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Role of *ruvAB* genes in UV- and γ-radiation and chemical mutagenesis in *Escherichia coli*

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Summary

Escherichia coli umuC122::Tn*5* was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine to isolate mutations that block the residual γ-radiation mutagenesis observed in *umuC* strains. Two of these mutations were shown by transductional mapping and plasmid complementation to map in the *ruvA* and *ruvB* genes (i.e., *ruvA200* and *ruvB201*). Whereas *ruvA200* was complemented by *ruvA*$^+$ plasmids, the only other known *ruvA* mutation, *ruvA59*::Tn*10* required both the *ruvA*$^+$ and *ruvB*$^+$ genes to show complementation. The *ruvA200*, *ruvB201*, *ruvA59*::Tn*10* and *ruvB60*::Tn*10* mutations all reduced γ-radiation-induced ochre reversion [*argE3*(Oc) → Arg$^+$] to about 30% of the wild-type level, and they all reduced UV-radiation-induced ochre reversion to about 15% of the wild-type level. The *ruvA200* and *ruvB201* mutants also showed reduced γ- and UV-radiation mutagenesis with two other assays [*hisG4*(Oc) → His$^+$ and Rif^s → Rif^r]. Streptozotocin mutagenesis (Rif^r) was reduced to about half of the wild-type level in *ruv* strains, but ethyl methanesulfonate mutagenesis was normal. While the *umuC* strain did not show the oxygen enhancement of γ-radiation mutagenesis, the *ruvA200* strain showed an oxygen effect that was similar to that shown by the wild-type strain. When the *ruvA200* mutation was combined with the *umuC* mutation, γ-radiation mutagenesis was further reduced to 5% of the wild-type level and cells showed a synergistic sensitization to UV- and γ-radiation-induced killing. A mutational spectrum analysis indicates a general depression of both *umuC*-dependent and *umuC*-independent γ-radiation mutagenesis in the *ruvA* strain, which is in contrast with the site-specific reduction in γ-radiation mutagenesis that is observed in the *umuC* mutant. The reduced radiation mutagenesis in the *ruvA* strain could not be correlated with a reduction in transcription of the *recA* or *umuC* genes.

While the function of the *recA–lexA* regulated *umuDC* operon seems to be essential for UV-radiation mutagenesis (reviewed in Walker, 1984), it is of special interest here that the *umuDC* operon is not essential for all of γ-radiation mutagenesis (Steinborn, 1978; Sargentini and Smith, 1984, 1989). In fact, for cells that are γ-irradiated in the absence of oxygen, mutagenesis (base substitution) at some chromosomal sites is virtually independent of the *umuC* gene, while mutagenesis at other chromosomal sites is strongly dependent upon the *umuC* gene; also the additional mutagenesis that is produced when cells are γ-irradia-

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TABLE 1

E. coli K12 STRAINS USED

Stanford Radiology No.	Genotype [a]	Source, derivation, or reference [b]
SR47	*recA56 ilv-318 thr-300 thi-1 rel-1 rpsE* Hfr λ^-	JC5088, J. Foulds
SR669	*recA56 ilv-318 thr-300 srlA300*::Tn*10 rpsE300* HfrKL16 λ^-	JC10240, A.J. Clark
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, ECGSC
SR894	*srlA300*::Tn*10* and as SR749	SR749 × P1::Tn*9c*ts·SR669, Tc^r
SR1165	*umuC122*::Tn*5* and as SR749	Sargentini and Smith, 1984
SR1310	*his pyrC46 thyA araD139 Δ(lac)U169 malA thi hag*::Tn*10 rpsL*	YK4516, Y. Komeda
SR1416	λd(*recA*::*lac*)c1 *ind* and as SR749	GC2375, R. D'Ari
SR1436	*recA441 sulA11 umuC121*::Mud(Ap,*lac*) *argE3 hisG4 ilv*(Ts) *leuB6 thr-1 galK2 ΔlacU169 malE*::Tn*5 rpsL31* F^- λ^-	GW1104, G.C. Walker
SR1438	*Δ(recA–srlR)306 srlR301*::Tn*10* λ(*recA*$^+$) and as SR749	EST945, E.S. Tessman
SR1467	*Δ(recA–srlR)306 srlR301*::Tn*10* and as SR749	SR749 × P1 *vir*a·SR1438, Tc^r
SR1697	*ruvA59*::Tn*10* and as SR749	N2058, R.G. Lloyd
SR1698	*ruvB60*::Tn*10* and as SR749	N2057, R.G. Lloyd
SR1701	*ruvB9*	BE5036, H.I. Ogawa
SR1749	*recA56* and as SR749	SR894 × P1::Tn*9c*ts·SR47, Srl^+
SR1947	*umuC122*::Tn*5 xmu-1062(ruvB201)* and as SR749	SR1165, MNNG
SR1993	*umuC122*::Tn*5 xmu-1138(ruvA200)* and as SR749	SR1165, MNNG
SR2067	*umuC122*::Tn*5 ruvA59*::Tn*10* and as SR749	SR1165 × P1 *vir*a·SR1697, Tc^r
SR2103	*umuC122*::Tn*5 ruvB201 hag*::Tn*10* and as SR749	SR1947 × P1 *vir*a·SR1310, Tc^r
SR2116	*eda-51*::Tn*10* IN(*rrnD-rrnE*)*1* λ^-	N3041, ECGSC
SR2125	*umuC122*::Tn*5 ruvA200 hag*::Tn*10* and as SR749	SR1993 × T4GT7·SR1310, Tc^r
SR2183	*umuC122*::Tn*5 ruvB201 eda-51*::Tn*10* and as SR749	SR1947 × P1 *vir*a·SR2116, Tc^r
SR2184	*umuC122*::Tn*5 ruvA200 eda-51*::Tn*10* and as SR749	SR1993 × P1 *vir*a·SR2116, Tc^r
SR2206	*eda-51*::Tn*10* and as SR749	SR749 × P1 *vir*a·SR2183, Tc^r
SR2207	*ruvB201 eda-51*::Tn*10* and as SR749	Same as SR2206
SR2209	*eda-51*::Tn*10* and as SR749	SR749 × P1 *vir*a·SR2184, Tc^r
SR2210	*ruvA200 eda-51*::Tn*10* and as SR749	Same as SR2209
SR2212	*umuC122*::Tn*5 xmu-1062(ruvB201) eda-51*::Tn*10* and as SR749	SR1165 × P1 *vir*a·SR2183, Tc^r
SR2214	*umuC122*::Tn*5 eda-51*::Tn*10* and as SR749	SR1165 × P1 *vir*a·SR2184, Tc^r
SR2215	*umuC122*::Tn*5 xmu-1138(ruvA200) eda-51*::Tn*10* and as SR749	Same as SR2214
SR2314	pFB502/*recA56* and as SR749	SR1749 × pFB502, Ap^r

