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Characterization of the *Pseudomonas aeruginosa recA* Analog and Its Protein Product: *rec-102* Is a Mutant Allele of the *P. aeruginosa* PAO *recA* Gene

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We cloned a 2.3-kilobase-pair fragment of the *Pseudomonas aeruginosa* PAO chromosome which is capable of complementing *recA* mutations of *Escherichia coli*. The *recA*-complementing activity was further localized to a 1.5-kilobase-pair *Pvu*II-*Hin*dIII fragment. Southern blot analysis under conditions of high stringency indicated that DNA sequence homology is shared by the *E. coli recA* gene and the *P. aeruginosa recA* analog. The cloned *recA* analog was shown to restore resistance to methyl methanesulfonate, nitrofurantoin, and UV irradiation to *E. coli recA* mutants. Upon introduction of the cloned *P. aeruginosa* gene, these mutants regained recombination proficiency in HfrH-mediated conjugation and the ability to induce lambda prophages and SOS functions (*din* gene transcription) after exposure to DNA-damaging agents. Lambda prophage carrying a cI *ind* mutation was not inducible, suggesting that the mechanism of induction of these SOS functions by the *P. aeruginosa* RecA analog is similar to that by the activated *E. coli* RecA protein. The product of the *recA* analog was identified in minicells as a protein of approximately 47,000 daltons. Western blot analysis using anti-*E. coli* RecA antibody demonstrated that this protein is antigenically cross-reactive with the *E. coli recA* protein. The *recA*-containing fragment was cloned into the broad-host-range vector pCP13 and introduced into Rec$^-$ strains of *P. aeruginosa* containing the *rec-102* allele. The plasmid was shown to restore recombination proficiency in FP5-mediated conjugations and to restore resistance to UV irradiation and methyl methanesulfonate to these Rec$^-$ mutants. It was shown that a wild-type allele of *rec-102* is necessary for UV-mediated induction of D3 and F116 prophages. The cloned *recA* analog restored the UV inducibility of these prophages in *rec-102* mutants. These data indicate that *rec-102* is a mutant allele of the *P. aeruginosa recA* gene and suggest that there has been considerable conservation of the *recA* gene in the evolution of the gram-negative bacteria.

In *Escherichia coli* the protein product of the *recA* gene is required for several processes. Homologous recombination requires the synaptase activity of this protein (10, 49). The exact role of the RecA protein in the process of recombination is complex. In vitro the protein has been demonstrated to possess ATPase activity and the ability to promote invasion of duplex DNA by single-stranded homologous regions (29, 44, 50). In addition to its structural and catalytic roles in homologous recombination, the *recA* gene product is required to initiate the response of the SOS network to DNA-damaging agents (23). Damage to DNA causes the RecA protein to become activated to a condition in which it promotes the rapid cleavage of the *lexA*-encoded repressor protein, relieving repression of the SOS network (47). Activated RecA protein also promotes the cleavage of the bacteriophage lambda cI repressor, causing induction of the lambda prophage to lytic growth (36, 37).

Analogs of the *E. coli recA* gene have been isolated from several diverse species of bacteria by complementation in *trans* of *E. coli recA* mutations (4, 18, 22, 24). These heterologous genes have been expressed at least nominally in *E. coli*, and Southern blot analysis has revealed DNA sequence homology among several isolates (22, 43). We have identified, from a random library of *Pseudomonas aeruginosa* PAO1 chromosomal DNA, sequences capable of complementing several of the pleiotropic effects of various *E. coli recA* mutations (24). It is therefore possible that the product of a *recA* analog executes the same functions in *P. aeruginosa* as does the *recA* gene product in *E. coli*. If so, a *recA* mutant of *P. aeruginosa* should exhibit a greater sensitivity to UV irradiation and agents that damage DNA such as mitomycin C (MMC) and methyl methanesulfonate (MMS). *P. aeruginosa recA* mutants would be expected to show a greatly reduced ability to support homologous recombination. In addition, it seems likely that temperate phages of *P. aeruginosa* capable of UV induction to lytic growth from the prophage state would require the presence of a functional *recA* analog.

Several Rec$^-$ mutants of *P. aeruginosa* PAO have been described which exhibit a number of these characteristics (9, 17, 31). Früh et al. (17) reported in 1983 the isolation and characterization of several Rec$^-$ strains of *P. aeruginosa* PAO. The origin of the nitrosoguanidine-mutagenized DNA used to construct these mutants was *P. aeruginosa* PAT. These strains were found to be markedly deficient in the ability to recombine DNA received by either transduction or R68.45-mediated conjugation, to be more sensitive to UV irradiation than is the parental strain, and to demonstrate MMC inhibition of growth. The allele contained in these strains, *rec-102*, was mapped to approximately 42 min on the *P. aeruginosa* PAO chromosomal map. Miller and Ku (31) reported the isolation of a number of mutant strains of *P. aeruginosa* PAO deficient in the establishment of lysogeny. One of the mutations isolated, *lesB908*, had several properties suggesting that it may be analogous to *recA* mutations of *E. coli*. Strains containing *lesB908* are sensitive to UV irradiation and are unable to undergo homologous recombination. This mutation was mapped to the 40- to 45-min

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region of the PAO chromosomal map. In addition, Ohman et al. (32), using a gene replacement technique, isolated a *recA* mutant of the clinical isolate *P. aeruginosa* FRD.

We have previously reported the isolation and characterization of a *P. aeruginosa recA* analog (24). In that report we described activity of the analog in homologous recombination and prophage induction. In this paper we extend these analyses, more precisely delineate the *recA*-complementing DNA sequences, and identify the protein product of the *P. aeruginosa recA* analog. This *recA* analog was subcloned into a broad-host-range plasmid and mobilized into *P. aeruginosa rec-102*-containing strains. The effect of this plasmid on the pleiotropic phenotype conferred by the *rec-102* mutation is also reported in this paper.

(Portions of this work will be submitted by T.A.K. to the Graduate School, Loyola University of Chicago, in partial fulfillment of the requirements for the Ph.D. degree.)

MATERIALS AND METHODS

Bacteria and bacteriophages. The bacterial strains used are listed in Table 1. The bacteriophages used are described in Table 2.

Plasmids. Plasmids pKML1 and pKML2 contain the *P. aeruginosa recA* analog within approximately 25- and 9.2-kilobase-pair (kbp) chromosomal DNA fragments, respectively (24). Plasmids pKML2003 and pKML2004 are subclones of pKML2 that contain the entire *P. aeruginosa recA* analog on smaller chromosomal DNA fragments in the vector pBR322. Plasmid pKML2006 is a Rec^- deletion derivative of pKML2003, with the segment between the *Bam*HI and *Bgl*II sites deleted.

Plasmid pKML302 is a Tn*5* insertion derivative of pKML2003, with the *recA*-complementing activity abrogated. Plasmid pKML301 is a Tn*5* insertion derivative of pKML2. This plasmid has a Tn*5* insertion in the same site and in the same orientation as does pKML302, with the concomitant loss of *recA*-complementing activity. Plasmid pKML303 is a Tn*5* insertion derivative of pKML2003. This construction inactivates the ampicillin resistance of the vector but leaves the *recA*-complementing activity of the clone intact. Plasmid pKML303 confers a phenotype of ampicillin sensitivity (Ap^s), kanamycin resistance (Km^r), and recombination proficiency ($RecA^+$).

