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Expression of the *recA* Gene of *Pseudomonas aeruginosa* PAO Is Inducible by DNA-Damaging Agents

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Western (immunoblot) analysis using *Escherichia coli* anti-RecA antiserum revealed that expression of the RecA protein of *Pseudomonas aeruginosa* PAO is induced upon exposure of the bacterium to UV irradiation or norfloxacin, a quinolone related to nalidixic acid.

In *Escherichia coli*, the RecA protein is required for the process of homologous recombination and for the induction of the SOS gene control network (8). We have cloned a functional analog of the *E. coli recA* gene from *Pseudomonas aeruginosa* PAO (3–5). The *P. aeruginosa recA* gene product is an effector of stress-induced gene expression capable of mediating several SOS-like responses to DNA-damaging agents. It allows both the induction of lambda prophage and *din* gene (10) expression in *E. coli recA* mutants (3, 4). In *P. aeruginosa*, the RecA$^+$ protein is required for the UV-mediated induction of temperate prophages (4, 5) and for the quinolone-mediated induction of stable DNA synthesis (1).

As part of the SOS network, expression of the *E. coli recA* gene itself is induced after exposure of the cell to a number of DNA-damaging agents, including UV irradiation and quinolones such as nalidixic acid (8). Several observations suggest that the *P. aeruginosa recA* gene might also be inducible and autoregulated. First, mutants of the *P. aeruginosa recA* gene which demonstrate a synaptase-proficient (Rec$^+$), protease-constitutive (Prt^c) phenotype exhibit a higher level of resistance to UV irradiation than does their wild-type (protease-inducible) parent (C.-M. C. Ku, M.S. thesis, University of Tennessee, Knoxville, 1978), a level similar to that exhibited by Rec$^+$ Prt^c *recA* mutants of *E. coli* (7). Second, wild-type *recA908* (Rec$^-$ Prt^c) merodiploids of *P. aeruginosa* exhibit higher levels of UV resistance than do their haploid, wild-type parents (5). Third, the DNA sequence of the *P. aeruginosa* gene contains an SOS consensus sequence (LexA repressor-binding site) at its putative 5′ control region (6).

Yarranton and Sedgwick (10) demonstrated that certain nonfunctional (truncated) forms of the *E. coli* RecA protein are capable of sensitizing wild-type cells to UV irradiation when present on a multicopy plasmid. This sensitization is dependent on the overproduction of the truncated RecA polypeptide fragments following induction of expression by a DNA-damaging agent. The extent of this negative complementation depends on the size of the truncated polypeptide produced. Full sensitization requires peptides of at least 25% of the full-length gene product, while peptides of less than 10% of the wild-type protein have no effect. A number of pBR322-derived plasmids (4) which produce inactive forms of the *P. aeruginosa* RecA protein were tested for the ability to sensitize Rec$^+$ *E. coli* AB1157 and JM103 to UV irradiation. Plasmid pKML2006, in which the *P. aeruginosa recA* promoter is deleted, did not alter the UV sensitivity of the Rec$^+$ host strains (data not shown). Plasmids with Tn*5* insertions in the *P. aeruginosa recA* gene sensitized the Rec$^+$ hosts only slightly (Fig. 1A). Mapping (4) and DNA sequence (6) data suggest that these Tn*5*-inactivated clones produce polypeptides too small to confer more than minimal UV sensitivity. Since Tn*5* insertions producing larger truncated polypeptides could not be isolated (4), another approach was taken.

Weisemann et al. (9) determined that certain fusions of β-galactosidase to the *E. coli recA* gene also sensitize cells to UV irradiation. This sensitization is dependent on induced expression of the fusion peptide following UV irradiation. Plasmid pKML2031 containing a Mu d(*lac*) protein fusion late in the *P. aeruginosa recA* gene (4) was introduced into the Rec$^+$ strains of *E. coli*. Following UV irradiation, a significant increase in UV sensitivity of the host strains was observed, apparently due to an increase in expression of the *P. aeruginosa recA*–β-galactosidase fusion product (Fig. 1B). Several interpretations of these data are possible. However, when interpreted in light of the finding of Sano and Kageyama that the *P. aeruginosa recA* gene contains an SOS consensus sequence (6), the data suggest that the expression of the *P. aeruginosa recA* gene is regulated in *E. coli*, presumably by the *lexA* gene product.

