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Autogenous Regulation and Kinetics of Induction of *Pseudomonas aeruginosa recA* Transcription as Analyzed with Operon Fusions

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A promoterless chloramphenicol acetyltransferase gene (*cat*) was used to construct *recA-cat* operon fusions to quantitatively examine the transcriptional regulation of the *Pseudomonas aeruginosa recA* gene in *P. aeruginosa* PAO. Wild-type *P. aeruginosa* containing the *recA8-cat* fusion was treated with methyl methanesulfonate (MMS) and showed immediate induction of chloramphenicol acetyltransferase (CAT) specific activity, whereas a *recA*::Tn*501* mutant of *P. aeruginosa* containing *recA8-cat* showed no induction with MMS. This indicated that a functional copy of *recA* was required for derepression of *recA* transcription and that *P. aeruginosa recA* protein was a positive regulatory factor promoting its own expression. Compared with that in the wild type, the uninduced level of CAT in *recA8-cat*-containing cells was reduced by approximately one-half in the *recA*::Tn*501* mutant, indicating that *recA*$^+$-dependent spontaneous induction contributes to the uninduced levels of *recA* expression in *P. aeruginosa*. MMS (0.012%) caused *recA*-directed CAT synthesis to increase almost immediately, with maximum CAT activity, fourfold higher than uninduced levels, attained at 60 min postinduction. The kinetics of *recA8-cat* fusion activity were shown to be directly related to the MMS doses used. Another fusion called *recAa1-cat*, where *cat* was located between the two transcriptional terminators of the *P. aeruginosa recA* gene, also showed dose-dependent induction by MMS, but the CAT activity from *recAa1-cat* was only one-half of that obtained with *recA8-cat* under the same conditions. Treatment of *recA*$^+$ *P. aeruginosa* containing *recA8-cat* with UV irradiation produced an immediate effect on *recA8-cat* transcription and showed little UV dose dependency at doses of 5 J/m^2 or greater. Treatment with 10 J/m^2 produced peak levels of *recA*-directed CAT activity, fivefold higher than background levels, by 60 min postirradiation; CAT activity remained at peak levels during the 120 min of the experiment. In contrast, nalidixic acid had a weak effect on *recA8-cat* expression in *P. aeruginosa*, although the response was dose dependent. Nalidixic acid (800 μg/ml) produced maximal CAT activity that was only twofold higher than background levels.

The *Escherichia coli recA* protein has been studied extensively and shown to be the major catalytic component of homologous recombination (for reviews, see references 11 and 40) and to have a positive regulatory role in the induction of various DNA repair pathways (for reviews, see references 18, 31, and 49). Transcription of the *E. coli recA* gene is normally repressed by the *lexA* protein (3). However, when *E. coli* cells are exposed to certain DNA-damaging agents or if a blockage to DNA replication occurs, then *recA* protein becomes activated and promotes autodigestion of the *lexA* repressor (30, 44). This inactivation of *lexA* repressor affects not only the derepression of *recA* but also numerous other unlinked genes which constitute the SOS system, many of whose products are involved in repairing DNA damage (24, 25, 31) and restoring stable DNA replication (26, 27). By using both mRNA hybridization and gene fusion techniques, the kinetics of *E. coli recA* induction have been studied under many different inducing conditions (4, 33–35, 45, 46, 50). The genetic regulation of the *recA* locus has been quantitatively examined only in *E. coli*, an organism which normally inhabits the specialized environment of the mammalian colon.

Gene products with activities similar to that of the *recA* protein of *E. coli* have been identified in several other bacterial species. It has been shown that synthesis of *recA* protein in the enteric soil dweller *Proteus mirabilis* is induced subsequent to UV irradiation (13), and its *recA* protein is capable of cleaving the *E. coli lexA* repressor (13, 51). Genetic studies suggest that the *Proteus mirabilis recA* gene is repressed by a *lexA*-like repressor in vivo (13). The *Shigella flexneri*, *Erwinia carotovora*, and *Proteus vulgaris recA* proteins also have the ability to promote *E. coli lexA* protein proteolysis, and all appear to be repressed by *lexA* protein when contained in an *E. coli* background (23). In *Bacillus subtilis*, the *recE* gene (a *recA* analog) is induced during competence development and treatments which promote recombinational repair (9); the *recE* gene may also be autogenously regulated and repressed by the *B. subtilis recA* protein (10).

We have previously reported the isolation of the *recA* gene from *Pseudomonas aeruginosa* and the construction and characterization of *P. aeruginosa recA* mutants (22, 37). *P. aeruginosa* is a common gram-negative organism which inhabits soil environments and is also an important opportunistic pathogen afflicting a variety of compromised human hosts. *P. aeruginosa recA* mutants are deficient in homologous recombination and are sensitive to DNA-damaging agents (22, 37). Such pleiotropic deficiencies are shared with *recA* mutants of *E. coli* (5). Since *P. aeruginosa recA* mutants show deficiencies that are qualitatively similar to those exhibited by *recA* mutants of *E. coli*, it has been proposed that their *recA* proteins have similar activities (37).