TABLE 1 (continued)

Stanford Radiology No.	Genotype [a]	Source, derivation, or reference [b]
SR2335	*umuC122* :: Tn*5* *ruvB60* :: Tn*10* and as SR749	SR1165 × P1 *vir*a · SR1698, Tc^r
SR2346	pHS202/Δ(*recA–srlR*)*306 srlR301* :: Tn*10* and as SR749	SR1467 × pHS202, Ap^r
SR2355	pNJS100/*umuC122* :: Tn*5* *ruvA200 eda-51* :: Tn*10* and as SR749	SR2215 × pNJS100, Ap^r
SR2384	*ruvA59* :: Tn*10* and as SR749	SR749 × P1 *vir*a · SR1697, Tc^r
SR2385	*ruvB60* :: Tn*10* and as SR749	SR749 × P1 *vir*a · SR1698, Tc^r
SR2388	*ruvB9 eda-51* : Tn*10*	SR1701 × P1 *vir*a · SR2116, Tc^r
SR2392	*umuC122* :: Tn*5* *ruvB9 eda-51* :: Tn*10*	SR1165 × P1 *vir*a · SR2388, Tc^r
SR2404	λd(*recA* :: *lac*) c I *ind eda-51* :: Tn*10* and as SR749	SR1416 × P1 *vir*a · SR2184, Tc^r
SR2405	λd(*recA* :: *lac*) c I *ind ruvA200 eda-51* :: Tn*10* and as SR749	Same as SR2404
SR2408	*eda-51* :: Tn*10* and as SR1436	SR1436 × P1 *vir*a · SR2184, Tc^r
SR2409	*ruvA200 eda51* :: Tn*10* and as SR1436	Same as SR2408

[a] Genotype nomenclature is that used by Bachmann (1983).

[b] Ap^r and Tc^r indicate resistance to ampicillin and tetracycline, respectively; MNNG is *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; ECGSC is *E. coli* Genetic Stock Center; P1 *vir*a was obtained from A.J. Clark (University of California, Berkeley) and is a reisolate of P1 *vir*.

ted in the presence of oxygen (e.g., in air) is completely *umuC*-dependent at all of the chromosomal sites studied (Sargentini and Smith, 1989). Both *umuC*-dependent and *umuC*-independent γ-radiation mutagenesis is absent in a strain deleted for the *recA* gene (Sargentini and Smith, 1989). This suggests that the UmuDC proteins function in the mutagenesis caused by only certain types of γ-radiation-induced lesions, and that other γ-radiation-induced lesions produce mutations by another *recA*-dependent mechanism.

To better understand the *umuC*-independent mechanism of γ-radiation mutagenesis, we isolated new mutations that block the γ-radiation mutability of the *umuC* strain. We call such mutations *xmu* (for X-ray mutability). In this work we show that two of these *xmu* mutations reside in the *ruvA* and *ruvB* genes, genes that are known for their involvement in the repair of DNA damage (Otsuji et al., 1974; Lloyd et al., 1984; Benson et al., 1988), and we characterize the role of the *ruvA* and *ruvB* genes in radiation and chemical mutagenesis.

