely by the dideoxy-chain termination method (19) by following the instructions of the supplier (U.S. Biochemical Corp.). DNA sequence analysis was performed by using the Pustell Sequence Analysis Software (International Biotechnology, Inc.) and the FASTP system described by Lipman and Pearson (12).

RESULTS

The effect of DNA damage on the *A. variabilis recA* transcript abundance is shown in Fig. 1. Total RNA was isolated from a control culture and from cyanobacterial cells exposed to the DNA-damaging agent mitomycin C (5.0 μg/ml) for 1, 3, and 6 h. Hybridization of the 1.5-kb *Sau*3A I fragment, which contains the entire *A. variabilis recA* gene, to a Northern blot of this RNA revealed an increase in the abundance of a 1.2-kb transcript above basal levels after 1 h of exposure to mitomycin C (Fig. 1, compare lanes 1 and 2). Maximal transcript abundance occurred within 3 h of exposure and remained constant for the following 3 h. Removal of the mitomycin C from the culture medium after a 3-h exposure resulted in a gradual decline in the abundance of the 1.2-kb transcript to basal levels over a 6-h time course (Fig. 2).

To determine the approximate molecular mass and the relative mobility of the *A. variabilis recA* polypeptide on a

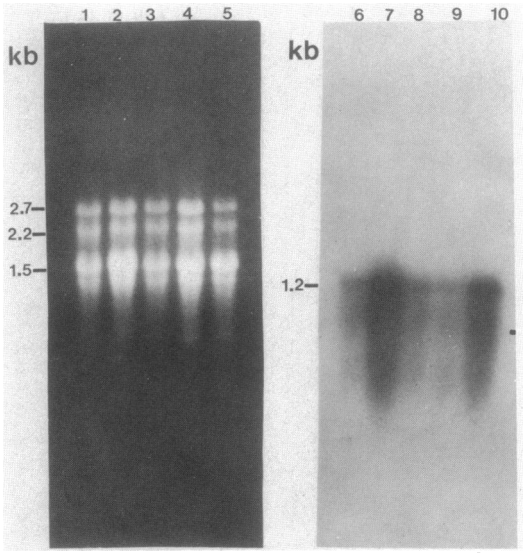

FIG. 5. Effect of other DNA-damaging agents on the *A. variabilis recA* transcript abundance. Total RNA was isolated from the cyanobacterial cultures and treated as described for Fig. 1. Shown are the ethidium bromide-stained gel (lanes 1 to 5) and the autoradiograph (lanes 6 to 10) after hybridization of the *recA* gene to RNA obtained from uninduced cells (lane 1 and 6) and cells exposed to 5 μg of mitomycin C per ml for 3 h (lanes 2 and 7), 0.015% MMS for 3 h (lanes 3 and 8), 80 μg of nalidixic acid per ml for 3 h (lanes 4 and 9), or UV light (120 J/m^2) followed by a 3-h dark incubation (lanes 5 and 10).

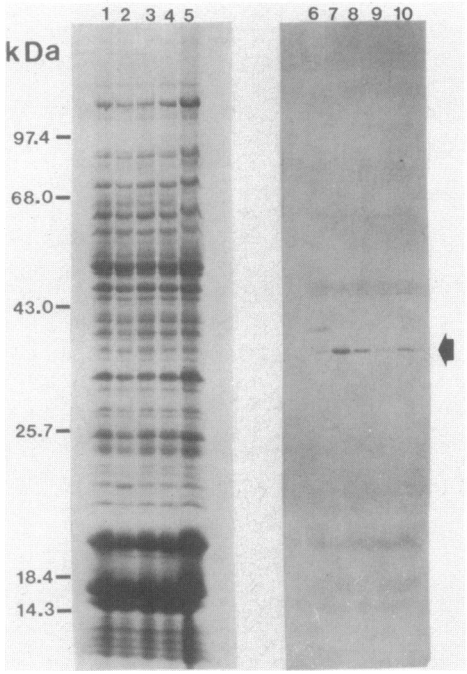

FIG. 6. Effect of DNA-damaging agents on induction of the *A. variabilis recA* protein. Total soluble proteins were isolated from the cyanobacterial cultures exposed to the DNA-damaging agents described in the legend to Fig. 5. After electrophoresis and transfer to nitrocellulose (as in Fig. 4), the blot was probed with polyclonal antiserum directed against the *E. coli recA* protein. Shown are the stained gel (lanes 1 to 5) and Western blot (lanes 6 to 10) profiles for proteins isolated from uninduced cells (lanes 1 and 6) and from cells exposed to mitomycin C (lanes 2 and 7), MMS (lanes 3 and 8), nalidixic acid (lanes 4 and 9), and UV irradiation (lanes 5 and 10).

