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Properties of R-plasmid pEB017, which confers both enhanced UV-radiation resistance and mutability to wild-type, *recA* and *umuC* strains of *Escherichia coli* K12

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Summary

The R-plasmid, pEB017, restored recombination ability to *recA56* and conferred enhanced resistance to UV-radiation and enhanced UV-radiation mutability to wild-type, *recA56* and *umuC36* strains of *Escherichia coli* K12. Comparatively, pEB017 enhanced UV-radiation mutability in a *umuC* strain, and also enhanced UV-radiation and nitrofuran mutability in a wild-type strain several-fold more than did another R-plasmid, pKM101. Plasmid pEB017 also mediated about a 3-fold enhancement of the SOS induction of β-galactosidase synthesis in a *recA* strain, compared with the normal *recA*$^+$ gene of *E. coli*. A *Bam*HI fragment of pEB017 DNA was cloned into plasmid vector pBR322 to yield pEB021. The *Bam*HI fragment in pEB021 (3.5 kb) is about 170 bp longer between the *Bam*HI and *Pst*I sites on the left end of the *recA*-like fragment, compared with published data on a similarly cloned *recA* gene from *E. coli*. Plasmid pEB021 conferred enhanced resistance to UV-radiation and enhanced UV-radiation mutability in wild-type and *recA* strains, and restored recombination ability in a *recA* strain. The introduction of pEB021 into a *umuC* strain made the cells slightly more resistant to killing by UV-irradiation, and promoted a small amount of UV-mutability in an otherwise nonmutable strain. These results suggest that R-plasmid pEB017 has a *recA*-like gene that mediates the enhanced resistance to UV-radiation and enhanced UV-radiation mutability, but which seems different in several important aspects from the normal *recA* gene in *E. coli*.

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R-plasmids such as R46, pKM101, R-Utrecht and R648 increase the survival of their host cells after damage by UV-irradiation and chemical mutagens (Siccardi, 1969; Marsh and Smith, 1969; MacPhee, 1973; Mortlemans and Stocker, 1976; Tweets et al., 1976; Monti-Bragadin et al., 1976). Plasmids R46 and pKM101 enhance UV-radia-

tion and chemical mutagenesis in wild-type and certain repair-defective strains of *Escherichia coli* and *Salmonella typhimurium*, but not in *recA* strains; in fact, the repair and mutability activities of these plasmids are dependent on the presence of a functional *recA* gene in the host strains (Mortlemans and Stocker, 1976; Walker, 1977; Venturini and Monti-Bragadin, 1978; Nunoshiba and Nishioka, 1987).

In contrast, R-plasmid pEB017 confers protection against killing by UV-irradiation and nitrofuran in *recA* host strains (Obaseiki-Ebor and Ak-