Materials and methods

Media

YENB was 0.75% yeast extract (Difco) and 0.8% nutrient broth (Difco). YENBG was YENB containing 1% glucose. LBt was 1% tryptone (Difco), 0.5% yeast extract, 1% NaCl and thymine at 10 μg/ml. MM was a 0.4% glucose–salts medium (Ganesan and Smith, 1968), supplemented with 1 mM L-arginine, L-histidine, L-leucine, L-proline and L-threonine; and with thiamine · HCl at 0.5 μg/ml. MM-1.5 was MM containing 1.5% YENB (v/v). To make plates, Bacto agar (Difco) was added at 1.5%, and medium was dispensed at 27 ml per petri dish. Arg-0 and His-0 plates were MM plates that were deficient in arginine and histidine, respectively. Arg-1.5 and His-1.5 plates were Arg-0 and His-0 plates that also contained 1.5% YENB (v/v). Rif plates were YENB agar containing rifampicin (Sigma) at 100 μg/ml (dissolved first in dimethyl sulfoxide at 10 mg/ml). LB-Ap50 plates contained 1% tryptone, 0.5% yeast extract, 0.5% NaCl, 1.5% Bacto agar,

and ampicillin (Sigma) at 50 μg/ml (dissolved first in dimethyl sulfoxide at 10 mg/ml). F-top agar was 0.8% NaCl and 0.8% Bacto agar. PB was Na_2HPO_4 at 5.83 g/l and KH_2PO_4 at 3.53 g/l, pH 7.0. MC (Miller, 1972) was 0.1 M $MgSO_4$ and 5 mM $CaCl_2$.

Bacteria, bacteriophage and plasmids

Bacterial strains used are listed in Table 1. Bacteriophage P1 transductions were performed as described by Miller (1972). Bacteriophage T4GT7 transductions were performed as described by Wilson et al. (1979) except that recipient cells were grown in LBt and resuspended in MC containing L-tryptophan at 25 μg/ml. All plasmids used in the complementation experiment were prepared by the "Boiling Method" for the "rapid, small-scale isolation of plasmid DNA" described in Maniatis et al. (1982). Plasmids were transformed into recipient cells according to Davis et al. (1980), except that cells were heated for 2 min at 45 °C and transformants were incubated for 60 min at 37 °C before plating on LB-Ap50.

Plasmids pHSG415, pPVA101, pPVA101::Tn*1000*-4, pPVA101::Tn*1000*-12, pFB502 and pGTI19 were obtained from the laboratory of Dr. Robert G. Lloyd (Nottingham), via Dr. Fiona Benson and Dr. Gary Sharples. Plasmid pHS202 was obtained from the laboratory of Dr. Hideo Shinagawa via Dr. Hiroshi Iwasaki. Plasmids pHS202 (stored in strain SR2346) and pFB502 (stored in SR2314) were isolated by the "Boiling" and "Lysis by SDS" methods, respectively, for the "large-scale isolation of plasmid DNA" described in Maniatis et al. (1982).

Unless specified, the procedures for plasmid subcloning are all from Maniatis et al. (1982). Plasmid pHS202 was cut with restriction enzymes *Eco*RI (BRL) and *Bam*HI (Toyobo) (under conditions suggested by the suppliers) to produce 2.7-kb and 1.0-kb (*ruvA*$^+$) fragments. The fragments were separated by 2.0% agarose (Bio-Rad) gel electrophoresis (4.3 V/cm, 120 min) in Tris-borate buffer (Tris, 10.8 g/l; Na_2EDTA, 2 mM) at pH 7.6. Plasmid pFB502 was similarly cut to yield 2.2-kb, 4.7-kb (ampicillin-resistance gene), and 7-kb DNA fragments, which were separated on a 0.5% agarose gel (150 min, otherwise as above). The 4.7-kb and 1.0-kb DNA fragments were recovered from the two gels by electroelution, phenol–chloroform extraction, and ethanol precipitation, and ligated [1.4 units of bacteriophage T4 DNA ligase (BRL) per 9 μl of DNA mixture]. After incubation overnight at room temperature, the ligation mixture was used to transform SR2215 cells (as above), using ampicillin-resistance selection. The resulting plasmid conferring ampicillin resistance in strain SR2355 was called pNJS100. A miniprep (as above) of strain SR2355 and 0.8% agarose gel electrophoresis (115 min) revealed a 5.7-kb plasmid cleavable by a mixture of *Eco*RI and *Bam*HI to produce 4.7-kb and 1.0-kb DNA fragments (not shown).