The 2.3-kbp *Bam*HI-*Hin*dIII fragment of pKML2003 was subcloned into the broad-host-range vector pCP13 (12) to produce pKML3001. The plasmids pKML2003 and pCP13 were digested with both *Bam*HI and *Hin*dIII and mixed in ligation buffer under conditions promoting the formation of hybrid molecules (34). The ligation mixture was used to transform *E. coli* HB101, with selection made for tetracycline resistance.

Plasmid pJC859 was the generous gift of A. J. Clark. It contains the *E. coli* K-12 *recA* gene on a 3.3-kbp DNA fragment inserted into the *Bam*HI site of pBR322.

Media and chemicals. Cells were grown in Luria broth (31) at 37°C with shaking. L agar was Luria broth containing 1.3% (wt/vol) Bacto-Agar (Difco Laboratories, Detroit, Mich.). Lysates of bacteriophages were prepared by using tryptone agar (24) for *E. coli* phages and L agar for *P. aeruginosa* phages. Antibiotics were used at the following concentrations: ampicillin, 50 μg/ml; kanamycin, 50 μg/ml unless otherwise stated; tetracycline, 12.5 μg/ml for *E. coli* and 250 μg/ml for *P. aeruginosa*. TM buffer (24) was used as phage diluent. TE buffer (24) was used to store DNA.

Agar, Casamino Acids, tryptone, yeast extract, *Pseudomonas* isolation agar, and lactose MacConkey agar were purchased from Difco. Agarose was purchased from FMC Corp., Rockland, Maine). Acrylamide and *N,N'*-methylenebisacrylamide were purchased from Bio-Rad Laboratories, Richmond, Calif.). All other chemicals and antibiotics were purchased from Sigma Chemical Co., St. Louis.

DNA techniques. *P. aeruginosa* PAO chromosomal DNA was isolated by a modification of the method of Marmur (28). Restriction endonucleases were purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind., and digestions were performed as recommended by the manufacturer. Deletion analysis of pKML2 and pKML2003 was performed by restriction digestion followed by religation using T4 ligase under conditions favoring intramolecular ligation (34). Deleted plasmids were transformed into *E. coli recA* mutants by a calcium chloride technique (13), and the RecA phenotype of the transformants was examined by testing the ability of the plasmid-containing cells to grow on L agar supplemented with 0.01% (vol/vol) MMS or 2 μg of nitrofurantoin (NF) per ml of medium (42).

Mobilization of pKML3001 from *E. coli* to *P. aeruginosa*. Triparental matings were performed by the method of Ruvkun and Ausubel (39), with the following modifications. pRK2013 (16) was used as the mobilizing plasmid. *P. aeruginosa* strains to be mated were grown for 16 h at 43°C and concentrated fivefold before conjugation to disable the restriction system of *P. aeruginosa* PAO (38). *Pseudomonas* isolation agar supplemented with 250 μg of tetracycline per ml of medium was used for selection of transconjugants. The plates were incubated for 2 days at 37°C.

UV sensitivity. Cells were grown to approximately 2×10^8 CFU/ml in Luria broth. Their sensitivity to UV irradiation was determined as previously described (24).

Bacterial conjugations. Conjugal matings of *E. coli* were performed as described by Kokjohn and Miller (24). *P. aeruginosa* conjugations were done as described by Okii et al. (33). *Pseudomonas* minimal medium (31) containing 1.3% (wt/vol) agar and 0.4% (wt/vol) glucose and supplemented with appropriate amino acids (25 μg/ml) was used for conjugations. For the determination of the acquisition of plasmid FP5, the mating procedure was identical to that described above; however, transconjugants were selected for resistance to $HgCl_2$ (2.5 μg/ml).

Prophage induction. (i) Lambda phages. The preparation of lambda lysogens and conditions for prophage induction using MMC have been described (24). *E. coli* A585 grown in YEM broth (2) was used as the indicator strain for all titrations.

(ii) D3 phages. Cells to be used for induction studies were grown to approximately 2×10^8 CFU/ml in Luria broth and harvested by centrifugation at $5,000 \times g$ for 5 min. The cells were suspended in an equal volume of saline (0.85% NaCl in water) and exposed to UV irradiation at a fluence of 10 J/m^2. The irradiated cells were incubated in the dark for 2 h and lysed by addition of a 1/10 volume of chloroform. Cell debris was removed by centrifugation, and the titers of the lysates were determined by using *P. aeruginosa* PAO1 as the indicator strain.

Tn*5* mutagenesis of pKML2003. Our procedure for Tn*5* mutagenesis of plasmid pKML2003 was similar to that of Bartlett and Matsumura (2). pKML2003 was introduced by transformation into nonsuppressing *E. coli* 594. *E. coli* 594(pKML2003) was grown to the mid-log phase in YEM (2) at 30°C, and Tn*5* was delivered by infection with lambda 467 (14) at a multiplicity of infection of approximately five. Phage absorption was allowed for 20 min at room tempera-