Because so little is known about *recA* gene regulation outside of *E. coli*, especially in organisms that are potentially exposed to a variety of mutagenic agents in soil and water

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TABLE 1. Bacterial strains and plasmids[a]

Strain or plasmid	Genotype or phenotype	Source (reference)
E. coli HB101	*proA2 leuB6 thi-1 lacY1 hsdR hsdM recA13 supE44 rpsL20*	This laboratory (2)
P. aeruginosa		
PAO1	Prototroph	D. Haas (20)
PDO2	*recA7*::Tn*501* Hg^r (PAO1 derivative)	This laboratory (22)
Plasmids		
pLAFR1	IncP1 λ *cos rlx* Tc^r	F. Ausubel (16)
pCP19	IncP1 λ *cos rlx* Tc^r	This laboratory (15)
pHC79ΔAp	pBR322 λ *cos* Ap^s Tc^r	This study
pUC8	Ap^r	This laboratory (47)
pCM4	pBR327 *cat* Ap^r	R. Rodriguez (6)
pHCAT4	pBR322 λ *cos cat* Ap^s Tc^r	This study
pJH2	pLAFR1 *recA*$^+$ (from *P. aeruginosa* PAO1) Tc^r	This laboratory (22)
pJH23	pJH2 *recAa1-cat* Tc^r Cm^r	This laboratory (22)
pORV2	pCP19 *recA*$^+$ (from *P. aeruginosa* PAO1) Tc^r	This study
pJH36	pORV2 *recA8-cat* Tc^r Cm^r	This study

[a] Abbreviations: r, resistant; s, sensitive; Tc, tetracycline; Ap, ampicillin; Cm, chloramphenicol; Hg, mercury.

environments, we have examined the regulation of the *P. aeruginosa recA* locus. Using Northern (RNA) and Western blot (immunoblot) analyses, we recently demonstrated that the *P. aeruginosa recA* gene is induced by a DNA-damaging agent (i.e., methyl methanesulfonate [MMS]) (22). Also, we have presented evidence for negative regulation of *P. aeruginosa recA*; when several *recA* mutants were characterized, one *recA* allele (*recA102*) showed apparent defects in its interaction with a *lexA*-like repressor (22). In the present study, operon fusions were used to examine the regulation of *P. aeruginosa recA* because they provide an accurate relative measurement of gene expression (50). The construction of operon fusions (using a promoterless chloramphenicol acetyltransferase [CAT] gene) allowed us to assay for the fusion gene product under various inducing treatments and in different genetic backgrounds. We demonstrate here that the *P. aeruginosa recA* gene product is a positive effector of *P. aeruginosa recA* transcription, just as the *E. coli recA* protein is in *E. coli*. However, significant differences were found when we compared the kinetics of transcription from the *P. aeruginosa recA* with those reported for *E. coli recA* under similar inducing conditions. Two operon fusions within the *P. aeruginosa recA* gene were also compared and shown to have distinct properties.

MATERIALS AND METHODS

Strains and plasmids. Bacterial strains and plasmids used in this study are shown in Table 1.

Media. L medium was 1% tryptone (Difco Laboratories), 0.5% yeast extract (Difco), and 0.5% NaCl. NY medium was 0.8% nutrient broth (Difco) and 0.5% yeast extract. Minimal medium (VB medium) was that described by Vogel and Bonner (48). Media were solidified with 1.5% agar (Difco). Antibiotics were used in selection media at the following concentrations (per milliliter): tetracycline, 20 μg for *E. coli* and 100 μg for *P. aeruginosa*; and chloramphenicol, 25 μg for *E. coli* and 100 μg for *P. aeruginosa*. Resistance to MMS was tested by patching bacteria onto an agar medium prepared daily by spreading 200 μl of 1.5% MMS on the surface of an L agar plate (i.e., ~0.012% MMS).

DNA techniques and mobilization of recombinant plasmids. Plasmid DNA was isolated from *E. coli* by the method of Birnboim and Doly (1) and purified on ethidium bromide-cesium chloride gradients. Plasmids were used to transform calcium chloride-treated (7) *E. coli*. Restriction endonucleases (Boehringer Mannheim Biochemicals), T4 DNA ligase (New England BioLabs, Inc.), and calf intestinal alkaline phosphatase (Boehringer Mannheim) were used in enzyme reactions as specified by the manufacturers. Restriction fragments were examined by agarose gel electrophoresis as described by Maniatis et al. (32). Recombinant plasmids were constructed in the broad-host-range IncP1 vector pLAFR1 (16) or in the related plasmid pCP19 (15) and were mobilized from *E. coli* to *P. aeruginosa* in triparental matings (17) by using the conjugative helper plasmid pRK2013 (14).