Mutagenesis with N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) and mutant selection

To isolate mutations that block *umuC*-independent γ-radiation mutagenesis, strain SR1165 (*umuC122*::Tn*5*) was mutagenized with MNNG (Aldrich) generally as described by Adelberg et al. (1965). Cells were shaken overnight in YENB at 37 °C, diluted 1:500 into 20 ml of YENB and shaken until the optical density at 650 nm (OD_{650}) reached 0.4 [$\sim 1 \times 10^8$ colony-forming units per milliliter (CFU/ml)]. Cells from a 10-ml sample were harvested by membrane filtration, washed and resuspended in 2 ml of TM buffer (Adelberg et al., 1965). A 1.8-ml sample of cells was added to 0.2 ml of MNNG at 1 mg/ml and shaken at 37 °C. Cell samples were diluted and spread on YENB plates every 5–10 min, and the plates showing about 50% lethality ($\sim$ 40 min treatment) were saved. [At this survival level, every cell can be assumed to contain one or more mutations (Adelberg et al., 1965).] Colonies were cloned on YENB plates and then clones were tested for their nutritional requirements. Of 646 clones, 286 (44%) grew very slowly on YENB plates or did not grow on MM plates, and were discarded. The remaining 360 clones were tested for γ-radiation mutability by aerating cells overnight at 37 °C in YENBG, irradiating with 40 krad and plating 0.2-ml samples on Arg-1.5 plates. Under these conditions, parental cells produced about 500 Arg$^+$ mutants per plate; 77 mutant clones that produced less than 50 Arg$^+$ mutants per plate were saved. γ-

Radiation-induced mutant frequencies were then measured to ensure that cell lethality was not compromising the mutability analysis. Ethyl methanesulfonate (EMS) mutagenesis was determined by placing 2 μl of EMS (Eastman) in the center of an Arg-1.5 plate spread with 0.2 ml of an overnight YENBG culture. EMS mutagenesis was largely *recA*-independent as previously reported (Ishii and Kondo, 1975) (we obtained 240 Arg^+ colonies for a *umuC* strain vs. 120 for a *recA56* strain), which allows this test to indicate whether the reversion system was working properly. That is, the inability to induce Arg revertants by EMS probably indicates that the MNNG had induced a second mutation either in the *argE* gene or in another *arg* gene. Clones giving no Arg revertants after EMS treatment were discarded. Of 360 clones, 16 passed these tests and were identified as *xmu* mutants. Two clones were shown to carry *recA* mutations (*recA2003* and *recA2004*) by the linkage (bacteriophage P1) between their *xmu* mutation and the *srl* locus, and by the restoration of γ-radiation mutability to these *xmu* mutants by a plasmid, pVW12, (provided by Dr. Tzu-chien V. Wang in our laboratory) carrying just the $recA^+$ gene (data not shown). Two clones, SR1947 (*xmu-1062*) and SR1993 (*xmu-1138*), are the focus of this study, and 12 other clones are in earlier stages of analysis.

Preparation and irradiation of cells

Log-phase cells were prepared by diluting overnight YENB or MM cultures 1:500 or 1:100, respectively, into homologous medium and shaking at 37°C until an OD_{650} of 0.4 was attained. For survival experiments, cells were harvested by filtration on membranes that had been treated with bovine serum albumin at 1 mg/ml (to prevent cells from sticking to the filters), washed 3 times and resuspended in PB at an OD_{650} of 0.2. For radiation-mutagenesis experiments, cells were pelleted by centrifugation (6 min, $6000 \times g$), washed twice, and resuspended in PB at an OD_{650} of 5 ($\sim 1 \times 10^9$ CFU/ml) for γ-irradiation, or at an OD_{650} of 0.2 for UV-irradiation. γ-Irradiation (^{137}Cs) was accomplished using a Mark I, model 25 irradiator (J.L. Shepherd and Associates); the dose rate was 3.9 krad min^{-1}. Cells were bubbled with air (for oxic irradiation) for 3 min or with N_2 (99.99%) for 10 min (for anoxic irradiation) before (and during) irradiation. Cells were UV-irradiated in open 10-cm glass petri dishes using an 8-W General Electric germicidal lamp (G8T5) emitting primarily at 254 nm; the dose rate was 1 J $m^{-2}sec^{-1}$, but was reduced to 1/5th or 1/25th (by neutral density filtration) for sensitive strains. Dosimetry procedures and irradiation details have been described (Sargentini and Smith, 1983). UV-Irradiated cells were concentrated tenfold by centrifugation before being assayed for mutants (see Mutation assays, below).

Treatment of cells with EMS or streptozotocin

For EMS treatment, log-phase cells were prepared as for irradiation, then a 1-ml volume of washed cells in PB at $OD_{650} = 0.4$ was added to 1 μl of EMS (Eastman) plus 19 μl of dimethyl sulfoxide, and the mixture was shaken for 60 min at 37°C. After the incubation with EMS, 7 ml of PB was added to the treated and untreated cells before assaying for mutants (see Mutation assays, below). For streptozotocin treatment, 1 ml of washed log-phase cells (as above) was centrifuged at $16000 \times g$ for 1 min, and the cells were resuspended in 1 ml of streptozotocin (Sigma) freshly dissolved at 10^{-5} M in PB. The cell–streptozotocin mixture was shaken at 37°C for 30 min before the cells were pelleted, washed and resuspended in 1 ml of PB before being assayed for mutants (see Mutation assays, below).