polyacrylamide gel, various plasmids containing the entire *recA* coding region as well as flanking sequences were used to prime a procaryotic transcription-translation system (16). An autoradiograph of the polyacrylamide gel is shown in Fig. 3. The transcription-translation of a 1.5-kb genomic *Sau*3A I fragment cloned in both orientations resulted in the synthesis of a novel polypeptide with an M_r of 37,000 to 38,000 (Fig. 3, lanes 2 and 3). In addition, transcription-translation of a 3.5-kb *Sau*3A I fragment (which spans the 1.5-kb *Sau*3A I fragment) also resulted in the synthesis of a 37- to 38-kilodalton (kDa) polypeptide (Fig. 3, lane 4). For comparison, a plasmid containing the *E. coli recA* coding region, pFK7, produced a 40- to 41-kDa polypeptide (Fig. 3, lane 5).

To detect the induction of the cyanobacterial *recA* protein, total soluble polypeptides were isolated from two cyanobacteria, *A. variabilis* and *Anacystis nidulans* R2, before and after exposure to mitomycin C for 3 and 9 h. The stained polyacrylamide gel profile is shown in Fig. 4 (lanes 1 to 6). No significant differences in the stained polypeptide profiles were apparent after exposure to the DNA-damaging agent. A replicate of this gel was electroblotted onto nitrocellulose and probed with a polyclonal antiserum directed against the *E. coli recA* protein. An *A. variabilis* polypeptide with an M_r of 37,000 to 38,000 cross-reacted antigenically with the *E. coli recA* antiserum and increased in abundance with increasing time of exposure to mitomycin C (Fig. 4, lanes 7 to 9). No specific cross-reactivity was obtained when *Anacystis nidulans* R2 polypeptides were probed with *recA* antiserum (Fig. 4, lanes 10 to 12).

We have also studied the impact of other DNA-damaging agents on the abundance of the *A. variabilis recA* transcript. Total RNA was isolated from cells exposed to mitomycin C, MMS, or nalidixic acid for 3 h and from cells subjected to UV irradiation (120 J/m^2) followed by a 3-h dark incubation. The total RNA profile and the autoradiograph of the Northern blot after probing with the entire *A. variabilis recA* gene are shown in Fig. 5. Both UV irradiation and exposure to MMS or mitomycin C significantly increased the abundance of the 1.2-kb transcript above basal levels (Fig. 5, compare lanes 7, 8, and 10 with 6). Exposure to nalidixic acid did not appear to increase the abundance of the cyanobacterial *recA* transcript under these conditions (Fig. 5, lane 9).

The exposure of *A. variabilis* to a variety of DNA-damaging agents also resulted in an increase in the abundance of the 37- to 38-kDa polypeptide above basal levels (Fig. 6). UV irradiation as well as MMS and mitomycin C exposure produced a significant accumulation of the polypeptide as detected by Western blotting. Nalidixic acid did not appear to stimulate synthesis of the *recA* protein. The Northern and Western blot data are in agreement with the observation that MMS, mitomycin C, and UV exposure at the indicated levels reduce the growth rate of the cyanobacteria (data not shown). Nalidixic acid reduced cell viability (as determined by plating) but appeared not to stimulate *recA* transcription or translation.

The complete DNA sequence of the 1.5-kb *Sau*3A I genomic DNA fragment is shown in Fig. 7. The deduced amino acid sequence of the 1,074-base-pair open reading frame contained within this sequence is presented below the DNA sequence. This open reading frame, without posttranslational processing of the N-terminal methionine, encodes a polypeptide of 358 amino acids with a calculated M_r of 38,403. This is in good agreement with the M_r of the protein