TABLE 1

BACTERIAL STRAINS USED

Stanford Radiology No. [a]	Genotype [b]	Source, derivation, or reference [c]
J53-1	pEB017/*met pro* Nal^r	Obaseiki-Ebor and Akerele, 1987
SR47	*recA56 ilv thr rpsE* HfrKL16 λ^-	JC5088, J. Foulds
SR248	*leuB19 metE70 thyA36 deo(C2?) bioA2 lacZ53 malB45 rha-5 rpsL151* IN(*rrnD* – *rrnE*) F^- λ^-	KH21, R.B. Helling
SR440	*recA56 ilv thr rpsE* HfrKL16 λ^-	JC5088, J. Gross
SR669	*recA56 ilv-318 thr-300 srlA300*::Tn*10* *rpsE300* HfrKL16	JC10240, A.J. Clark
SR712	*Δ(uvrB301-pgi) umuC36*, otherwise as SR749	TK501, T. Kato
SR749	*argE3 hisG4 leuB6 Δ(gpt-proA)62 thr-1 thi-1 ara-14 galK2 lacY1 mtl-1 xyl-5 tsx-33 rfbD1 mgl-51 kdgK51 rpsL31 supE44 rac* F^- λ^-	AB1157, B.J. Bachmann
SR894	*srlA300*::Tn*10*, otherwise as SR749	SR749 × P1::Tn*9*cts · SR669, Tc^r
SR1159	*recB21*, otherwise as SR749	Sargentini and Smith, 1986
SR1252	*polA5*, otherwise as SR749	N.J. Sargentini
SR1416	λd(*recA::lac*) *c*Iind, otherwise as SR749	GC2375, R. D'Ari
SR1516	*umuC36*, otherwise as SR749	N.J. Sargentini
SR1691	*his-4 pro trpC3 thyA*::Tn*5 deo thi ara-9 galK2 lac-114 malA1 mtl-1 ton rpsL* F^-	KD2173, H. Nakayama
SR1749	*recA56*, otherwise as SR749	SR894 × P1::Tn*9*cts · SR47, Srl^+
SR2316	pEB017/*recA13*, otherwise as SR749	SR2319 × P1 vira · SR2325 NT^r Kn^r
SR2318	pEB017/otherwise as SR749	SR749 × SR2342, Kn^r
SR2319	*recA13*, otherwise as SR749	AB2463, A.S. Breeze
SR2320	pEB017/*recA13*, otherwise as SR749	SR2319 × SR2342, Kn^r
SR2321	pEB017/*recB21*, otherwise as SR749	SR1159 × SR2342, Kn^r
SR2325	pEB017/*polA5*, otherwise as SR749	SR1252 × SR2342, Kn^r
SR2337	F^- λ^-	W3110, A.S. Breeze
SR2339	pEB017/*umuC36*, otherwise as SR749	SR1516 × SR2342, Kn^r
SR2342	pEB017/F^- λ^-	SR2337 × J53-1, Kn^r
SR2343	pKM101/otherwise as SR749	SR749 × SR2456, Ap^r
SR2456	pKM101/*Δ(uvrB) hisD6610 his01242 rfa*	TA97, B.N. Ames
SR2478	pEB017/*recA56*, otherwise as SR749	SR1749 × SR2342, Kn^r
SR2479	pKM101/*umuC36*, otherwise as SR749	SR1516 × SR2456, Ap^r
SR2480	pEB018/*recA56*, otherwise as SR749	SR1749 × pEB017 DNA, Kn^r
SR2481	pEB019/*recA56*, otherwise as SR749	Same as SR2480
SR2482	λd(*recA::lac*) *c*Iind *thyA*::Tn*5*, otherwise as SR749	SR1416 × P1 *vira* · SR1691, Kn^r
SR2483	*recA56* λd(*recA::lac*) *c*Iind, otherwise as SR749	SR2482 × SR440, Thy^+

TABLE 1 (continued)

Stanford Radiology No. [a]	Genotype [b]	Source, derivation, or reference [c]
SR2484	pEB017/*recA56* λd(*recA::lac*) *c*Iind, otherwise as SR749	SR2483×SR2342, Kn^r
SR2511	pEB021/*recA56*, otherwise as SR749	SR1749×pEB021, Ap^r
SR2514	pEB021/otherwise as SR749	SR749×pEB021, Ap^r
SR2515	pEB021/*umuC36*, otherwise as SR749	SR1516×pEB021, Ap^r

[a] All strains are derived from *E. coli* K12, except SR2456 which is derived from *Salmonella typhimurium*.

[b] UV^r, NT^r, Kn^r, Tp^r, Tc^r and Ap^r indicate resistance to UV-irradiation, nitrofurantoin, kanamycin, trimethoprim, tetracycline and ampicillin, respectively.

[c] Bacteriophage P1 *vira* was obtained from A.J. Clark (University of California at Berkeley) and is a reisolate of P1 *vir*. Strains constructed by N.J. Sargentini: SR894, SR1252, SR1465, SR1467, SR1516 and SR1749.