Construction of the *recA8-cat* operon fusion in pJH36. An operon fusion located in the *P. aeruginosa recA* protein-coding region was constructed (Fig. 1) by replacing a 0.7-kilobase (kb) segment from the 3′ end of *recA* with a promoterless *cat* gene, which encodes CAT. pHC79ΔAp was a derivative of pHC79 (19), in which the 0.65-kb *Pst*I-*Eco*RI fragment was replaced with a 26-base-pair *Pst*I-*Eco*RI multiple cloning site from pUC8 (47). pHC79ΔAp was digested with *Bam*HI in the presence of ethidium bromide to cut only one of its two potential sites (38) and ligated to a *Bam*HI *cat* cartridge obtained from pCM4 (6). pHCAT4 (Fig. 1) had the *cat* gene in the multiple cloning site of pHC79ΔAp, with the direction of *cat* transcription oriented *Pst*I to *Hin*dIII. pORV2 (Fig. 1) was constructed by ligating a 2.4-kb *Bam*HI-*Hin*dIII fragment containing the *P. aeruginosa* PAO1 *recA* gene from pJH2 (22) into the broad-host-range vector pCP19. The *cat*-containing *Pst*I-*Hin*dIII fragment of pHCAT4 was then used to replace the terminal 0.7-kb *Pst*I-*Hin*dIII fragment of the *recA* gene in pORV2 to form pJH36. HB101(pJH36) displayed weak chloramphenicol (10 μg/ml) resistance and sensitivity to MMS. pJH36 retained the proximal 0.54 kb of the *P. aeruginosa recA*-coding sequence, thus putting the promoterless *cat* gene under the transcriptional control of the *recA* promoter (Fig. 1). The *recA* allele in pJH36 was termed *recA8-cat*.

Procedures for induction of *recA*. To measure the induction responses of the *P. aeruginosa recA* promoter and operator due to treatment with MMS or nalidixic acid, cells were grown at 37°C with aeration in NY medium containing tetracycline to a density of 5×10^8 cells per ml. A 20-ml sample was removed (time zero), and the remaining culture

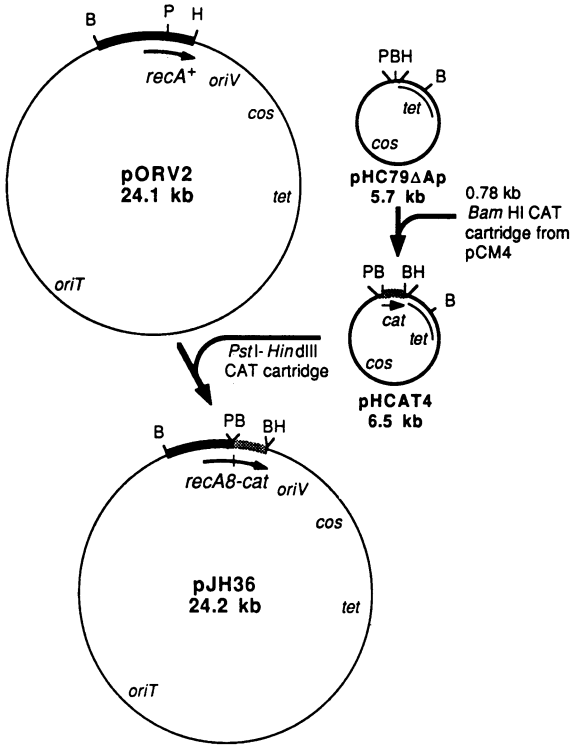

FIG. 1. Construction of pJH36 containing the *P. aeruginosa recA8-cat* operon fusion. A promoterless *cat* gene (0.78 kb) from pCM4 was inserted into a *Bam*HI site of pHC79ΔAp, with the direction of *cat* gene transcription as shown, to form pHCAT4. pORV2 was an IncP1 (broad-host-range) replicon containing 2.4 kb of *P. aeruginosa* PAO1 which included the *P. aeruginosa recA* gene. The *cat* cartridge was excised from pHCAT4 with *Pst*I and *Hin*dIII and ligated into pORV2, which had been cut with the same enzymes. pJH36 contained the *cat* gene in replacement of the terminal 0.7 kb of the *P. aeruginosa recA* gene, and *cat* was in the same transcriptional direction as *recA*. Restriction endonuclease cleavage sites shown: B, *Bam*HI; H, *Hin*dIII; and P, *Pst*I. Symbols: ▭, *cat* cartridge.