Mutation assays

For assaying the induction of rifampicin-resistant (Rif^r) mutants, treated and untreated cells were diluted into MM-1.5 to obtain $\sim 1 \times 10^6$ CFU/ml, and they were shaken 16–20 h at 37°C to produce saturated cultures. Samples (0.1 or 0.2 ml) of these saturated cultures were spread on Rif plates, or 1-ml samples were mixed with 2.5 ml of F-top agar and poured over Rif plates to determine the number of Rif^r mutants per milliliter. Cells were also plated on YENB to determine the CFU/ml. The Rif^r mutant frequency for nontreated cells was subtracted from that for treated cells to determine the induced mutant frequency.

For assaying Arg^+ or His^+ mutants, 0.2-ml cell samples of nontreated cells were spread on Arg-0 and Arg-1.5, or on His-0 and His-1.5 plates (usu-

ally 4, but 6–8 for less mutable strains); treated cells were spread only on Arg-1.5, or on His-1.5 plates (the broth supplement in these plates allows mutation fixation and expression, and a limited amount of cell growth). Diluted cells were plated on Arg-1.5 or on His-1.5 to determine the CFU/ml. The induced mutant frequencies were calculated from:

$$\left[M_t - M_{po} + M_o(1 - \text{surviving fraction})\right](1 \times 10^8)/$$

(viable cells per plate)

where M_t is the average number of mutant colonies arising from irradiated cells on YENB-supplemented plates, M_{po} is as M_t but for nonirradiated cells, and M_o is as M_{po} but for plates without broth; see Bridges (1972) for details.

Indirect sequence determination of Arg$^+$ mutants

In general this involved testing Arg$^+$ mutants for their sensitivity to a set of 6 bacteriophage T4 strains; the pattern of phage sensitivity determines the site and base change of the mutation conferring the Arg$^+$ phenotype. The procedure is described in Sargentini and Smith (1989), except that we now employ phage suspensions of 10^6 plaque-forming units/ml for the wild-type, B17, oc427, and ps205 strains, and we now only use L-Broth Agar plates that are less than three days old (as this consistently gives unambiguous results).

β-Galactosidase assay

The method was derived from that of Miller (1972). Cells grown to log phase in YENB at 37°C (strains SR2404 and SR2405) or at 30°C (for the temperature-conditional strains SR2408 and SR2409) were washed and resuspended in DTM at an OD_{650} of 0.4, and then were UV-irradiated and diluted 28-fold into MM-1.5 at the preirradiation growth temperature. The β-galactosidase activity in the cultures (which is related to transcription of the *recA* or the *umuC* gene in the appropriate strain) was determined over a 2-h incubation period. Samples (35-ml) were removed at various times and centrifuged at $6000 \times g$ for 6 min at room temperature, and the pellet was resuspended in 2 ml of Z buffer containing chloramphenicol at 100 μg/ml (CZ) (this harvesting procedure took about 12 min). For *recA* induction, 0.5-ml (nonirradiated) or 0.1-ml (irradiated) samples were diluted to 1 ml with CZ; for *umuC* induction, 1 ml of nondiluted cells was used. These 1-ml samples of cells in CZ were placed on ice, and the remainder of the cells in CZ were added to 20 μl of formaldehyde solution (37%), and the OD_{600} of this suspension was determined later. Within 3 h after irradiation, 40 μl of 0.1% sodium dodecyl sulfate and 40 μl of $CHCl_3$ were added to the cell samples on ice, and the mixtures were vortexed for 10 sec and then placed at 28°C for 10 min. Then 200 μl of *o*-nitrophenyl-β-D-galactoside at 4 mg/ml in Z buffer was added to start the color reaction. When a faint yellow color appeared, the reaction was terminated by mixing in 0.5 ml of 1 M Na_2CO_3 to give a reaction volume of 1.7 ml. The mixtures were centrifuged at $3000 \times g$ for 10 min to remove cell debris, and the A_{420} was determined. β-Galactosidase (units/OD_{600}) was determined according to Miller (1972) from:

$$(A_{420})(\text{reaction volume})(1000)/(OD_{600})$$

(sample volume)(reaction time, min)