Plasmid pEB017 is the original isolate, and confers UV^r, NT^r, Kn^r and Tp^r (Obaseiki-Ebor and Akerele, 1987). Plasmid pEB021 is the *Bam*HI fragment from pEB017 cloned into the vector pBR322, and confers UV^r, NT^r and Ap^r. Plasmid pEB018 was isolated following transformation of SR1749 with DNA from pEB017 to give strain SR2480, and whose phenotype is UV^s NT^s Kn^r Tp^r. Plasmid pEB019 was isolated as for pEB018 to give strain SR2481, whose phenotype is UV^r NT^r Kn^r Tp^r, i.e., as for pEB017.

erele, 1987). This paper presents further results on the properties of plasmid pEB017, and reports (i) that pEB017 exceeds pKM101 in enhancing UV-radiation and nitrofuran mutability in *E. coli* K12 strains, and (ii) that there is a *recA*-like gene in plasmid pEB017 that, when cloned into pBR322 (to produce pEB021), confers enhanced resistance to UV-irradiation and enhanced UV-radiation mutagenesis to wild-type and *recA* strains, and confers a small increase in resistance to UV-irradiation, and a small amount of UV-radiation mutability in an otherwise nonmutable *umuC* strain.

Materials and methods

Media

YENB broth, LBt broth, YENB agar, and phosphate buffer (67 mM, pH 7) have been described (Sargentini and Smith, 1981). Supplemented minimal media (SMM) and DTM buffer were as described by Wang and Smith (1982). Selective media for resistance to nitrofurantoin, kanamycin sulfate, ampicillin, tetracycline and streptomycin sulfate were YENB agar containing 2.5, 50, 40, 15 and 50 μg/ml of the respective antibacterial agents. SMM agar containing trimethoprim (20 μg/ml) was used to select trimethoprim-resistant mutants. Arg-0 was SMM agar without arginine. Arg-1.5 was Arg-0 agar containing YENB broth at 1.5% (v/v).

Bacteria, plasmids and bacteriophage

The *E. coli* strains and phages used in this study are listed in Table 1. For conjugation, cultures of donor and recipient strains were grown overnight in LBt broth. The recipient culture (0.01 ml) was added to 10 ml of LBt in a 250-ml flask previously warmed to 37°C. The donor culture was diluted 10-fold in 67 mM phosphate buffer, pH 7, and 0.01 ml was added to the recipient suspension in the 250-ml flask, and mixed. The flask was incubated for 24 h at 37°C in an incubator without shaking. Various dilutions of the 24-h culture were spread on selective plates and incubated at 37°C.

Transformation employed the method of Lederberg and Cohen (1974). Transduction using bacteriophage P1 was essentially as described by Miller (1972).

Plasmid DNA extraction

50-ml overnight LBt cultures were harvested for the extraction of plasmid DNA using the SDS-alkaline lysis protocol of Takahashi and Nagano (1984). The air-dried plasmid DNA pellet was dissolved in 10 mM Tris (pH 8), 1 mM disodium EDTA, and RNAase at 10 μg/ml. An

average yield of 5–10 mg of plasmid DNA was obtained from a 50-ml culture. The plasmid DNA solution was stored at −20°C.

Agarose gel electrophoresis

Electrophoresis of the plasmid DNA preparations was carried out on a horizontal 0.5% w/v agarose gel [0.04 M Tris–acetate buffer (pH 8) (Maniatis et al., 1982) with 0.002 M EDTA] at 2.0 V/cm for about 4 h. DNA molecular weight markers, i.e., a 1-kb DNA ladder and lambda DNA/*Hin*dIII fragments were included. The gel was stained with ethidium bromide (0.5 μg/ml) in water for 30 min. Photographs were taken with the stained gels sitting on a UV transilluminator (300 nm).

Restriction endonuclease analysis and cloning into plasmid vector pBR322

The plasmid DNA was cut with restriction endonucleases using the conditions recommended for each enzyme by the supplier (Bethesda Research Laboratories).

The putative *recA* gene was cloned, using *Bam*HI to digest plasmid pEB017 DNA and the plasmid vector pBR322. The restriction mixture was ligated with T4 DNA ligase at room temperature overnight (Maniatis et al., 1982), and was used to transform an *E. coli recA* strain (SR1749) to ampicillin resistance (Ap^r), i.e., carrying the pBR322 vector plasmid. The Ap^r clones that had lost tetracycline resistance were streaked on nitrofurantoin plates to determine their resistance to nitrofurantoin, and on YENB agar for determining their resistance to 20 J/m^2 of UV-radiation. Strain SR2511 was selected for further study.