continued incubating with either MMS or nalidixic acid at the doses indicated below; samples (20 ml) were removed at regular intervals. To measure *recA* induction due to treatment with UV light, cells were grown in VB glucose medium containing tetracycline to a density of 5×10^8 cells per ml, and a 20-ml sample was removed (time zero). The remainder of the culture (180 ml) was transferred to a baking dish (30 by 20 cm) and then irradiated in a dark room with UV light (predominantly 254 nm) from a germicidal lamp with the doses indicated below at a rate of 1 J/m^2 per s. The cultures were immediately transferred to foil-covered flasks and incubated at 37°C with aeration, with samples (20 ml) removed at regular intervals under dim light.

CAT assays. Cells were removed from samples by centrifugation (6,000 × *g* for 15 min), washed with 5 ml of cold saline (0.85% NaCl), and suspended in 2.0 ml of cold 30 μM dithiothreitol–50 mM Tris hydrochloride (pH 7.8). The cells were lysed by sonication (30 W, two 30-s pulses), and insoluble proteins were removed by centrifugation (9,000 × *g* for 15 min). The supernatant was assayed for CAT activity by the method of Shaw (43), and values were converted to CAT activity (nanomoles per minute per milliliter) by the method of Rodriguez and Tait (41). Protein concentrations were determined by the method of Larson et al. (28), and specific CAT activity (nanomoles per minute per milligram of total protein) was determined by normalizing the CAT activity to total protein concentration.

RESULTS

Autogenous regulation of the *P. aeruginosa recA* gene. A *recA* operon fusion, pJH36 (Fig. 1), was constructed by replacing 0.7 kb from the terminus of *recA* with a *cat* cartridge (*recA8-cat*; see Materials and Methods), thus inactivating the plasmid-borne *recA* gene product and putting *cat* transcription under the control of the upstream *recA* promoter and operator. The *recA8-cat* construct in pJH36 was contained on an IncP1 (i.e., broad-host-range and low-copy-number [2 to 4 copies per chromosome]) replicon. CAT specific activity was used as an indicator of the relative level of transcription originating from the *recA* promoter, and the *recA8-cat* operon fusion in pJH36 was used to determine whether *recA* expression in *P. aeruginosa* was autoregulatory. PDO2 is a *recA7*::Tn*501* mutant of *P. aeruginosa* PAO1 that we recently constructed which contains no detectable *recA* protein and shows increased sensitivity to DNA-damaging agents such as MMS and UV irradiation (22). PAO1(pJH36) was Cm^r and MMS^r, indicating that the plasmid-borne *recA8-cat* fusion was expressed in this host. In contrast, PDO2(pJH36) was Cm^r but remained MMS^s, indicating that there was some uninduced transcription of the plasmid-borne *recA8-cat* fusion in the absence of a functional copy of *recA*.

The relative transcriptional activities of the *recA8-cat* fusion in *recA*$^+$ and *recA7* mutant hosts were determined by assaying for CAT specific activities in growing cultures of PAO1(pJH36) and PDO2(pJH36) following treatment with MMS. The untreated PAO1(pJH36) cells showed a constant basal level of CAT specific activity during logarithmic-phase growth (Fig. 2A). The MMS (0.012%)-treated PAO1(pJH36) cells showed almost immediate induction of *recA8-cat* and attained a specific CAT activity fourfold greater than the uninduced basal level (Fig. 2A). In contrast, *recA8-cat* in the *recA7* mutant PDO2(pJH36), when treated in an identical fashion, remained uninduced for CAT (Fig. 2A). These results demonstrated that *P. aeruginosa* required a functional copy of *recA* for *recA* transcription to be induced by MMS. Thus, the *recA* protein in *P. aeruginosa* is a positive regulatory factor that promotes its own expression, as does the *recA* protein in *E. coli* (35). Also, the uninduced level of CAT activity in PDO2(pJH36) was approximately one-half of that seen in PAO1(pJH36) (Fig. 2A), indicating that *recA*$^+$-dependent spontaneous induction contributes to the uninduced level of *recA* expression in *P. aeruginosa*.