In *E. coli*, induction of lambda lysogens takes place upon activation of the proteolysis-promoting activity of the RecA protein following exposure either to UV irradiation or to quinolones such as nalidixic acid (8). *P. aeruginosa* prophages D3 and F116L are induced following exposure of lysogens to UV irradiation. This induction requires that the lysogen have a RecA$^+$ phenotype (4, 5). To determine if quinolones might also elicit an inducible response in *P. aeruginosa*, the ability of norfloxacin to induce phage production in the Rec$^+$ D3 lysogenic RM5003 (5) was assessed. A culture of RM5003 was treated with norfloxacin (2 μg/ml), and the levels of spontaneous and norfloxacin-induced phage production were determined as previously described (3). Exposure to norfloxacin increased phage production 30-fold above the spontaneous level. This is approximately the same rate of induction as that produced by UV irradiation of this lysogen (5). As it appeared that exposure to either UV irradiation or norfloxacin elicited a stress-induced response in *P. aeruginosa*, these agents were assessed for the ability to induce increased expression of the *P. aeruginosa* RecA protein.

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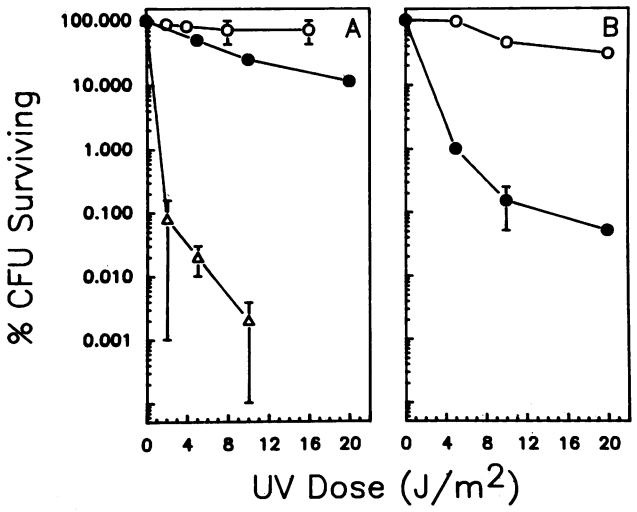

FIG. 1. UV irradiation sensitivity of Rec$^+$ strains of *E. coli* containing various forms of the inactivated *P. aeruginosa recA* gene. Cells were grown to a density of approximately 10^8 CFU/ml in Luria broth, pelleted, suspended in saline, and exposed to various fluences of UV irradiation. After appropriate dilution in saline, the irradiated cells were plated in duplicate on L agar (3) and incubated at 37°C overnight in the dark. The mean and the range of values of at least two repetitions are plotted. (A) Symbols: ○, AB1157 (Rec$^+$); ●, AB1157 containing pKML301 (*P. aeruginosa recA*::Tn*5*); △, JC2926 (*recA13*). (B) Symbols: ○, JM103 (Rec$^+$); ●, JM103 containing pKML2031 [*P. aeruginosa recA*::Mu d(*lac*)].