TABLE 1. Bacterial strains

Strain	Plasmid	Relevant genotype[a]												Other markers[a]	Prophage[b]	Source or reference
		rec	*arg*	*his*	*hsd*	*ilv*	*leu*	*lys*	*pro*	*pur*	*sup*	*trp*	*thi*			
E. coli																
594		+	+	+	+	+	+	+	+	+	+	+	*-1*	*lac-3350rpsL*		48
A585		*B1009*	+	+	+	+	*B6*	+	+	+	*E44*	*-1*	*-1*	*fhuA21*		F. W. Stahl
AB1157		+	*E3*	*-4*	+	+	*B6*	+	A2	+	*E44*	*-1*	*-1*			10
χ1488		+	+	*-53*	*R2*	*-277*	+	*C65*	+	*E41*	+	+	*-1*	*minA1 minB2*		11
GW1031		*A56*	*E3*	*-4*	+	−	*B6*	+	+	+	+	*-1*	*-1*	*lac(U169) sulA11 dinB1*::Mu d(Amp^r *lac*)		G. W. Walker
HB101		*A13*	+	+	*S20*	+	−	+	A2	+	*E44*	+	*-1*			5
JC158		+	+	+	+	+	+	+	+	+	+	+	*-1*	*serA6* HfrH		10
JC2926		*A13*	*E3*	*-4*	+	+	*B6*	+	*A2*	+	E44	*-1*	*-1*			A. J. Clark
JC11372		*A56*	*E3*	*-4*	+	+	*B6*	+	*A2*	+	*E44*	*-1*	*-1*	*srl-310*::Tn*10*		24
JC14773	pJC859	*A56*	*E3*	*-4*	+	+	*B6*	+	*A2*	+	*E44*	*-1*	*-1*			24
JM103	F'*proAB lacI*^{q}Z	+	+	+	+	+	+	+	*AB*	+	*E44*	+	*-1*	*lac sbcB*		S. Kaplan
RM1086	pKLM2	*A56*	*E3*	*-4*	+	+	*B6*	+	*A2*	+	*E44*	*-1*	*-1*	*srl*-310::Tn*10*		24
RM1139	PKML303	*A56*	*E3*	*-4*	+	−	*B6*	+	+	+	+	*-1*	*-1*	*lac(U169) sulA11 dinB1*::Mu d(Amp^r *lac*)		GW1031
RM1184		+	*E3*	*-4*	+	+	*B6*	+	*A2*	+	*E44*	*-1*	*-1*		(λR)	AB1157
RM1185		+	*E3*	*-4*	+	+	*B6*	+	*A2*	+	*E44*	*-1*	*-1*		(λ207)	AB1157
RM1186		*A56*	*E3*	*-4*	+	+	*B6*	+	*A2*	+	*E44*	*-1*	*-1*	*srl-310*::Tn*10*	(λR)	JC11372
RM2310	pKML2003	*A13*	+	+	*S20*	+	−	+	*A2*	+	*E44*	+	*-1*			HB101
RM2311	pKML301	*A13*	+	+	*S20*	+	−	+	*A2*	+	*E44*	+	*-1*			HB101
RM2312	pKML302	*A13*	+	+	*S20*	+	−	+	*A2*	+	*E44*	+	*-1*			HB101
RM2313	pKML303	*A13*	+	+	*S20*	+	−	+	*A2*	+	*E44*	+	*-1*			HB101
RM2314	pKML2004	*A13*	+	+	*S20*	+	−	+	*A2*	+	*E44*	+	*-1*			HB101
RM2315	pKML2003	*A56*	*E3*	*-4*	+	+	*B6*	+	*A2*	+	*E44*	*-1*	*-1*	*srl-310*::Tn10		JC11372
RM2316	pKML2003	*A13*	*E3*	*-4*	+	+	*B6*	+	*A2*	+	*E44*	*-1*	*-1*			JC2926
RM2317	pKML2004	*A13*	*E3*	*-4*	+	+	*B6*	+	*A2*	+	*E44*	*-1*	*-1*			JC2926
RM2318	pKML2003	*A56*	*E3*	*-4*	+	+	*B6*	+	*A2*	+	*E44*	*-1*	*-1*	*srl*-310::Tn*10*	(λR)	JC11372
RM2319	pKML2003	*A56*	*E3*	*-4*	+	+	*B6*	+	*A2*	+	*E44*	*-1*	*-1*	*srl-310*::Tn*10*	(λ207)	JC11372
RM2320	pKML3001	*A13*	+	+	*S20*	+	−	+	*A2*	+	*E44*	+	*-1*			HB101
RM2325	pKML2003	+	+	*-53*	*R2*	*-277*	+	*C65*	+	*E41*	+	+	*-1*	*minA1 minB2*		χ1488
RM2326	pKML2006	+	+	*-53*	*R2*	*-277*	+	*C65*	+	*E41*	+	+	*-1*	*minA1 minB2*		χ1488
POII1681		+	*F*	+	+	+	+	+	*AB*	+	+	+	*-1*	*lac rpsL*	(Mu dII1681)	6
P. aeruginosa																
PAO1		+	+	+	+	+	+	+	+	+	+	+	+			31
PAO25		+	*F10*	+	+	+	*-10*	+	+	+	+	+	+			17
PAO303		+	*B21*	+	+	+	+	+	+	+	+	+	+			31
PAO832	FP5	+	+	*-151*	+	*-261*	+	+	+	*-66*	+	+	+	*pyr-21*		B. W. Holloway
PTO66		*-102*	+	*-4*	+	*-1118*	+	*-12*	*-82*	+	+	*-6*	+	*fon*		17
PTO6003		*-102*	+	+	+	+	+	+	+	*-67*	+	+	+			17
RM187	FP5	+	*B21*	+	+	+	+	+	+	+	+	+	+	*nalA901*		PAO25
RM247		+	*F10*	+	+	+	*-10*	+	+	+	+	+	+		(D3)	PAO25
RM265		*-102*	+	+	+	+	*-10*	+	+	+	+	+	+			PAO25
RM276		*-102*	+	+	+	+	*-10*	+	+	+	+	+	+		(D3)	RM265
RM2321	pKML3001	*-102*	+	*-4*	+	*-1118*	+	*-12*	*-82*	+	+	*-6*	+			PTO66
RM2322	pKML3001	*-102*	+	+	+	+	+	+	+	*-67*	+	+	+			PTO6003
RM2323	pKML3001	*-102*	+	+	+	+	*-10*	+	+	+	+	+	+			RM265
RM2324	pKML3001	*-102*	+	+	+	+	*-10*	+	+	+	+	+	+		(D3)	RM265

[a] Genotypic symbols follow the conventions recommended by Demerec et al. (15). Designations are as specified by Bachmann (1), except *fon*, which indicates resistance to phage F116L.

[b] Prophages are indicated by giving the name of the phage in parenthesis when present.

ture, and the infected cells were then incubated for 2 h at 30°C.

Cells were plated on L agar containing 400 μg of kanamycin per ml to enrich for Tn*5* insertions into the plasmid (20). After incubation for an additional 16 to 20 h, the plates were scraped and plasmid DNA was isolated by a rapid clone analysis technique (27). This plasmid DNA was used to transform *E. coli* HB101. Selection for transformed clones was made by plating on L agar containing ampicillin and kanamycin and incubating overnight at 37°C. Transformants were screened for the RecA$^-$ phenotype by testing the ability of cells to grow on L agar containing MMS or NF, as described above. The positions of the Tn*5* insertions abolishing *recA*-complementing activity were mapped by restriction analysis.

Southern blot analysis of the *recA* analog. Plasmids pKML2 and pJC859 were digested with *Bam*HI, electrophoresed on a 0.7% agarose gel (13), denatured, and blotted onto nitrocellulose filters (B85; pore size, 0.45-μm; Schleicher & Schuell, Inc., Keene, N.H.) by using capillary transfer (27).

TABLE 2. Bacteriophages

Phage	Relevant genotype	Source or reference
E. coli		
λR	*R*am5	24
λmms813	*vir*	24
λ207	cI *ind*	F. W. Stahl
λ467	*b*221 *rex*::Tn*5O*am29 *P*am80	14
Mu dII1681	cts62::IS*121* d(Kan^r *lacZYA*)	6
P. aeruginosa		
D3	Wild type	7
D3c	*c13*	Spontaneous clear-plaque mutant of D3

Plasmid pKML2 was labeled with the nonradioactive nucleotide biotin-11-dUTP (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) by using a nick translation kit purchased from Enzo Scientific Co., New York, N.Y. DNA hybridization and detection of bound probe were performed in accordance with the recommendations of Bethesda Research, using a modification of the method of Wahl et al. (46).

Mini-Mu d(Kan^r *lac*) insertions into the *recA* analog. Plasmid pKML2003 was introduced by transformation into *E. coli* POII1681 (6). Transposition of the Mu lysogens was initiated by thermal induction. The positions and orientations of the mini-Mu insertions in pKML2003 abolishing *recA*-complementing activity were determined by restriction endonuclease mapping.

Plasmid DNA from *recA*-inactivating insertions were isolated from clones and used to transform *E. coli* JM103. The Lac phenotype of *E. coli* JM103 containing mini-Mu insertion derivatives of pKML2003 in either orientation was examined by plating cells on lactose MacConkey agar (13) or on M9 agar (11) containing melibiose (2 mg/ml) as the sole carbon source and incubating the plates at 42°C (45).

Minicell analysis of pKML2003. Plasmids to be analyzed were introduced into the minicell-producing strain *E. coli* χ1488. Minicells were handled as detailed by Clark-Curtiss and Curtiss (11) and Goldberg and Mekelanos (18), with minor modifications. Sucrose gradients were prepared by freezing a solution of 22% (wt/vol) sucrose in buffered saline-glucose (11) and allowing the mixture to thaw at 4°C for 16 h (D. H. Bartlett, Ph.D. dissertation, University of Illinois at Chicago, Chicago, 1985).