MMS dosage-dependent induction kinetics of two *recA-cat* fusions in *P. aeruginosa*. When cultures of PAO1(pJH36) were grown in the presence of different concentrations of MMS, both the magnitude and the rate of *recA8-cat* expression were affected. As described above, MMS at a concentration of 0.012% caused *recA*-directed CAT synthesis to increase almost immediately; the specific CAT activity was twofold greater than the basal level by approximately 15 min after induction (Fig. 2A). Maximum CAT activity was fourfold greater than the uninduced level by 60 min after the initial inducing treatment, and then CAT activity declined (Fig. 2A). At one-half the MMS concentration (i.e., 0.006%), a lower rate of *recA8-cat* transcription was observed. There was a 15-min delay in the onset of induction, and the time required for CAT activity to increase to twofold higher than the basal level was about twice as long (i.e., 35 min) as that

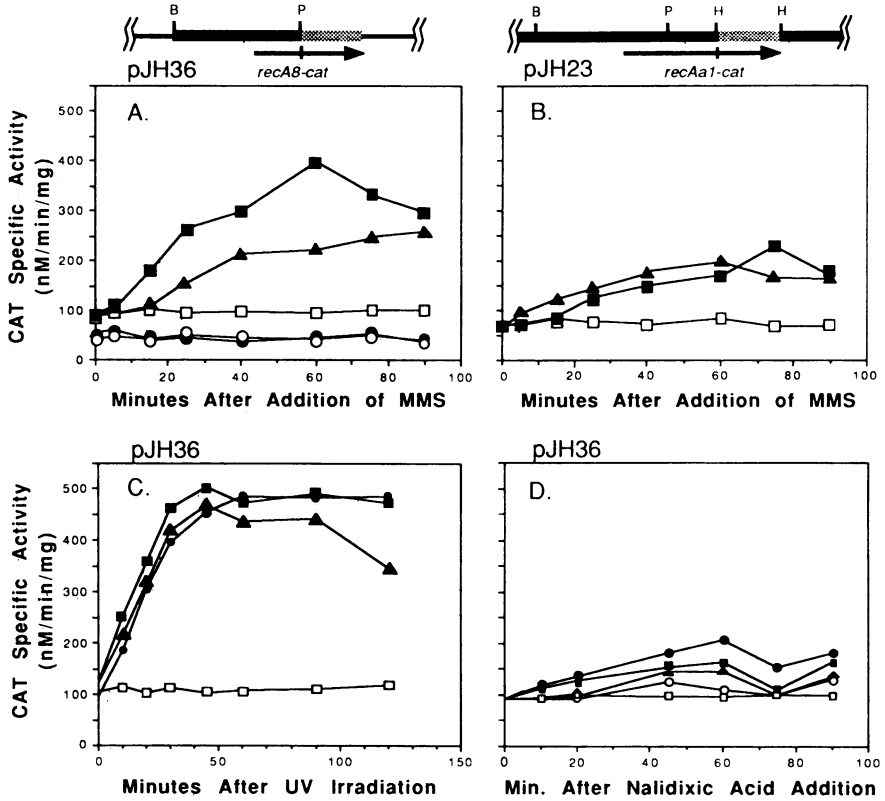

FIG. 2. CAT specific activity in *P. aeruginosa recA*$^+$ PAO1 or *recA7* mutant PDO2 that contained *recA8-cat* (pJH36; top, left) or *recAa1-cat* (pJH23; top, right) after treatment with MMS, UV, or nalidixic acid. Periodic samples were taken from cultures during logarithmic growth and assayed for CAT specific activity. Values represent the averages of three or more comparable experiments. (A) Strains harboring *recA8-cat* (pJH36). PDO2 with no treatment (○) or treated with 0.012% MMS (●) and PAO1 with no treatment (□) or treated with 0.006% (▲) or 0.012% (■) MMS. (B) PAO1 harboring *recAa1-cat* (pJH23): no treatment (□) or treatment with 0.012% (■) or 0.006% (▲) MMS. (C) PAO1 harboring *recAa1-cat* (pJH36): no treatment (□) or treatment with 5 (▲), 10 (■), or 30 (●) J of UV light per m^2. (D) PAO1 harboring *recA8-cat* (pJH36): no treatment (□) or treatment with 50 (○), 200 (▲), 500 (■) or 800 (●) μg of nalidixic acid per ml.

for cultures treated with twice as much (0.012%) MMS. By 90 min after the addition of 0.006% MMS to the culture, CAT specific activity attained a peak level that was just 2.5-fold greater than the uninduced level (Fig. 2A). Thus, the kinetics of *recA8-cat* fusion activity were directly related to the MMS doses used. However, at reduced concentrations of MMS (equal to or less than 0.0012%) in the culture, there was no detectable effect on *recA8-cat* expression (data not shown).