Results

To identify genes that are involved in *umuC*-independent γ-radiation mutagenesis, strain SR1165 (*umuC122*::Tn*5*) was mutagenized with MNNG (see Methods), and two mutants were selected for further study on the basis of their poor γ-radiation mutability. Whereas the parental *umuC* strain (an overnight YENBG culture resuspended in PB at $OD_{650} = 5$) irradiated with 40 krad yielded a mutant frequency of 140 Arg$^+$ mutants per 10^8 survivors and a surviving fraction of 4.2×10^{-1}, the *umuC xmu-1062* strain (SR1947) and the *umuC xmu-1138* strain (SR1993) showed no induction of mutants, and they showed surviving fractions of 7.2×10^{-3} and 1.4×10^{-3}, respectively. Conjugational mapping showed that both of these mutations were located near the *hisG* gene at 44 min on the *E. coli* K-12 chromosomal map (Bachmann, 1983). Bacteriophage transductions in this region

TABLE 2

TRANSDUCTIONAL MAPPING OF THE *xmu-1062* AND *xmu-1138* MUTATIONS

Donor lysate (relevant marker)	Recipient (relevant marker)	Fraction of recipients with change in Xmu phenotype [a]	Calculated distance between *xmu* and selected marker (min) [b]
T4·SR1310 (*hag*::Tn*10*)	SR1947 (*xmu-1062*)	10/60	2.0
T4·SR2103 (*hag*::Tn*10 xmu-1062*)	SR749 (+)	14/52	1.4
	SR1165 (+)	11/52	1.6
P1·SR2116 (*eda*::Tn*10*)	SR1947 (*xmu-1062*)	27/39	0.23
P1·SR1947 (*eda*::Tn*10 xmu-1062*)	SR749 (+)	46/101	0.46
T4·SR1310 (*hag*::Tn*10*)	SR1993 (*xmu-1138*)	9/48	1.7
T4·SR2125 (*hag*::Tn*10 xmu-1138*)	SR749 (+)	22/51	1.0
	SR1165 (+)	14/50	1.4
P1·SR2116 (*eda*::Tn*10*)	SR1993 (*xmu-1138*)	96/142	0.24
P1·SR2184 (*eda*::Tn*10 xmu-1138*)	SR749 (+)	56/107	0.39

[a] The Xmu phenotype was determined by scoring the number of Arg^+ mutants per Arg-1.5 plate spread with 0.2 ml of an overnight YENBG culture irradiated with 40 krad. $Xmu^+ = \sim 900$ Arg^+ colonies per plate; $Xmu^- = \leqslant 50$ Arg^+ colonies per plate.

[b] The distances listed were calculated according to Wu (1966) as equal to $L(1 - F^{1/3})$, where L = the length of the DNA carried by the transducing bacteriophage, i.e., 2.0 min for P1 *vir*a (Bachmann, 1983) and 4 min for T4GT7 (e.g., Wilson et al., 1979), and F is the fraction of recipients changing their Xmu phenotype.

showed linkage between both *xmu* mutations and the *hag* (42.45 min) and *eda* (40.7 min) genes (Table 2). Averaging the P1 transduction data shown in Table 2 suggests that the *xmu* locus is 0.33 min away from the *eda* locus, while averaging the T4GT7 transduction data suggests that the *xmu* locus is 1.5 min away from the *hag* locus. Taken together, these data suggest that the *xmu* locus is at 41.0 min.

Since the *ruv* locus (about 41.2 min; Bachmann, 1983) seems to be near the *xmu* site, plasmids were obtained that carry bacterial DNA that

TABLE 3

SURVIVAL OF UV-IRRADIATED STRAINS HARBORING PLASMIDS CARRYING THE *ruvA* AND/OR *ruvB* GENES

Plasmid carried [a]	Surviving fraction of UV-irradiated wild-type or mutant cells [b]				
	+ (SR2214)	*ruvA59*::Tn*10* (SR2067)	*ruvB9* (SR2392)	*xmu-1138* [c] (SR2215)	*xmu-1062* [c] (SR2212)
None	0.1	0.0002	0.0002	0.0001	0.00004
pHSG415 (no insert)	0.2	0.0005	0.0002	0.00009	0.00002
pPVA101 (*ruvA*$^+$ *ruvB*$^+$)	0.1	0.2	0.06	0.1	0.1
pPVA101::Tn*1000*-4 (*ruvA*$^+$ *ruvB*$^-$)	0.09	0.0009	0.003	0.2	0.0009
pPVA101::Tn*1000*-12 (*ruvA*$^+$ *ruvB*$^-$)	0.1	0.0006	0.004	0.1	0.003
pFB502 (*ruvA*$^+$)	0.2	0.001	0.00007	0.2	0.0004
pGTI19 (*ruvB*$^+$)	0.2	0.0001	0.04	0.0002	0.1
pNJS100 (*ruvA*$^+$)	0.09	0.0007	0.00009	0.1	0.0004

[a] Plasmids are further described in Fig. 1 and Results.

[b] Surviving fractions are for YENB-grown (30 °C) log-phase *umuC122*::Tn*5* cells, diluted 100-fold in PB, irradiated at a dose of 30 J m^{-2}, plated on YENB at 30 °C. Pre-irradiation growth media contained ampicillin at 50 μg/ml for plasmid-carrying strains.