UV-irradiation and mutagenesis assay procedures

The UV-radiation source (254 nm), the measurement of fluence rate, and the UV-radiation mutagenesis procedure and methods of quantitation have been described (Sargentini and Smith, 1979).

Determination of chemical mutagenesis

Nitrofurantoin and nitrofurazone mutagenesis was determined as follows. *E. coli* cultures were grown overnight in YENB broth, harvested by centrifugation, washed once and resuspended in 67 mM phosphate buffer (pH 7) to an optical density at 650 nm (OD_{650}) of 0.2 in a Zeiss PMQII spectrophotometer. This OD corresponded to $1–3 \times 10^8$ colony-forming units (CFU) per ml, depending upon the strain. A 0.1-ml aliquot of the culture suspension was inoculated into 2 ml of soft-agar (0.6% Bacto agar, Difco) kept at 45°C, and containing the test mutagen and YENB broth at 1.5% (v/v). This mixture was immediately poured on Arg-0 plates. The mutant colonies were counted after incubation at 37°C for 3 days.

β-Galactosidase assay

A 1-ml aliquot of a log phase culture (YENB) was added to 9 ml of 67 mM phosphate buffer (pH 7). A 3-ml aliquot of this suspension in a 6 cm petri dish was UV-irradiated (254 nm) with 10 J/m^2. A 2-ml aliquot of the irradiated cells was added to 2 ml of double-strength YENB in a 50-ml flask, and incubated in a shaking waterbath for 60 min at 37°C. This solution was then centrifuged ($3000 \times g$ for 10 min) in small glass tubes, and the pellets were resuspended in 2 ml of Z Buffer (Miller, 1972). The alternative protocols (i.e., chloroform/SDS and centrifugation) of Miller (1972) were then used for the determination of β-galactosidase activity.

Results

R-plasmid pEB017 restriction digests and agarose gel electrophoresis

Plasmid pEB017 DNA was cleaved by the following restriction enzymes: *Acc*I, *Ala*I, *Ava*I, *Bgl*I, *Bam*HI, *Cla*I, *Eco*RI, *Hin*dIII, *Pst*I, *Sal*I and *Pvu*II. *Kpn*I did not cleave the plasmid. The size of plasmid pEB017 was about 60 Kb, based on *Eco*RI restriction and comparison with λ*Hin*dIII fragments in an 0.5% agarose gel (data not shown).

Cloning of the recA-like gene and restriction mapping

A *Bam*HI digest of pEB017 DNA was ligated into *Bam*HI restricted plasmid vector pBR322, and was used to transform *recA* strain SR1749. 420 cloned Ap^r transformants were obtained, and 56 were tetracycline-sensitive. 4 of the 56 tetracy-

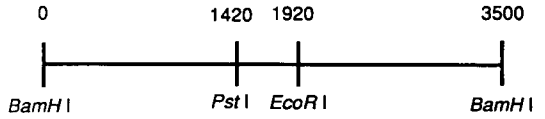

Fig. 1. Restriction map of the *Bam*HI fragment from pEB017 that was cloned into the *Bam*HI site of pBR322 to give pEB021.

cline-sensitive transformants were resistant to UV-irradiation and nitrofurantoin, e.g., SR2511. The plasmid (pEB021) DNA extracted from strain SR2511 was digested with various restriction endonucleases, and electrophoresed through agarose gels. The size of the cloned *recA*-like fragment from pEB021 restricted with *Bam*HI was 3.5 kb, and was calculated to be 3.5 kb by *Pst* I, and 3.4 kb by *Eco*RI restriction. The restriction map is shown in Fig. 1.