We recently constructed an alternate *recA-cat* operon fusion, pJH23, which contains the promoterless *cat* gene inserted in the *Hin*dIII restriction site located near the 3′ end of the *recA* gene (Fig. 2B) (22). Like pJH36, pJH23 is an IncP1 replicon and therefore has the same low copy number. pJH23 retains its *recA*$^+$ activity because the *cat* insertion is downstream of the *recA* protein-coding sequence but upstream of a *recA* transcriptional terminator (22). We have identified two transcriptional terminators in the *P. aeruginosa recA* gene, one upstream (*recAt1*) and one downstream (*recAt2*) of the *Hin*dIII site; therefore, the *cat* gene in pJH23 is located between these two termination sites (22). We called the fusion in pJH23 *recAa1-cat* to indicate that the *cat* gene is in an mRNA trailer of *recA*. When CAT activities in PAO1(pJH23) cells were determined under both noninduced and MMS-induced conditions, both the basal and induced levels of *recAa1-cat* expression were reduced compared with those for PAO1(pJH36) under the same conditions. PAO1(pJH23) displayed a basal level of CAT synthesis that was ~25% lower than that of PAO1(pJH36) (Fig. 2A and B). When treated with 0.012% MMS, the *recAa1-cat* in PAO1(pJH23) showed induction, but the peak level of CAT activity was approximately one-half the peak level of *recA8-cat* expression that was observed in PAO1(pJH36) as described above (Fig. 2A and B). At half the concentration of MMS (i.e., 0.006%), PAO1(pJH23) cells contained approximately the same specific CAT activity as the cells treated with the higher concentration of MMS. Thus, the transcriptional activity at the *cat* insertion in pJH23, which is downstream of the first termination site, is less active than the transcriptional activity of the CAT insertion in pJH36, which is located nearer the 5′ end of the *recA* coding sequence. Alternatively, the stability or efficiency of translation of *recAa1-cat* mRNA may be reduced compared with that of *recA8-cat* mRNA.

Rapid induction of *recA8-cat* in *P. aeruginosa* following UV irradiation. The treatment of *P. aeruginosa* PAO1(pJH36 *recA8-cat*) with moderate doses of UV light produced an immediate effect on *recA* transcription that showed little UV dose dependency at doses of 5 J/m^2 or greater (Fig. 2C). When cultures were irradiated with 5, 10, or 30 J of UV light per m^2, the level of *recA*-directed CAT synthesis increased at a uniformly high rate compared with the rate following induction with MMS. For all UV doses tested, CAT activity was twofold higher than the basal level by only 10 min after induction, and no delay in the onset of CAT activity was observed. The peak level of CAT activity was observed at about 45 min following UV treatment and was 4.5- to 5-fold

greater than the uninduced level of activity. The level of *recA*-directed CAT activity remained at peak levels during the 120 min of the experiment when cells were treated with a higher dose (10 or 30 J/m^2) of UV; however, when cells were treated with a lower dose (5 J/m^2), CAT levels had fallen by approximately one-third at 120 min postirradiation (Fig. 2C).

Nalidixic acid a poor inducing agent of *recA* transcription in *P. aeruginosa*. Nalidixic acid had a weak effect on *recA8-cat* expression in PAO1(pJH36) compared with MMS and UV as inducing agents, although the response was dose dependent (Fig. 2D). The highest concentration of nalidixic acid used (800 μg/ml) produced the highest level of CAT activity, but maximal CAT activity was only twofold greater than that at the uninduced level. Nalidixic acid at 500 and 200 μg/ml produced a slightly lower response, and the increase in *recA8-cat* expression was barely detectable with nalidixic acid at 50 μg/ml (Fig. 2D). The highest level of *recA8-cat* expression was generally observed 60 min after addition of the inducing agent. An initial lag in *recA8-cat* induction was observed at doses of 50 and 200 μg/ml. For all doses of nalidixic acid tested, a noticeable decrease in CAT activity was observed shortly after cells had attained their highest level of induction, but this decrease was always followed by a second increase in CAT activity that began at about 75 min after addition of the inducing agent (Fig. 2D).

Because treatment of *P. aeruginosa* with such high concentrations of nalidixic acid was required to elicit even a mild induction response of *recA8-cat*, we tested the effect of nalidixic acid on the ability of *P. aeruginosa* PAO1(pJH36) to grow in culture (NY medium with tetracycline). Compared with the growth rate of untreated culture, which had a doubling time of approximately 35 min, treatment with 200 μg of nalidixic acid per ml reduced the growth rate only about 15%; treatment with 800 μg of nalidixic acid per ml reduced the growth rate by approximately 60% (data not shown). The fact that log-phase cells were still capable of limited growth in the presence of high concentrations of nalidixic acid indicated that some functional DNA gyrase, the target site of nalidixic acid, was still present. *P. aeruginosa* is known to be generally more resistant to antibiotics than enteric bacteria are, and this resistance has been linked to the relative impermeability of its outer membrane (36). Therefore, it is likely that the mild effects of nalidixic acid treatment on *recA8-cat* induction were due to lower effective intracellular concentrations of this drug, which are related to the slow transport of the acid into the cell.