Minicells were labeled with [^{35}S]methionine (specific activity, 1,114 Ci/mmol; New England Nuclear Corp., Boston, Mass.). The minicells were suspended at an A_{595}^{1cm} of 1.0 in M9 minimal medium supplemented with 0.4% (wt/vol) glucose and histidine at 25 μg/ml. The cells were labeled by incubation with [^{35}S]methionine (50 μCi/ml) for 45 min at 37°C and chased with cold methionine (100 μg/ml) for 5 min. The labeled minicells were recovered by centrifugation in an Eppendorf Microfuge and prepared for electrophoresis on sodium dodecyl sulfate-polyacrylamide gels.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography of labeled proteins. The discontinuous system of Laemmli (25) was used for analysis of proteins, with a stacking gel of 4% acrylamide–2.7% bisacrylamide and a resolving gel of 12% acrylamide–2.7% bisacrylamide. The molecular weight standards (Bio-Rad) used were as follows: lysozyme (M_r, 14,400); soybean trypsin inhibitor (M_r, 21,500); carbonic anhydrase (M_r, 31,000); ovalbumin (M_r, 45,000); bovine serum albumin (M_r, 66,200); and phosphorylase *b* (M_r, 92,500). After electrophoresis, the gels were impregnated with sodium salicylate (pH 7) by soaking them in a 1 M solution for 30 min; they were then dried and autoradiographed (8).

Induction of *dinB1*::Mu d(Amp^r *lac*) transcription. Our procedure for induction of *dinB1*::Mu d(Amp^r *lac*) transcription was similar to that of Kenyon et al. (23), with minor modifications. *E. coli* GW1031 and RM1039 were grown in M9 medium containing 1% Casamino Acids to a density of approximately 2×10^8 CFU/ml. The cells were harvested by centrifugation at 5,000 × *g* for 5 min and suspended in an equal volume of saline. The cells were then exposed to UV irradiation at a fluence of 2 J/m^2 for strain GW1031 or 10 J/m^2 for strain RM1039. The irradiated cells were incubated in the dark, and samples were assayed for β-galactosidase activity at various times after irradiation.

RESULTS

Restriction mapping and deletion analysis of pKML2 and pKML2003. Plasmid pKML2 contains the *P. aeruginosa recA* analog on an approximately 9-kbp fragment of DNA cloned into the *Bam*HI site of pBR322 (Fig. 1). This DNA fragment is able to complement several *E. coli recA* mutants in *trans*, allowing growth of cells containing the cloned DNA in medium containing MMS or NF (24). When nick-translated pKML2 DNA was used to probe a Southern blot of the *E. coli recA*-containing plasmid pJC859 under high-stringency conditions, significant DNA base sequence ho-

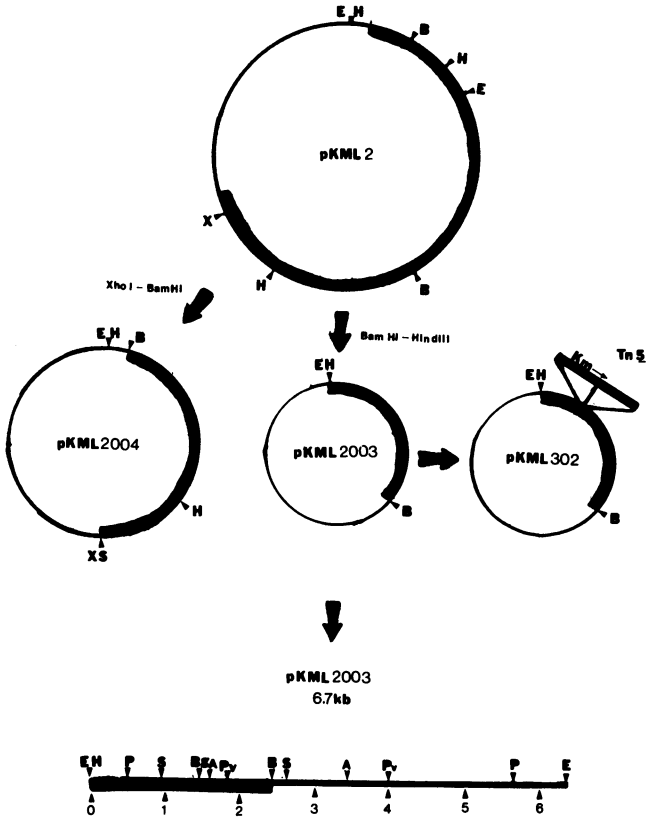

FIG. 1. Restriction endonuclease maps of *P. aeruginosa recA*-containing plasmids. The thin lines represent pBR322 DNA; the thick lines represent *P. aeruginosa* chromosomal DNA. The bottom line is a detailed map of pKML2003. A, *Ava*I; B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I; X, *Xho*I. Size is expressed in kilobase pairs.

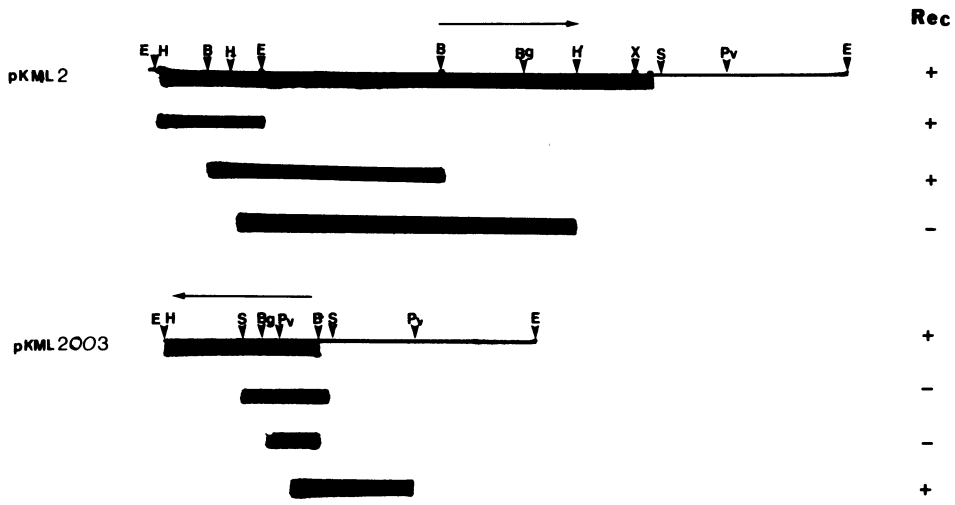

FIG. 2. Localization of the *P. aeruginosa recA* analog. The ability to complement *E. coli recA* mutations was correlated to the physical map to localize the position of the *recA* analog on plasmids pKML2 and pKML2003. Deletions were generated by restriction endonuclease digestion and religation of the deleted plasmid. The region deleted is indicated by the line immediately below the maps of pKML2 and pKML2003. The ability of the resultant construction to complement the *E. coli* RecA$^-$ phenotype was tested and is indicated in the column to the right. The arrows indicate the direction of transcription of the *recA* analog (see the text and Fig. 3 for details). B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; Pv, *Pvu*II; S, *Sal*I; X, *Xho*I.

mology between the *E. coli recA* gene and the *P. aeruginosa recA* analog was revealed.