Test for recombination

Recipient strains were transduced (Miller, 1982) with a P1 *vira* lysate of strain SR962 (*argE*$^+$ *leuB*$^+$). Both Leu$^+$ and Arg$^+$ transductants were obtained at either wild-type levels or higher in a *recA56* strain carrying either pEB017 or pEB021 (Table 2).

Plasmid pEB017 and pEB021 protection of wild-type, recA, and umuC strains against killing by UV-irradiation

Plasmid pEB017 conferred enhanced resistance to the wild-type strain against UV-irradiation. The protection by pEB017 was similar to that by plasmid pKM101 (Fig. 2). When the

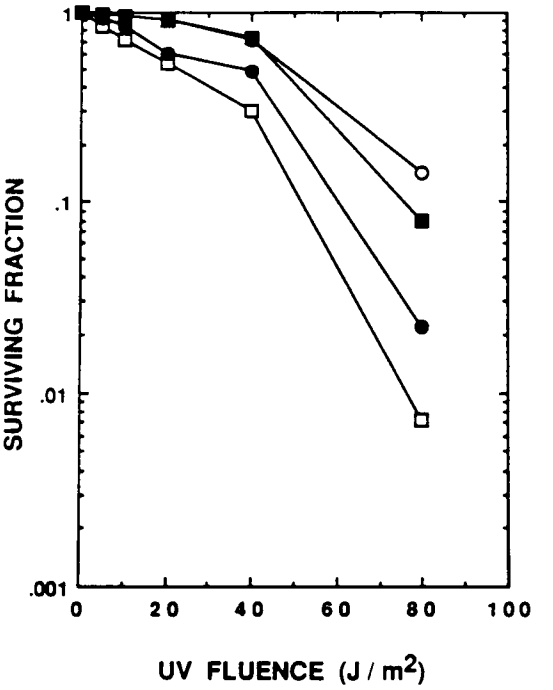

Fig. 2. UV-radiation survival curves for wild-type *Escherichia coli* K12 without (□, SR749) and with plasmid pEB017 (○, SR2318), plasmid pKM101 (■, SR2343), or plasmid pEB021 (●; SR2514).

recA-like gene of plasmid pEB017 was cloned into pBR322 to give pEB021, it also enhanced the resistance of the wild-type strain to a small extent (Fig. 2).

Plasmid pEB017 enhanced the resistance of *recA* strains, i.e., *recA56* (Fig. 3) and *recA13* and *ΔrecA306* (data not shown), more than that of the

TABLE 2

RECOMBINATION ABILITY AS DETERMINED BY A TRANSDUCTIONAL CROSS [a]

Recipient strains	Leu$^+$ transductants		Arg$^+$ transductants	
	Leu$^+$/ml	Recombination deficiency	Arg$^+$/ml	Recombination deficiency
SR749 (*recA*$^+$)	42,930	1.00	20,620	1.00
SR1749 (*recA56*)	0	< 0.00002	20	0.001
SR2478 (pEB017/*recA56*)	41,290	0.96	29,580	1.43
SR2511 (pEB021/*recA56*)	38,000	0.89	37,790	1.83

[a] Recipient strains were transduced with a P1 *vira* lysate of strain SR962 (*argE*$^+$ *leuB*$^+$) as described in Methods. Transductants/ml have been corrected for spontaneous revertants (0–30/ml). Deficiency values are the transductants/ml for each strain divided by the value for strain SR749.