[c] Based upon the data in this Table (as discussed in the text), the *xmu-1138* and *xmu-1062* mutations have been renamed as *ruvA200* and *ruvB201*, respectively.

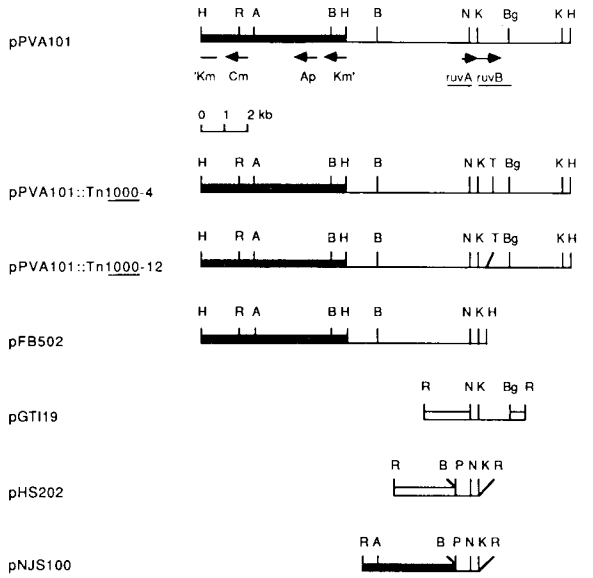

Fig. 1. Partial restriction map of plasmids used. The closed boxes indicate plasmid DNA derived from pHSG415. The open boxes indicate plasmid DNA derived from pUC19. The horizontal lines indicate bacterial DNA; the vertical lines indicate DNA restriction sites or Tn*1000* insertion sites. Some plasmid maps are derived from Attfield et al. (1985). The plasmid maps are aligned at their *Nru*I restriction sites. Abbreviations: A, *Ava*I; B, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; K, *Kpn*I; N, *Nru*I; R, *Eco*RI; T, Tn*1000* insertion site; 'Km, Km', the interrupted kanamycin-resistance gene; Cm, chloramphenicol-resistance gene; Ap, ampicillin-resistance gene.

includes the *ruv* region (Attfield et al., 1985). These plasmids (Fig. 1) were tested for their ability to enhance UV-radiation resistance in purified *umuC xmu* strains. Plasmid pPVA101 is known to complement several *ruv* mutations (including *ruvA59*::Tn*10*), but pFB502 and the two Tn*1000* insertion derivatives of pPVA101 are not supposed to complement any *ruv* mutations (*ruvA59*::Tn*10* was not tested; Attfield et al., 1985). Since the *xmu-1062* mutation was complemented by pPVA101 but not by pPVA-101::Tn*1000*-4, pPV101::Tn*1000*-12, or pFB502 (Table 3), it shows the same complementation pattern as all of the other *ruv* mutations, which are now deduced to occur in *ruvB* (Shurvinton et al., 1984; Attfield et al., 1985; Benson et al., 1988), and therefore *xmu-1062* must be a *ruvB* mutation. This is also supported by the complementation of the *xmu-1062* and *ruvB9* mutations by pGTI19 (Table 3), a plasmid that should carry just the *ruvB* gene (cf., Benson et al., 1988 and Fig. 1).

The *ruvB* gene is located between the *Kpn*I and *Bgl*II sites near the *Nru*I site on pPVA101 and derived plasmids (Benson et al., 1988 and Fig. 1). Since all of the above plasmids, except pGTI19, complemented the *xmu-1138* mutation (Table 3), we conclude that the *xmu-1138* gene is not *ruvB*, and that it is located to the left of the *Kpn*I site that is near the *Nru*I site in these plasmids. To locate the *xmu-1138* gene more precisely, we obtained plasmid pHS202, which should contain just the *ruvA* gene (Fig. 1). Because pHS202 [a relaxed plasmid derived from pUC19 (Yanisch-Perron et al., 1985)] afforded very little UV-radiation protection (data not shown) vs. pFB502, we recloned the putative *ruvA* gene from pHS202 into a 4.7-kb plasmid derived from pSC101 via pHSG415 and pFB502 (see Methods and Fig. 1). This plasmid, pNJS100, is expected to exhibit a stringent control of its replication due to its derivation from pSC101 (Hashimoto-Gotoh et al., 1981). Since pNJS100 (Fig. 1) restored the wild-type level of UV-radiation resistance to the *xmu-1138* strain (Table 3), we conclude that the *xmu-1138* gene resides just to the left of the *Kpn*I site (near the *Nru*I site) in all of the plasmids shown (except pGTI19), and that it is the *ruvA* gene. [It should be noted that the map position of the Tn*1000*-12 insertion shown in Attfield et al. (1985) must be in error, because pPVA101::Tn*1000*-12 produces the RuvA protein (Attfield et al., 1985; Benson et al., 1988). In Fig. 1 we indicate where we believe Tn*1000*-12 should map.] We will hereafter refer to the *xmu-1138* and *xmu-1062* mutations as *ruvA200* and *ruvB201*, respectively.