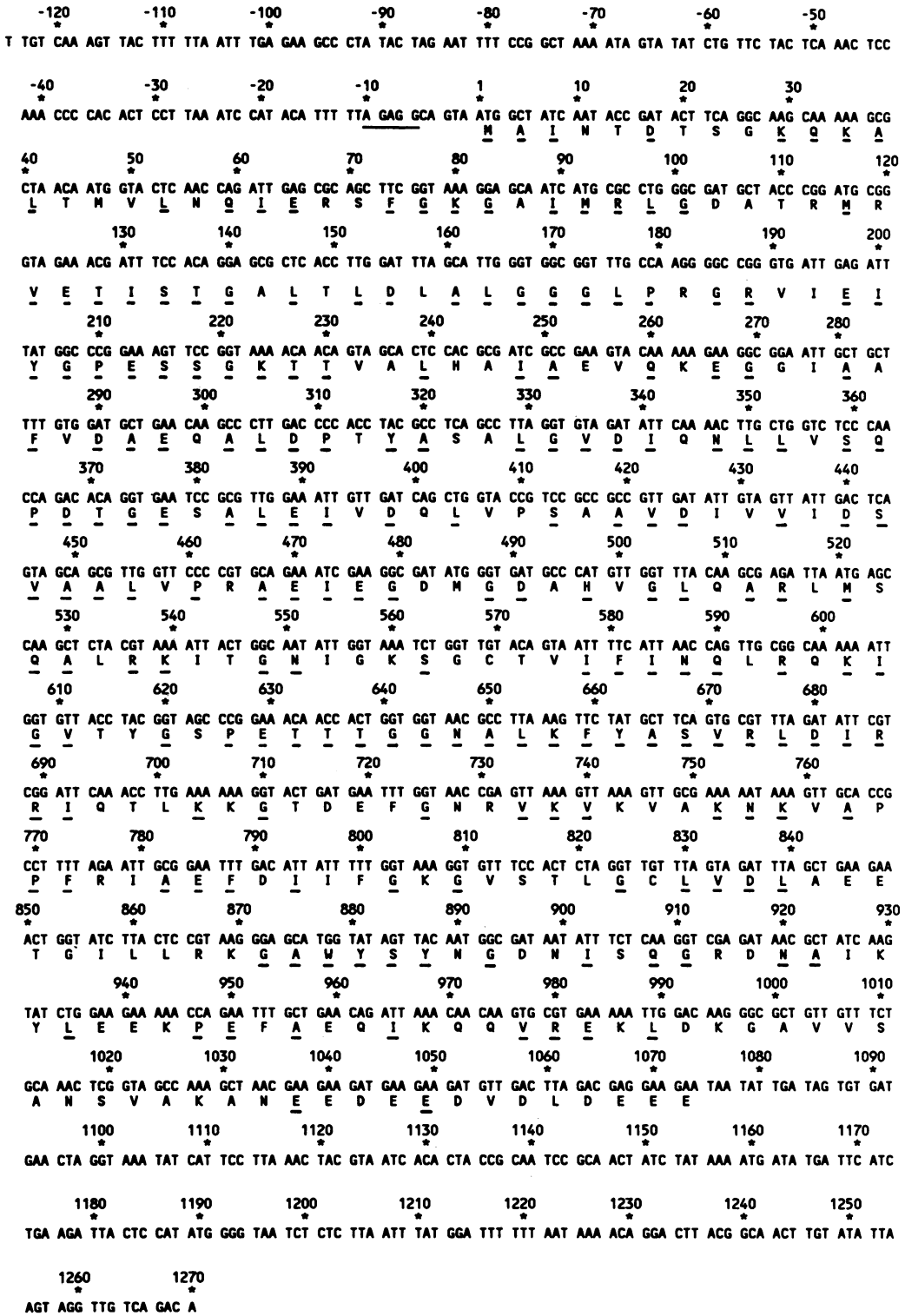

FIG. 7. Nucleotide sequence and derived amino acid sequence of the *A. variabilis recA* gene. The nucleotides are numbered above the sequence starting from the ATG initiation codon of the *recA* protein. The deduced amino acid sequence is shown below the nucleotide sequence. Those amino acids sharing identity after alignment with the amino acid sequence of the *E. coli recA* protein are underlined. A putative Shine-Dalgarno sequence is also underlined.

determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and identified by immunological techniques. A putative Shine-Dalgarno ribosome-binding site is located 5 base pairs upstream of the open reading frame (Fig. 7). Numerous −10 and −35 consensus sequences are also present upstream of the initiation codon. However, which if any of these signals are recognized in vivo is not yet known. An *E. coli*-like *lexA* repressor-binding site, termed an SOS box, does not occur within the putative promoter region of the *A. variabilis recA* gene.