To delineate more precisely the location and extent of the coding and control sequences of the analog, a number of subclones were constructed and tested for the ability to allow growth of cells in medium containing MMS or NF (Fig. 1). Deletion of restriction fragments from pKML2 followed by religation and transformation into *E. coli* HB101 was used to generate several clones. Deletion analysis of pKML2 indicated that the *recA*-complementing activity is contained within the *Bam*HI-*Xho*I fragment of the insert DNA (Fig. 2). Subclones of this region were generated and examined for the RecA$^+$ phenotype. The *Bam*HI-*Xho*I fragment was cloned into pBR322 by replacing the *Bam*HI-*Sal*I fragment of the vector. This clone, pKML2004, is RecA$^+$ and has the insert DNA in the same orientation as that in pKML2. A subclone containing the 2.3-kbp *Bam*HI-*Hin*dIII fragment of *P. aeruginosa* DNA was constructed by replacing the *Bam*HI-*Hin*dIII fragment of pBR322 (Fig. 1). This subclone, pKML2003, is also RecA$^+$ and has the insert DNA in an orientation opposite to that in pKML2 and pKML2004.

Thus, deletion analysis and subcloning experiments revealed that the *recA* analog is contained within a 2.3-kbp *Bam*HI-*Hin*dIII DNA fragment. This fragment was physically mapped and subjected to deletion analysis (Fig. 1 and 2). This analysis indicated that the *recA*-complementing activity is contained in the *Hin*dIII-*Pvu*II fragment and that the gene extends at least to the *Bgl*II site. Restriction analysis of pKML2003 revealed no cleavage sites for *Hpa*I or *Xba*I.

Tn*5* mutagenesis of pKML2003. To more precisely identify the site of the *recA*-complementing activity, Tn*5* insertion derivatives of pKML2003 were generated and physically mapped (Fig. 1). Several independently isolated clones which had lost the MMS and NF resistance phenotype normally conferred by the plasmid were examined. All were found to contain an insertion in the same region and in the same orientation. One of these plasmids, pKML302, was used for further experimentation. The Tn*5* insertion present in pKML302 caused the simultaneous loss of all activities associated with a RecA$^+$ phenotype in *recA* mutants of *E. coli* (see below).

Mini-Mu d(Kan^r *lac*) fusions with the *recA* analog. To further localize the *recA*-complementing sequences and to determine the direction of transcription of the *recA* analog, several independent mini-Mu d(Kan^r *lac*) fusions in both orientations were isolated in pKML2003 (Fig. 3). Two independently generated clones representing mini-Mu insertions in opposite orientations were introduced into *E. coli* JM103 to determine which construction was transcriptionally active, i.e., expressed β-galactosidase activity. It was determined that both constructions expressed β-galactosidase at very low levels. Since these mini-Mu phages make translational fusions, it was possible that the fusion tested was not in the proper reading frame. In these constructions *lacY* is translated regardless of the reading frame generated by the fusion event since it has an independent translational start site. The product of the *lacY* gene is required by *E. coli* for growth at 42°C on medium with melibiose as the sole carbon source (45). We therefore assayed the transcriptional expres-

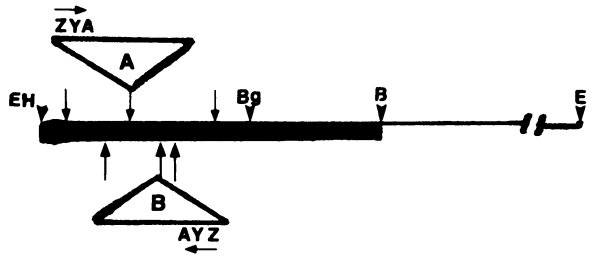

FIG. 3. Location of mini-Mu dII1681 insertions in pKML2003 which eliminated *recA*-complementing activity. The thick line represents *P. aeruginosa* chromosomal DNA; the thin line represents pBR322 DNA. The downward arrows denote insertions in orientation A, with *lac* transcription from left to right on the map. The upward arrows denote insertions in orientation B, with *lac* transcription proceeding from right to left. Insertions in orientation B were found to be transcriptionally active (see the text). Abbreviations are defined in the legend to Fig. 2.

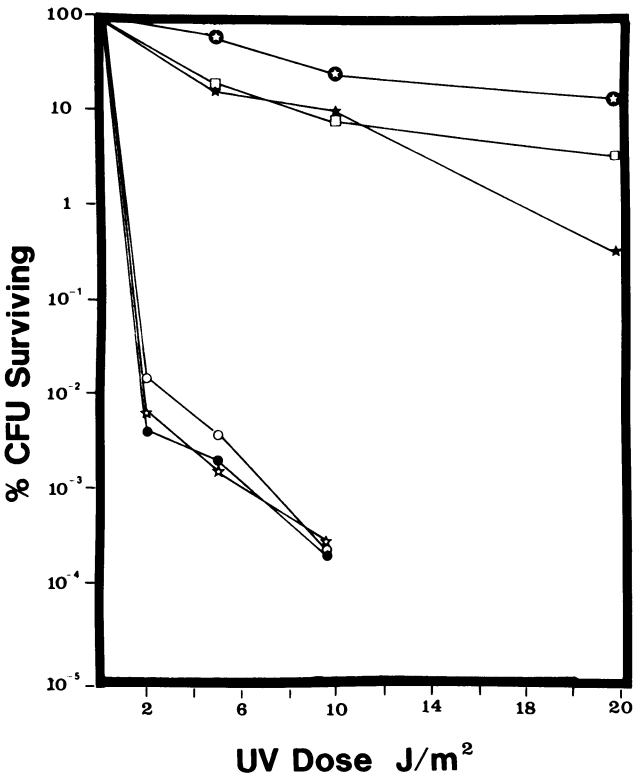

FIG. 4. Restoration of resistance by the *P. aeruginosa recA* analog to killing by UV irradiation of *recA* mutants of *E. coli*. Cells were grown to a density of approximately 10^8 CFU/ml in Luria broth, pelleted, suspended in saline, and exposed to various fluences of UV irradiation. After appropriate dilution in saline, the irradiated cells were plated on L agar and incubated at 37°C overnight in the dark. A representative experiment is shown. Symbols: ●, *E. coli* HB101 *recA13*; ○, *E. coli* RM2312 *recA13*(pKML302); ☆, *E. coli* JC2926 *recA13*; ★, *E. coli* RM2316 *recA13*(pKML2003); □, *E. coli* RM2317 *recA13*(pKML2004); ✪, *E. coli* AB1157 *recA*$^+$.

sion of the fusion by testing for *lacY* activity. Inserts with orientation B were found to express *lacY* at high levels.

Determination of UV irradiation sensitivity in *E. coli recA* mutants. The ability of the various subclones to restore resistance to UV irradiation in *E. coli recA* mutant strains was determined. pKML2003 and pKML2004 were found to restore the UV resistance of strains containing *recA* mutations (Fig. 4). Plasmid pKML302 was unable to confer resistance to UV irradiation to *recA* strains.

Determination of conjugational proficiency in *E. coli recA* strains. To test the recombinational proficiency of clones containing the *P. aeruginosa recA* analog, matings between the HfrH strain JC158 and RecA$^-$ recipients were performed. Plasmids pKML2003 and pKML2004 restored homologous recombination proficiency to nearly wild-type levels after conjugation of various *E. coli recA* mutants (Table 3). The Tn*5* insertion derivative of pKML2003, pKML302, was unable to support homologous recombination in these *recA* mutants.