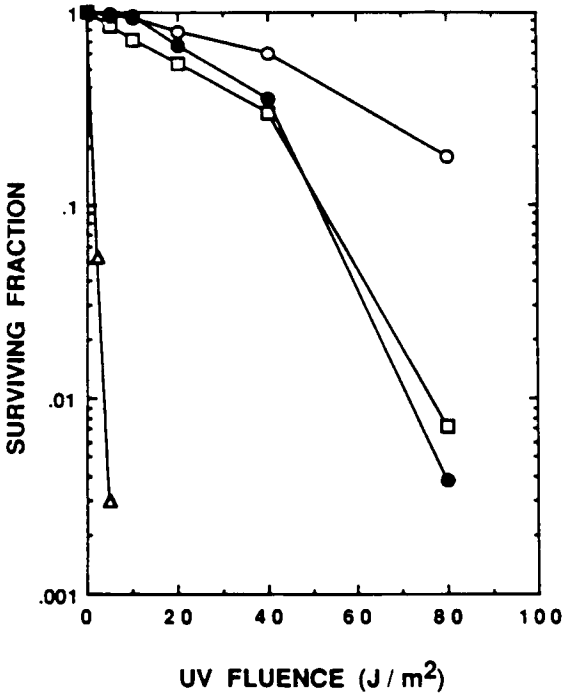

Fig. 3. UV-radiation survival curves for *recA56* strains of *Escherichia coli* K12 without (Δ, SR1749), and with plasmid pEB017 (○, SR2478) or plasmid pEB021 (●, SR2511). The curve for the wild-type strain (□, SR749) is taken from Fig. 2.

recA$^+$ strain without the plasmid (Fig. 3). Plasmid pEB021 also conferred resistance to UV radiation to *recA* strains up to the wild-type level, i.e., *recA*$^+$ without plasmid pEB017 (Fig. 3).

The *umuC36* strain was somewhat more sensitive to UV-irradiation than the wild-type strain (Fig. 4). Plasmids pEB017 and pKM101 conferred enhanced resistance to UV irradiation to the *umuC* strain, and the resistance was more than that for a wild-type strain without a plasmid. Plasmid pEB021 enhanced the survival of the *umuC* strain to that of the wild-type strain without a plasmid (Fig. 4). It would have been ideal to conduct these experiments with the "NUL" mutation, *umuC222* :: Tn*5*, but a Tn*10* insert in *uvrA* proved to be unstable in the presence of pEB017. Therefore, we chose to use *umuC36*.

Enhancement of UV-radiation mutagenesis

Plasmid pEB017 enhanced UV-radiation mutagenesis in the wild-type, *recA*, and *umuC* strains (Table 3). Comparatively, plasmid pEB017 enhanced the UV radiation mutagenicity in *E. coli* several-fold more than did plasmid pKM101. The enhanced mutagenesis produced by plasmid pEB017 could be readily observed after 1 day of incubation, increasing to a maximum level in 3 days; while the mutants were observed after 3 days with pKM101, and increased to a maximum level after 5 days.

Plasmid pEB021 markedly enhanced the UV mutability of wild-type and *recA* strains, and even in the *umuC* strain there was a significant enhancement of UV-mutagenesis by pEB021 compared to the *umuC* strain without the plasmid. Note that pEB017 probably contains the *mucAB* genes, but pEB021 does not.

Nitrofuran mutagenesis

Table 4 shows the results of experiments in which nitrofurantoin and nitrofurazone were tested for mutagenicity in *E. coli* AB1157, i.e., strain SR749 with and without the plasmids

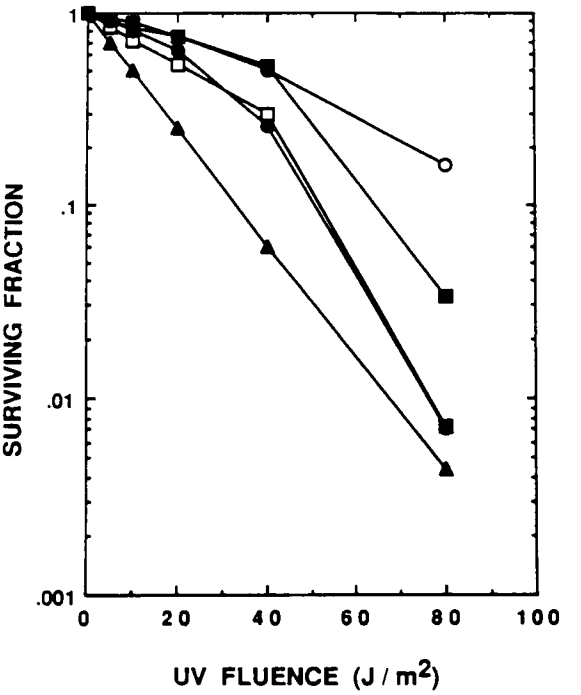

Fig. 4. UV-radiation survival curves for *umuC36* strains of *Escherichia coli* K12 without (Δ, SR1516) or with plasmid pKM101 (■, SR2479), plasmid pEB017 (○, SR2339), or plasmid pEB021 (●, SR2515). The curve for the wild-type strain (□, SR749) is taken from Fig. 2.