The deduced amino acid sequences of the *E. coli* and cyanobacterial *recA* proteins are identical at 58% of the residues (Fig. 7). The cyanobacterial initiation codon was selected on the basis of significant homology existing between the two amino acid sequences as well as the size of the cyanobacterial open reading frame generated by this selection. The DNA sequences of the two genes were not similar either 5′ or 3′ of the cyanobacterial open reading frame. Hydrophobicity plots of the *A. variabilis* protein suggest that the polypeptide is highly hydrophobic with the exception of a hydrophilic carboxy-terminal region (data not shown).

DISCUSSION

In this study, we have examined the transcriptional and translational expression of the *A. variabilis recA* analog. It is apparent that exposure of the cyanobacteria to a variety of DNA-damaging agents results in an increase in *recA* transcript abundance. The exposure of *A. variabilis* to DNA damage also results in an increase in the abundance of a 37- to 38-kDa polypeptide which cross-reacts antigenically with antiserum raised against the *E. coli recA* protein. The cyanobacterial polypeptide detected by Western blotting is of the same molecular mass as the polypeptide synthesized in vitro by a DNA-directed procaryotic transcription-translation system primed with the *A. variabilis recA* gene. Sequence analysis of this DNA fragment followed by comparison with the *E. coli recA* gene confirmed the identity of the *A. variabilis* gene and provided a deduced molecular weight of the gene product. Taken in concert, these data clearly show that the 37- to 38-kDa polypeptide is the *A. variabilis recA* protein. In addition, it has recently been demonstrated that *recA* analogs isolated from other procaryotic organisms are also antigenically similar to the *E. coli recA* protein (8, 10, 13). In this study, the *E. coli* antiserum did not cross-react with proteins isolated from another cyanobacterium, *Anacystis nidulans* R2. This is perhaps not unexpected, as the *Anacystis nidulans* R2 gene, on the basis of Southern hybridization and nucleotide sequence data, is different from the *A. variabilis recA* gene, particularly within the hydrophilic carboxy-terminal region of the protein (unpublished data).

It has been previously reported that a short period of UV exposure increases the ability of *Synechocystis* sp. strain PCC 6803 to be transformed by nonhomologous DNA (2). Our data suggest that the UV exposure would result in the induction of the cyanobacterial *recA* protein and thus increase the recombinational capacity of the cell. It is possible that during the repair of the UV-induced DNA damage nonhomologous DNA is integrated illegitimately into the genome at the observed elevated frequency (2).

The kinetics of *A. variabilis recA* induction and deinduction during and after exposure to DNA-damaging agents are similar to those observed for SOS processing in *E. coli* (21, 22). In cyanobacterial cells in which DNA has not been damaged or DNA replication has not been inhibited, a basal level of *recA* transcription exists. The basal level of *recA* expression in normally growing cells is presumably responsible for the high level of homologous recombination observed in these organisms (23). The onset of DNA damage results in a rapid increase (relative to generation times) in both *recA* transcript and protein abundance, and these levels remain elevated while DNA-damaging conditions persist. Once damage ceases and the DNA is repaired, *recA* transcript abundance returns to basal levels.

In *E. coli*, this pattern of *recA* expression is initiated by a variety of DNA-damaging agents, including mitomycin C, MMS, and UV irradiation. These agents, which generate different types of DNA lesions, were also found to increase the cyanobacterial *recA* transcript and protein levels. Nalidixic acid, however, while a potent inducer of *recA* transcription in *E. coli*, had little impact on *A. variabilis*. The low permeability of this compound, as reported for other procaryotic organisms, may have reduced its effect (9). Alternatively, the cyanobacterial DNA gyrase may not be the site of nalidixic acid toxicity in these organisms.

Although the cyanobacteria and *E. coli recA* genes exhibit similar transcriptional and translational patterns in response to DNA damage, we have shown previously that the *A. variabilis recA* gene does not appear to complement the inducible SOS-processing system in *E. coli recA* mutants (16). Furthermore, we have observed that expression of the cyanobacterial *recA* gene is not induced in *E. coli recA* mutants as a result of DNA damage (unpublished data). The absence of an *E. coli*-like SOS box in the 5′ upstream region of the *A. variabilis recA* gene may very well explain these observations. The inability of the *lexA* repressor to bind to the cyanobacterial gene would result in constitutive expression that is unaffected by changing levels of DNA damage. It seems, therefore, that although the cyanobacteria possess an inducible, *recA*-dependent DNA repair system, the mechanism of induction-repression is not identical to the *E. coli lexA*-based system. Whether the cyanobacteria have a unique *lexA*-like repressor system or utilize a novel mechanism for regulation of *recA* expression is currently under investigation.

ACKNOWLEDGMENTS

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