Lambda prophage induction in *E. coli recA* strains. In *E. coli*, the induction to lytic growth of resident lambda prophage after DNA damage requires the presence of a functional and specifically activated *recA* gene (35, 47). The ability of the *P. aeruginosa recA* analog-containing clones to support spontaneous and MMC-stimulated induction of lambda prophage was investigated (Table 4). The presence

TABLE 3. Recombinational proficiency of Hfr crosses[a]

Recipient	Relevant characteristic(s)	Recombinational proficiency (*proA*$^+$ recombinants/100 donors)
HB101	*recA13*	3.5×10^{-5}
RM2310	*recA13*(pKML2003)	0.75
RM2312	*recA13*(pKML302)	$<3 \times 10^{-6}$
AB1157	Rec$^+$	7.3
JC2926	*recA13*	4.4×10^{-4}
RM2316	*recA13*(pKML2003)	2.9
RM2317	*recA13*(pKML2004)	3.0

[a] *E. coli* JC158 was used as the donor strain. Cells were mixed in a donor-to-recipient ratio of 1:10. Matings were performed for 2 h at 37°C.

of the *P. aeruginosa recA* analog stimulated lambda prophage induction greatly. The ability of pKML2003 to induce a lambda cI *ind* prophage was also tested. The *P. aeruginosa recA* analog was unable to induce this prophage to lytic growth after MMC treatment (Table 4).

Identification of the protein product of the *P. aeruginosa recA* analog. Minicells were used to estimate the size of the *P. aeruginosa recA* analog protein product (Fig. 5). Comparison to size markers run in parallel revealed that the protein has a molecular weight of approximately 47,000. A Rec$^-$ deletion derivative of pKML2003 (pKML2006) did not produce this protein in minicells nor did a Tn*5* insertion derivative of pKML2, pKML301. Plasmid pKML2004 was found to produce a protein of the same molecular weight as that produced by pKML2003 (data not shown).

Induction of *dinB1*::Mu d(Amp^r *lac*) expression. Transcription of the *dinB* gene has been shown to be inducible by UV irradiation (20, 47). This induction of expression depends upon the *recA* gene product to promote cleavage of the *lexA*-encoded repressor. Induction of this gene can be monitored by assaying β-galactosidase activity in a *dinB1*::Mu d(Amp^r *lac*) fusion mutant. The *P. aeruginosa recA* analog was capable of supporting the UV induction of β-galactosidase expression in a *recA dinB1*::Mu d(Amp^r *lac*) mutant (Fig. 6). This induction of expression was specifically dependent upon UV irradiation of the cell; no induction of expression above the nominal base-line level was observed in its absence.

Construction of pKML3001. Several Rec$^-$ mutants of *P. aeruginosa* PAO have been described (9, 17, 31) which have some characteristics similar to *recA* mutants of *E. coli*. To determine whether the chromosomal fragment contained in pKML2003 would complement the Rec$^-$ phenotype in *P. aeruginosa*, we introduced the 2.3-kbp *Hin*dIII-*Bam*HI fragment of the PAO1 chromosome from pKML2003 (Fig. 1) into the broad-host-range vector pCP13. This construction was transformed into *E. coli* HB101. The ability of tetracy-

TABLE 4. Lambda prophage induction

Lysogen	Relevant characteristics	Phage produced (PFU/ml)		Induction ratio
		Spontaneous	Induced[a]	
RM1184	Rec$^+$ (λR)	4×10^5	2×10^9	5×10^3
RM1186	*recA56* (λR)	60	60	1.0
RM2318	*recA56*(pKML2003) (λR)	4×10^4	1×10^8	2.5×10^3
RM1185	Rec$^+$ (λ207)	40	40	1.0
RM2319	*recA56*(pKML2003) (λ207)	200	500	2.5

[a] Cells were incubated with mitomycin C at 5 μg/ml for 150 min.

cline-resistant clones to restore the resistance of HB101 to NF and UV irradiation was confirmed. Positive isolates were shown to contain plasmid DNA of the appropriate restriction pattern. The plasmid from one such clone, pKML3001, was mobilized into several *rec-102*-containing *P. aeruginosa* strains by a triparental mating technique. Transconjugants were examined for suppression of the pleiotropic Rec$^-$ phenotype conferred by the *rec-102* mutation.

UV sensitivity of *P. aeruginosa rec-102* mutants containing pKML3001. The sensitivity to UV irradiation of *rec-102* mutant strains with and without pKML3001 was compared to that of various Rec$^+$ strains of *P. aeruginosa*. The presence of the plasmid was found to confer considerably greater resistance to UV irradiation on the *rec-102* mutants examined, with restoration to essentially wild-type levels in most strains (Fig. 7). In addition, pKML3001 was found to restore the resistance of *rec-102* mutants to the radiomimetic drug MMS.

Conjugational and recombinational proficiency of *P. aeruginosa rec-102* strains. The ability of *rec-102* mutants containing pKML3001 to undergo homologous recombination after FP5-mediated conjugation was examined and compared with the efficiency of recombination in the absence of the plasmid (Table 5). The presence of the plasmid dramatically increased the number of recombinants recovered from Rec$^-$ recipient strains.

The ability of the various strains to acquire exogenous DNA through conjugation was assessed by determining the frequency of inheritance of the fertility plasmid FP5 (Table 5). Both Rec$^-$ and Rec$^+$ strains were able to receive and maintain FP5 at essentially the same levels.

Induction of prophage D3 from *rec-102*-containing strains.

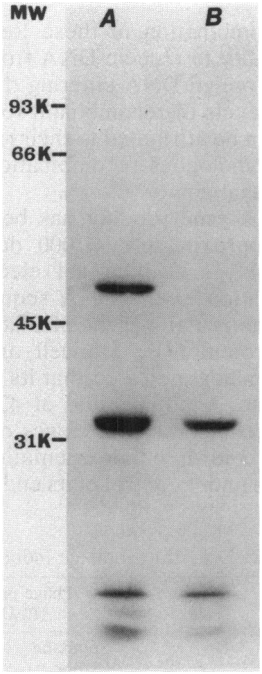

FIG. 5. Identification of the *P. aeruginosa recA* analog gene product. ^{35}S labeling of plasmid-encoded proteins was performed as described in the text. Lane A, *E. coli* RM2325, which contains pKML2003; Lane B, *E. coli* RM2326, which contains pKML2006, a Rec$^-$ deletion derivative of pKML2003 with the *Bam*HI-*Bgl*II fragment removed. The migration of standard molecular weight markers is indicated on the left.