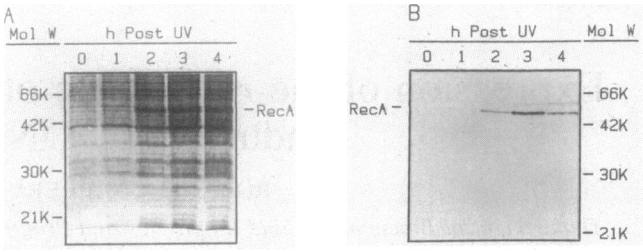

FIG. 2. Induction of the *P. aeruginosa* RecA protein by UV irradiation. PAO303 (Rec$^+$) was grown in Luria broth and exposed to 10 J/m^2 of UV irradiation as described in the text. All samples contained 8 Klett$_{660}$ units of cells. (A) Coomassie brilliant blue stained gel. (B) Immunoblot of identical gel. The larger-molecular-weight (Mol W) band visible in the induced cell lysates appears to be an aggregate of RecA protein, as extended boiling in sodium dodecyl sulfate was found to chase it into the lower RecA band. K, Kilodalton.

The *P. aeruginosa recA* gene product, a protein of an apparent molecular weight of 47,000 (4), is known to cross-react antigenically with anti-*E. coli* RecA antisera (4, 6). This cross-reaction was used to identify the *P. aeruginosa* RecA protein in cell lysates of the Rec$^+$ strain PAO303 (4) and to determine if either UV irradiation or norfloxacin could induce increased expression of this protein.

To test for induction by UV irradiation, cultures of PAO303 (Rec$^+$) were grown to mid-log phase, pelleted, suspended in saline, and exposed to 10 J/m^2 of UV irradiation as described previously (4). Following irradiation, the cells were again pelleted, suspended in Luria broth, and incubated in the dark at 37°C. Samples were taken at various times after irradiation. All samples were adjusted to contain a constant concentration of cells, pelleted, and suspended in 50 μl of 2× sample buffer (4). The samples were then boiled for 5 min, and 10 μl of each was loaded onto each of two identical 12.5% sodium dodecyl sulfate-polyacrylamide gels. Electrophoresis was carried out as previously described (4). After electrophoresis, one gel was stained with Coomassie brilliant blue and the second was transferred to a polyvinylidene difluoride membrane (Millipore Corp., Bedford, Mass.) as previously described (2). These membranes were subjected to Western (immunoblot) analysis using a biotinylated-streptavidin-horseradish peroxidase-conjugated antibody detection system (Bethesda Research Laboratories, Gaithersberg, Md.) as recommended by the manufacturer. *E. coli* anti-*recA* antibody (gift of S. Kowalczykowski, Northwestern University Medical School, Chicago, Ill.) was used as the primary antibody. This analysis revealed that the expression of the *P. aeruginosa recA* gene was inducible by exposure to UV irradiation (Fig. 2). The amount of RecA protein increased relative to other cellular proteins, reaching a maximum at 3 h postirradiation.

Similar experiments were carried out using norfloxacin as the inducing agent. Mid-log-phase cultures of PAO303 (Rec$^+$) and the isogenic RecA$^-$ strain RM8 (5) were divided, various amounts of norfloxacin (0, 1, 2, or 4 μg/ml) were added, and incubation was continued. Samples were withdrawn at various times and subjected to Western analysis as described above. Exposure to norfloxacin induced a specific increase in the concentration of the RecA protein in the Rec$^+$ strain PAO303. Maximum induction was observed at a concentration of 1 μg of norfloxacin per ml of medium, a concentration which is twofold higher than the MIC for this strain (1). When RM8 (RecA$^-$) was exposed to norfloxacin, Western analysis revealed no increase in the accumulation of RecA protein. These observations suggest that induction of the expression of the *P. aeruginosa recA* gene is autoregulated and dependent on the RecA$^+$ phenotype.

These data demonstrate that expression of the *P. aeruginosa* RecA protein is induced after exposure of the cell to DNA-damaging agents. The mechanism leading to this apparent induction has not been elucidated. Whether *P. aeruginosa* contains an analog of the *lexA* gene is now under investigation. In any case, the similarities between the *E. coli* and *P. aeruginosa* responses reported in this and other studies (1, 3–6) suggest that as in *E. coli* (8), induction of the *P. aeruginosa recA* gene is due to an increased rate of transcription.

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