TABLE 3

EFFECT OF PLASMIDS pEB017 AND ITS DERIVATIVES, AND pKM101 ON UV RADIATION MUTAGENESIS IN WILD-TYPE, *recA* AND *umuC* STRAINS OF *E. coli* K12 [a]

Strain designation	Relevant genotype	UV dose (J/m^2)	Arg^+ mutants/ 10^8 survivors	Ratio: strain/ SR749
SR749	wild-type (WT)	10	254	1
		20	666	1
SR2318	pEB017/WT	10	2191	8.6
		20	6371	9.6
SR2343	pKM101/WT	10	814	3.2
		20	1693	2.5
SR1467	*ΔrecA*	10	0	0
SR2329	pEB017/*ΔrecA*	10	3132	12.3
		20	7574	11.4
SR1749	*recA56*	10	0	0
SR2478	pEB017/*recA56*	10	3247	12.8
		20	9542	14.3
SR2480	pEB018/*recA56*	10	0	0
		20	0	0
SR2316	Transductant (UV^r NT^r)	10	1345	5.3
		20	5501	8.3
SR1516	*umuC36*	10	1.5	5.9×10^{-3}
		20	15.5	2.3×10^{-2}
SR2339	pEB017/*umuC36*	10	1814	7.1
		20	7157	10.7
SR2479	pKM101/*umuC36*	10	609	2.4
		20	1546	2.3
SR2514	pEB021/WT	10	1362	5.4
		20	5505	8.3
SR2511	pEB021/*recA56*	10	1078	4.2
		20	3772	5.7
SR2515	pEB021/*umuC36*	10	93	3.6×10^{-1}
		20	171	2.6×10^{-1}

[a] Data are the average of three independent experiments.

pKM101 and pEB017. Plasmid pEB017 was several-fold more effective in promoting revertants than was pKM101.

Induction of the synthesis of β-galactosidase

Strain SR1416 has a *recA*::*lac*$^+$ fusion gene in addition to its normal *recA* gene, and β-galactosidase can be induced in this strain by UV-irradiation at 10 J/m^2. The introduction of a *recA56* mutation into strain SR1416, to produce strain SR2483, blocked the induction of β-galactosidase. The introduction of plasmid pEB017 into strain SR2483 produced transconjugants that were, UV^r NT^r Kn^r Tp^r, e.g., strain SR2484. Strain SR2484 displayed more than 3 times the β-galactosidase activity of strain SR1416 after induction, i.e., 7280 and 2240 units of β-galactosidase, respectively.

Discussion

Plasmid pEB017 possesses a gene(s) that confers enhanced resistance to UV-irradiation, enhanced UV-radiation mutability, and enhanced resistance to nitrofurantoin to wild-type, *recA* and *umuC* strains, and restores recombination ability to a *recA* strain.

Thus, several pieces of experimental evidence suggest that there is a functional *recA*-like gene in plasmid pEB017, but it appears to differ in several important aspects from the normal *recA* gene of *E. coli*.

TABLE 4

Arg$^+$ REVERTANTS PER PLATE PRODUCED BY NITROFURANTOIN AND NITROFURAZONE IN *Escherichia coli* AB1157 DERIVATIVES [a]

Mutagen	Strain		
	WT (SR749)	pKM101/WT	pEB017/WT
Nitrofurantoin (μg/ml per plate)			
0.63	0	7	22
1.25	5	9	59
2.5	12	20	109
Nitrofurazone (μg/ml per plate)			
0.63	3	8	31
1.25	9	16	72
2.5	22	36	161

[a] Spontaneous revertants in the control plates were subtracted from the data presented.