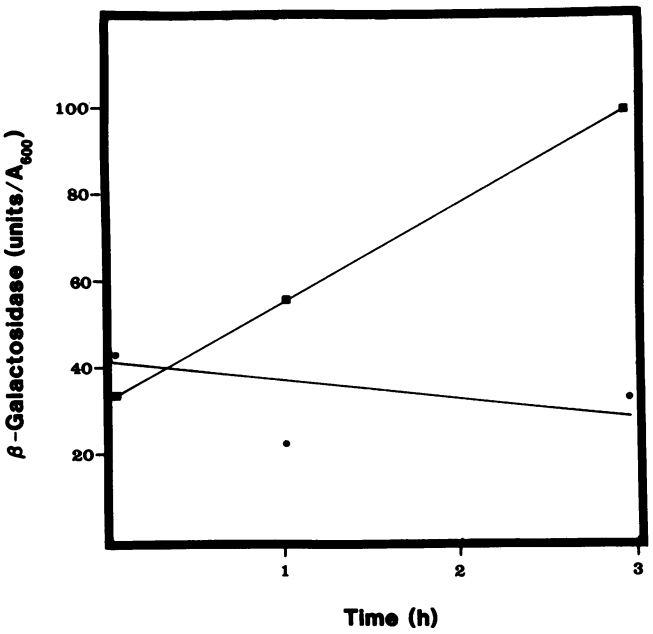

FIG. 6. Kinetics of UV induction of β-galactosidase activity by the *P. aeruginosa recA* analog in a *recA dinB1*::Mu d(Amp^r *lac*) strain of *E. coli*. Cells were grown in supplemented M9-glucose medium at 30°C. The cells were UV irradiated (10 J/m^2 for RM1139 and 2 J/m^2 for GW1031) at time zero. Samples (1 ml) were removed periodically, and total β-galactosidase activity in the culture was determined as described by Kenyon et al. (23). Cell density was determined by measuring A_{600}. A representative experiment is shown. Symbols: ●, *E. coli* GW1031 *recA56 dinB1*::Mu d(Amp^r *lac*; ■, *E. coli* RM1139 *recA56 dinB1*::Mu d(Amp^r *lac*)(pKML303).

The D3 prophage is inducible by UV irradiation in wild-type (Rec$^+$) lysogens of *P. aeruginosa* (7, 19). To determine whether UV induction of prophage D3 was dependent on functions encoded by plasmid pKML3001, the ability of various D3 lysogens of *rec-102* mutants to release phage spontaneously and subsequent to UV irradiation was determined (Table 6). Whereas both *rec-102*-containing and wild-type strains were capable of spontaneously releasing D3 phage, the UV induction of prophage was inhibited by the presence of the *rec-102* allele. Plasmid pKML3001 was capable of restoring the UV inducibility of D3 prophage from *rec-102*-containing lysogens.

DISCUSSION

The protein product of the *E. coli recA* gene is required for the process of homologous recombination. In this process it acts as a synaptic protein (29, 30, 49). The RecA protein is also activated by various DNA-damaging agents to promote the cleavage of the *lexA* and lambda *c*I repressors in the initiation of the SOS response (23, 26, 37).

We isolated a clone of *P. aeruginosa* PAO chromosomal DNA capable of complementing, in *trans*, *E. coli recA* mutants so as to restore UV resistance and recombinational proficiency. The location of the *recA*-complementing sequences was determined by deletion analysis. A 2.3-kbp DNA segment present in pKML2003 contains the entire *recA* analog. That this segment contains the endogenous promoter of this gene was suggested by the observation that this segment was expressed when inserted in either orientation into pBR322. Translational fusions of the *recA* analog

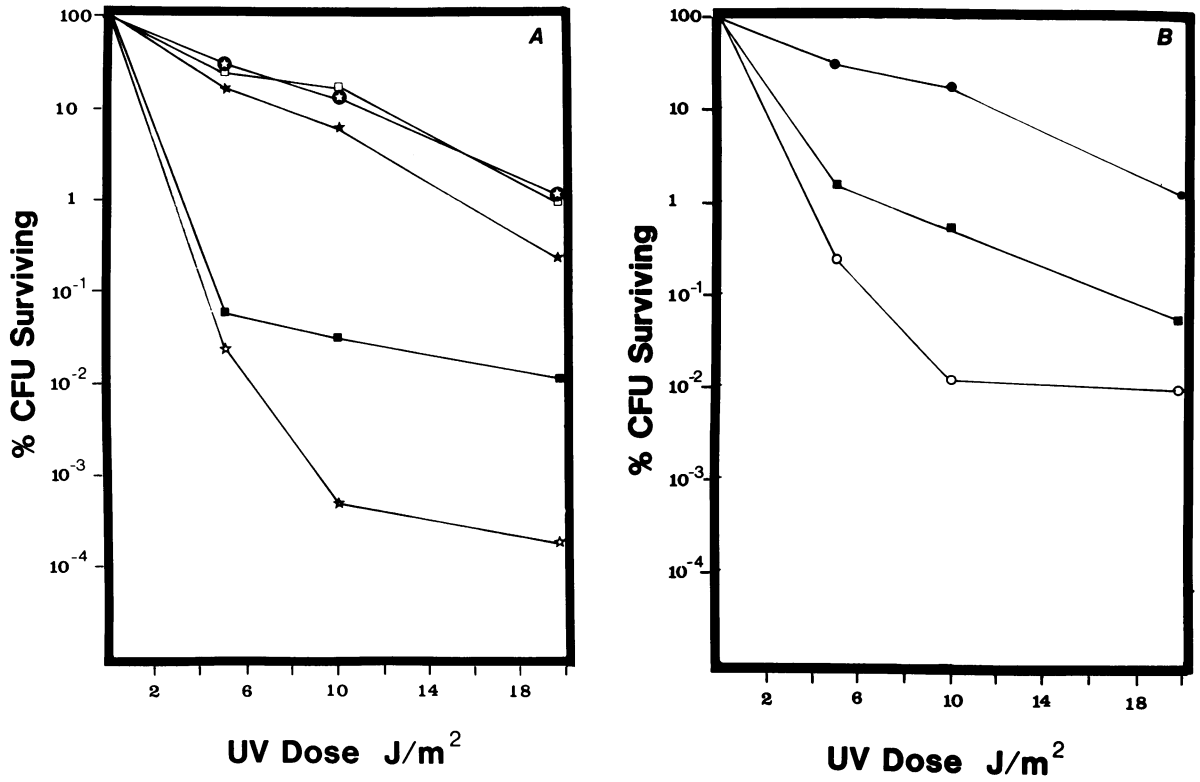

FIG. 7. Restoration by pKML3001 of resistance to killing by UV irradiation of various *rec-102* mutants of *P. aeruginosa*. Experiments were performed as described in the legend to Fig. 4. A representative experiment is shown. (A) Strains: ■, PTO6003 *rec-102*; □, RM2322 *rec-102*(pKML3001); ☆, PTO66 *rec-102*; ★, RM2321 *rec-102*(pKML3001); ✪, PAO25 *rec*$^+$. (B) Strains: ●, PAO25 *rec*$^+$; ○, RM265 *rec-102*; ■, RM2323 *rec-102*(pKML3001).

with *lacZYA* were constructed by using mini-Mu phage. The pattern of expression of this gene fusion indicated that the *P. aeruginosa recA* analog is transcribed in the direction from *Bam*HI toward *Hin*dIII on the physical map (Fig. 1).

When this fragment was subcloned into a broad-host-range vector and mobilized into several *P. aeruginosa* strains containing the *rec-102* allele (17), the pleiotropic effects of this mutation were complemented by the cloned fragment. The presence of pKML3001 in *rec-102*-containing mutants conferred greatly increased resistance to UV irradiation and restored recombinational proficiency in FP5-mediated crosses. Strains with and without the plasmid were capable of receiving and maintaining plasmid DNA through conjugation at essentially the same level. We conclude that the defect in recombination in these Rec$^-$ strains is due neither to an inability to receive DNA from donor cells nor to destruction of foreign DNA entering the cell. Therefore, the difference in levels of recombinant formation exhibited by these strains can be attributed to their relative proficiency in carrying out homologous recombination and not to any difference in conjugal ability.