DISCUSSION

When *E. coli* is exposed to DNA damage or when blockage to DNA replication occurs, a signal is produced that activates the *recA* protein to stimulate autodigestion of the *lexA* repressor protein (44), thereby derepressing transcription of the *recA* gene (18, 31, 49). The signals that activate *recA* protein to induce *lexA* protein proteolysis are undefined but may include nucleotides, oligonucleotides, and damaged single- or double-stranded DNA (8, 33, 39). It has been suggested that the concentration of activated *recA* protein is a function of the linear density of DNA lesions, which is determined by the dose of inducing agent (49).

It is likely that the *recA* gene in *P. aeruginosa* is similarly repressed by a *lexA*-like repressor protein and undergoes a release of negative regulation involving cleavage of that repressor. The nature of the *recA102* allele in *P. aeruginosa* appears to be to interact with a *lexA*-like repressor (22). Sequence analysis of a region upstream of the *P. aeruginosa* *recA*-coding sequence revealed consensus to the *lexA*-repressor-binding sites of a number of *lexA* protein-repressed genes in *E. coli* (42). Furthermore, the *P. aeruginosa recA* protein is capable of cleaving the λ phage cI repressor (J. M. Horn and D. E. Ohman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, H63, p. 118), which shares the same Ala-Gly cleavage site as *lexA* protein (21), although where *recA* protein binds to these repressors is not known.

In this study, we used operon fusions in which the *P. aeruginosa recA* regulatory region controlled the transcriptional activity of a promoterless *cat* gene to examine the expression of *recA* in *P. aeruginosa* following treatment with various inducing agents. Although the mRNA stability and translational efficiency of an operon fusion may not exactly mimic those of the target gene, assaying *recA-cat* fusion activity can provide an accurate relative measure of the rates of *recA* expression (50). We found that *recA8-cat* in a *recA* mutant background was uninducible upon treatment with MMS, whereas it was clearly MMS inducible when contained in a *recA*$^+$ background. This indicated that, as in *E. coli*, the *P. aeruginosa recA* protein was a positive regulatory factor affecting the derepression of its own transcription. This finding corroborates evidence which we have previously reported on the transcriptional activation of the mutant chromosomal *recA2* and *recA102* alleles by a plasmid-borne *recA*$^+$ gene in *P. aeruginosa* PAO (22). We also observed in the present study that the basal level of *recA* transcription in a *recA* mutant was half the specific activity found in an isogenic *recA*$^+$ background, suggesting that spontaneous activation of *recA* protein contributes to the uninduced level of transcription of the *P. aeruginosa recA* gene. This correlates with studies done with *E. coli* by Casaregola et al. (4) in which the basal level of *recA* transcription in an *E. coli recA* mutant background, as measured by a *recA-lac* operon fusion activity, was 65% of the uninduced activity observed in an *E. coli recA*$^+$ background. Also, Little (29) has demonstrated that there is some cleavage of *lexA* protein in uninduced *recA*$^+$ *E. coli*.

P. aeruginosa recA gene expression was dose dependent for treatment with both MMS and nalidixic acid, and as the extracellular drug concentration increased, the *recA*-controlled CAT activity was induced coordinately. Induction of the *E. coli recA* gene by 0.025% MMS or only 40-μg/ml nalidixic acid treatments resulted in a 10-fold induction of *recA* transcriptional activity (4), whereas the *P. aeruginosa recA* gene showed markedly reduced induction ratios for the two drugs. However, the impermeability of *P. aeruginosa* to antibiotics has been documented (36), and the actual intracellular concentrations of these drugs may be low compared with the concentrations in the medium. Also, delays seen in the onset of *recA* induction at the lower concentrations of MMS and nalidixic acid may reflect the time needed to accumulate enough signal, or activated *recA* protein, to derepress the *recA* gene. By contrast, in *E. coli*, no lag times are associated with nalidixic acid-induced *recA* expression (33, 35).

When *P. aeruginosa recA*$^+$-containing *recA8-cat* (pJH36) was continuously treated with 0.012% MMS, the peak level of CAT activity was seen 60 min after addition of the inducing agent, and then a decrease in *recA* CAT expression was observed. This drop in *recA*-directed CAT activity, even though the inducing agent was presumably still present, may have been due to the rapid repair of DNA lesions by high concentrations of induced *recA* protein and other DNA repair enzymes within the cell. As the density of DNA

lesions decreased, the level of signal for activation of *recA* protein declined, resulting in a depression of the level of *recA* transcription. In addition, time-dependent degradation of MMS in the medium under these experimental conditions may also contribute to the decrease in *recA* expression.