(a) Both plasmids pEB017 and pEB021, which is pBR322 that contains a *Bam*HI fragment from pEB017, restore the recombination ability of a *recA56* strain (Table 2).

(b) When pEB017 was transferred into a *recA* strain of *E. coli*, it conferred protection against UV-radiation (Fig. 3), and enhanced UV-radiation mutability (Table 3) more so than did the chromosomal *recA* gene in a wild-type strain.

(c) Plasmid pEB017 produced more than 3-fold the quantity of SOS-mediated synthesis of β-galactosidase in a *recA* strain compared with that induced in a wild-type strain (see Results). This suggests that plasmid pEB017 may not have just picked up an ordinary *E. coli recA* gene. This conclusion is also supported by the fact (Fig. 1) that, the cloned *Bam*HI fragment in pEB021 seems to be slightly longer (3500 bp) than the *Bam*HI fragment (3300 bp) cloned by Madiraju et al. (1988) from the *E. coli* chromosome. In addition, in the left half of the cloned *Bam*HI fragment from pEB017, the *Bam*HI site to *Pst*I site fragment is about 170 bp longer than that reported by Madiraju et al. (1988) for the *recA* gene cloned from *E. coli*, although the restriction sites at *Pst*I and *Eco*RI, when measured from the right end of the map agree with the data of Madiraju et al. (1988). Another *Eco*RI site may be present in the *Bam*HI fragment containing the putative *recA* gene, since the sum of the *Eco*RI fragments is 100 bp shorter than the single cut vector, whereas this problem was not observed with *Pst*I. It will be important to sequence this *recA*-like gene from pEB017 for comparison with the *recA* gene from *E. coli* and from other sources (e.g., Kenner et al., 1984; Pierre and Paoletti, 1983; Koomey and Falkow, 1987; Barrows, 1989; Dalrymple et al., 1989).

The ability of plasmid pEB021 to confer a small but significant UV-mutability in *umuC* cells could help to elucidate the mechanism of the enhanced UV-radiation mutagenesis in *umuCD*$^+$ strains. It is known that the RecA protein interacts with UmuCD′ and Pol III proteins to induce mutations (Walker, 1984; Peterson et al., 1988; Woodgate et al., 1989; Echols and Goodman, 1990). The UmuC protein is thought to act in a complex with the UmuD′ protein as a fidelity-relaxing factor to allow Pol III to continue DNA synthesis past lesions. The *recA*-like gene from pEB017 may enhance the activity of the UmuCD proteins in the host cell, thereby enhancing mutagenesis. In *umuC36* cells, the putative RecA-like protein from pEB017 could have some fidelity-relaxing properties to enable Pol III protein to continue synthesis past a lesion in the absence of the UmuCD′ complex.

Plasmid pEB017 has properties different from those of plasmid pKM101, which is used in the Ames Salmonella mutation assay. The *S. typhimurium* tester strains have no functional UmuDC proteins (Podger and Hall, 1984; Walker, 1984; Herrera et al., 1988), therefore, plasmid pKM101 complements the requirement for UmuDC proteins to promote mutagenicity (Little et al., 1989). Plasmid pEB017 exceeded pKM101 in the induction of UV-radiation and nitrofuran mutagenesis in *E. coli* K12 wild-type and *umuC* strains (Tables 3, 4), although it was not quite as effective as pKM101 in the Ames *S. typhimurium* tester strains for mutagenesis by nitrofurantoin and nitrofurazone (data not shown). The differential mutability effects of plasmids pEB017 and pKM101 could be influenced by the host cell. Because plasmid pEB017 produced a significant enhancement in mutagenesis in *E. coli* (Tables 3, 4), it may prove to be very useful in enhancing

mutations in characterized *E. coli* mutation tester strains, e.g., *E. coli* K12 ND-160 (Clarke and Wade, 1975).

Further studies to elucidate the nature of the RecA-like, UmuC-like and UmuD-like proteins produced by plasmid pEB017 will be of interest for the proper understanding of the survival enhancing and mutagenesis enhancing action of plasmid pEB017.

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