The *E. coli recA* gene product has been identified as a polypeptide of approximately 42,000 daltons by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (40). Based on examination of the DNA sequence of the *recA* gene, a smaller molecular weight of 37,842 has been predicted for the protein (41). Minicell analysis of the *P. aeruginosa recA* analog indicated that its protein product is considerably larger. A polypeptide of 47,000 daltons was produced in minicells containing clones of the *recA* analog inserted into the vector in either orientation, indicating that the gene product is under control of its endogenous promoter

TABLE 5. Recombinational and conjugal proficiency of FP5 crosses[a]

Recipient	Relevant characteristic(s)	Recombinational proficiency[b] (recombinants/100 donors)	Plasmid acquisition[c] (Hg^r transconjugants/100 donors)
PTO66	*rec-102*	4.0×10^{-6}	1.6×10^{-1}
RM2321	*rec-102*(pKML3001)	1.4×10^{-3}	5.0×10^{-1}
PAO303	Rec$^+$	4.4×10^{-3}	2.4×10^{-2}

[a] RM187 was used as the donor in PTO66 and RM2321 matings, and PAO832 was used in the PAO303 mating. Cells were mixed in a donor-to-recipient ratio of 1:1.2. Matings were performed for 2 h at 37°C in liquid medium.

[b] *his-4*$^+$ recombinants were selected in PTO66 and RM2321 matings, and *argB21*$^+$ recombinants were selected in the PAO303 mating.

[c] Transfer of FP5 was quantified by selection for Hg^r.

TABLE 6. D3 prophage induction

Lysogen	Relevant characteristics	Phage produced (PFU/ml)		Induction ratio
		Spontaneous	UV induced[a]	
RM247	Rec$^+$ (D3)	2×10^7	5×10^8	25
RM276	*rec-102* (D3)	3×10^6	2×10^6	0.7
RM2324	*rec-102* (pKML3001) (D3)	1.5×10^7	2×10^8	13

[a] Cells were suspended in 0.85% saline and UV irradiated at a fluence of 10 J/m^2. The cells were suspended in Luria broth and incubated for 2 h at 37°C.

in these constructions. Tn*5* insertional inactivation of the *P. aeruginosa recA* analog specifically abolished the presence of this protein in minicell extracts, confirming that it is the product of the *P. aeruginosa recA* gene. Furthermore, Northern blot analysis of mRNA isolated from *P. aeruginosa* PAO1 has indicated that the chromosomal sequences contained in pKML2003 are expressed in *P. aeruginosa* PAO (T. A. Kokjohn and R. V. Miller, manuscript in preparation).

Two characteristics associated with the SOS network in *E. coli* are the induction of error-prone DNA repair and the induction of prophage lambda by DNA-damaging agents (47). The cloned *P. aeruginosa recA* analog was capable of induction of resident lambda prophage to lytic growth in response to DNA-damaging agents. It also allowed the expression of β-galactosidase from a *dinB1*::Mu d(Amp^r *lac*) fusion. Hence, the *recA*-complementing activity was capable of inducing SOS functions under control of both the *c*I and *lexA* repressors. A number of lambda phage mutants have been isolated that are not inducible by agents such as UV irradiation or MMC (37). One such uninducible mutant has been shown to encode a *c*I repressor resistant to *recA*-mediated cleavage (37). We demonstrated that the *P. aeruginosa recA* analog was incapable of inducing to lytic growth a resident uninducible lambda prophage. This suggests that the mechanism of elimination of *c*I-mediated repression of lytic functions of prophage lambda is likely to be very similar or identical for both the *E. coli* and *P. aeruginosa recA* gene products.

Error-prone DNA repair is not induced by nalidixic acid (3) or UV irradiation (C. S. Simonson and R. V. Miller, manuscript in preparation) in either Rec$^+$ or Rec$^-$ strains of *P. aeruginosa* PAO. However, the induction of D3 prophage was observed upon exposure of a Rec$^+$ D3 lysogen to UV irradiation. Whereas the spontaneous release of phage was essentially the same in Rec$^+$ and Rec$^-$ strains, the induction of D3 prophage by UV irradiation was dependent on the presence of a wild-type allele of *rec-102*. The presence of the cloned *P. aeruginosa recA* analog within the cell allowed the induction of D3 prophage by UV irradiation at levels equivalent to those in isogenic Rec$^+$ strains. Thus, the UV induction of D3 prophage was dependent on the functions encoded in the DNA contained in pKML3001. We also determined that the UV induction of *P. aeruginosa* prophage F116L (19) was dependent on the functions encoded on pKML3001 (data not shown). This prophage induction provided the first clear demonstration that at least a subset of the DNA damage-inducible phenomena of *E. coli* occurs in *P. aeruginosa* PAO and is dependent on the RecA$^+$ phenotype.

The *P. aeruginosa* RecA protein was capable of inducing prophages of three totally unrelated bacteriophages, D3 and F116L of *P. aeruginosa* and lambda of *E. coli*. Southern blot analysis revealed that there is no overall DNA sequence homology among them. The repressors of phages D3 and F116 do not show sequence homology to each other or to the lambda *c*I repressor (R. V. Miller and T. A. Kokjohn, J. Bacteriol., in press). This may indicate that evolution of these temperate phages has occurred to take advantage of the potential of the RecA protein of gram-negative bacteria to respond to the level of DNA damage in the host cell.

On the basis of the data discussed above, we conclude that the *P. aeruginosa* PAO chromosomal fragment which is contained in pKML3001 carries a gene whose protein product performs functions in *P. aeruginosa* analogous to the functions of the RecA protein of *E. coli*. Therefore, the *P. aeruginosa* PAO chromosomal fragment present in pKML3001 contains the *P. aeruginosa recA* gene. The data also support the conclusion that the *rec-102* mutation is an allele of the *P. aeruginosa recA* gene, and we propose renaming this marker *recA102*. In addition, preliminary results indicate that *lesB908* (31) represents a second mutant allele of the *P. aeruginosa recA* gene.

The *recA* gene of *P. aeruginosa* seems to execute the same functions as its *E. coli* counterpart. It is clearly required for homologous recombination and is probably the major synaptic protein normally operating in *P. aeruginosa*, judging from the consequences of its loss to the cell. *P. aeruginosa* genes are usually only poorly expressed in *E. coli* (21); however, the *recA* analog was expressed at levels adequate to allow complementation of *E. coli recA* mutants both in recombination and in SOS induction phenomena. Southern blot analysis of pKML2 demonstrated DNA base sequence homology to the *E. coli recA* gene. Western blot analysis of the protein product of this gene with anti-*E. coli* RecA antibody revealed that a protein of 47,000 daltons shares antigenic cross-reactivity with the *E. coli* RecA protein. This protein is expressed both in *E. coli*, as revealed by the presence of a plasmid-specific cross-reactive protein in the *E. coli recA*-deletion mutant JC13551 (S. Kowalczykowski, personal communication), and in *P. aeruginosa*, as revealed in the presence of a 47,000-dalton cross-reactive protein in cell extracts of *P. aeruginosa* PAO1 (Kokjohn and Miller, in preparation). The data reported here suggest that the *recA* gene, whatever its origin, has been well conserved both structurally and functionally throughout the gram-negative bacteria.

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