After more than 60 min following the addition of nalidixic acid, decreases in *recA8-cat* expression were observed for all concentrations tested. Decreases in levels of *E. coli recA* mRNA were also observed during the same interval after the addition of nalidixic acid to the medium (33). In our studies on *P. aeruginosa*, these decreases were soon followed by an increase in *recA*-directed CAT activity. Although the reason for the decrease and subsequent increase in *recA* expression by nalidixic acid cannot be explained from these data, it is possible that nalidixic acid has transcriptional effects on the *recA* gene in *P. aeruginosa*. Nalidixic acid treatment has been shown to cause transcriptional effects on some genes in *E. coli* (12). Alternatively, *P. aeruginosa* cells may become more permeable to nalidixic acid when exposed for more than 60 min.

We have also examined the induction of *recA* by MMS by using pJH23, a plasmid that contains the *recAa1-cat* fusion in which the *cat* gene is inserted between the two transcriptional terminators (*recAt1* and *recAt2*) of the *P. aeruginosa recA* gene. We had previously identified two lengths of *recA* mRNA corresponding to transcription terminating at either the upstream (1.2-kb) or downstream (1.4-kb) termination sites (22). We also had noted that the shorter transcript was in higher concentration, especially during uninduced conditions (22). Here, we compared the CAT specific activities of pJH23 and pJH36, noting that transcriptional activity was greater from the fusion (pJH36) that contains the *cat* gene near the 5′ end of *recA*. This finding corroborates our previous observations that the downstream terminator seems to be used less frequently than the upstream terminator. Positional effects of the fusion in relation to the *recA* promoter could account for these observations, and such positional effects have been described elsewhere (4). However, it has also been observed that different *recA-lacZ* fusions with the *E. coli recA* gene, while displaying different basal levels of β-galactosidase activity, maintain the same induction ratios (4). Thus, the lower induction ratio displayed by *recAa1-cat* compared with *recA8-cat* may reflect a decreased level of transcription in this region of the *P. aeruginosa recA* gene.

In contrast to the situation observed with MMS or nalidixic acid induction, both the rates and extent of activity from *recA-cat* were largely independent of the UV doses were tested. This observation indicates that a low level of UV-generated signal is sufficient to fully activate *recA* protein. Because there are no transport effects associated with UV treatment, it is possible to directly compare the *E. coli* and *P. aeruginosa recA* induction responses at similar UV doses. Operon fusion assay techniques in *E. coli* have shown a dose-dependent response of the *E. coli recA* gene to UV treatments. Using a *recA-lac* operon fusion, Weisemann et al. (50) have shown a marked difference in *E. coli recA* induction kinetics at 5 versus 30 J of UV per m^2. Smith (45), using a *recA-trpED* operon fusion, has also shown that the extent of *E. coli recA* induction varies widely from 5 to 30 J of UV exposure per m^2, and Vericat et al. (46) have shown a similar dose dependency in the extent of *E. coli recA* induction over the same dose range.

It is apparent that the *P. aeruginosa recA* induction response to UV-induced damage is much more sensitive than that of *E. coli*. Our data showed that *P. aeruginosa recA* is expressed at the same high level, and with nearly the same high rate of induction, at UV doses of 5 to 30 J/m^2. *P. aeruginosa* is a soil-dwelling organism that is presumably exposed to high levels of UV daily. Therefore, it is reasonable to expect that *P. aeruginosa* would evolve mechanisms to cope with a higher level of UV-induced damage than a coliform organism like *E. coli*. Among these adaptations, the ready induction of DNA-repair enzymes would be most advantageous. However, in light of the data presented here, which indicate that the induction of *recA* by UV irradiation is more sensitive in *P. aeruginosa* than in *E. coli*, it is curious that *recA*$^+$ *P. aeruginosa* FRD is killed more readily than *recA*$^+$ *E. coli* K-12 when exposed to equivalent UV doses (37).

The length of persistence in the induction response is another aspect of the kinetics of *recA* UV induction that differs in *E. coli* and *P. aeruginosa*. Two studies measuring the increase in *recA* mRNA in *E. coli* after exposure to 10 J of UV per m^2 have shown that the level of *recA* transcripts decreases approximately 20 min after the UV treatment (33, 34), although the actual rate of *lexA* protein cleavage may not return to normal until much later (29). At UV doses of both 5 and 30 J/m^2, Weisemann et al. (50) have likewise demonstrated a decrease in the β-galactosidase activity of an *E. coli recA-lacZ* fusion after the peak level of specific activity had been reached. However, we observed that the *P. aeruginosa recA8-cat* fusion (pJH36) maintained peak-level CAT specific activity at doses of 10 and 30 J of UV per m^2 for at least 120 min after the UV pulse. Only with a low dose of UV (5 J/m^2) did the *recA8-cat* fusion start to decrease by 45 min postirradiation. Thus, activation of the *P. aeruginosa recA* protein apparently requires fewer UV lesions than does activation of the *recA* protein in *E. coli*.

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