Since our project was designed to identify new genes that are involved in *umuC*-independent γ-radiation mutagenesis, we assayed mutagenesis in clean strains possessing the two new *ruv* mutations as well as two *ruv* mutations that were previously known, *ruvA59*::Tn*10* and *ruvB60*::Tn*10* (Shurvinton et al., 1984; Benson et al., 1988). For log-phase, YENB-grown, oxically-irradiated cells, the four *ruv* mutations reduced γ-radiation mutagenesis (*argE3* → Arg^+) to about the same degree as the *umuC* mutation, i.e., to about 30% of the wild-type level; however, mutagenesis was dramatically lower in the *umuC*

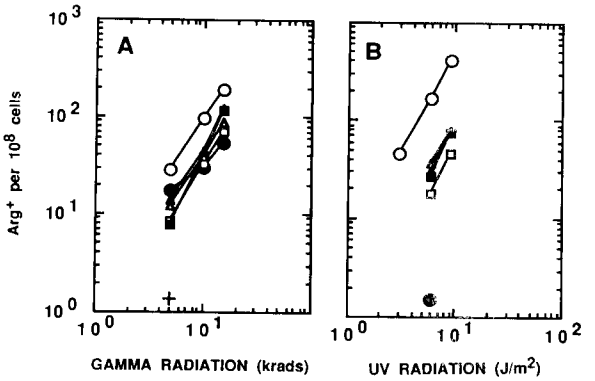

Fig. 2. UV- and γ-radiation mutagenesis [*argE3*(Oc) → Arg$^+$] in *E. coli ruv* and *umuC* strains. Cells were grown in YENB in log phase, γ- (A) or UV- (B) irradiated (oxic), and plated on Arg-1.5 to assay the radiation-induced mutant frequency. Data points are averaged from 2–4 Expts. per strain. No mutagenesis was detected in the *umuC* strain at 3 and 9 J m^{-2}, or in the *ruvA200* or *ruvB201* strains at 3 J m^{-2}; the *umuC ruvA* double mutant was not tested. Symbols: ○, wild-type (data combined for SR749, SR2206, SR2209); ●, *umuC* (SR2214); □, *ruvA200* (SR2210); ■, *ruvB201* (SR2207); △, *ruvA59*::Tn*10* (SR2384); ▲, *ruvB60*::Tn*10* (SR2385); +, *umuC ruvA200* (SR2215).

ruvA200 double mutant (Fig. 2A). No mutagenesis was detected at 2.5 krad for the *umuC ruvA200* strain (data not shown), and higher doses were not tested because the surviving fraction of cells would have been less than 0.20. For UV-irradiated cells, all of the *ruv* mutations reduced UV-radiation mutagenesis to about 15% (or less) of the wild-type level, while, as expected, the *umuC* mutation by itself caused a large reduction (> 99%) in mutagenesis (Fig. 2B). When wild-type (Fig. 3A) and *ruvA200* (Fig. 3B) cells were γ-irradiated in the absence of oxygen, they showed less mutagenesis than in its presence, while *umuC* cells did not show this oxygen effect (Fig. 3C).

To test whether the observed deficiency in mutability (*argE3* → Arg$^+$) seen in the *ruv* strains was a general property, other mutation assays were performed. The *ruvA200* and *ruvB201* strains gave similar results with another ochre reversion assay (*hisG4* → His$^+$) as had been obtained with the *argE3* ochre assay (Table 4). A forward mutation assay for the induction of rifampicin-resistance (which detects at least 15 different base substitutions and three in-frame additions or deletions; Jin and Gross, 1988) showed the *ruvA* and *ruvB* strains to be substantially less mutable by UV-radiation while their γ-radiation mutability was only slightly reduced (Table 4). The *umuC* strain gave the expected result with both mutation assays and for both radiations, i.e., partial γ-radiation mutability but little or no UV-radiation mutability (Table 4).

To help understand the nature of the *ruvAB* deficiency in mutagenesis, two chemical mutagens were employed: EMS and streptozotocin; both are monofunctional alkylating agents. While the *ruvA200* and *ruvB201* strains showed a normal level of EMS mutagenesis (*argE3* → Arg$^+$), they both showed reduced streptozotocin mutagenesis

TABLE 4

EFFECT OF *ruvA, ruvB* OR *umuC* MUTATIONS ON UV- AND γ-RADIATION MUTAGENESIS

Radiation	Mutants per 10^8 survivors [a]			
	Strain [b]	*argE3*(Oc) → Arg$^+$	*hisG4*(Oc) → His$^+$	Rif^s → Rif^r
γ-Radiation (15 krad, oxic)	Wild-type	192 ± 31	45 ± 5	23 ± 9
	ruvA200	71 ± 19	15 ± 7	12 ± 7
	ruvB201	114 ± 44	10 ± 9	20 ± 5
	umuC	52 ± 9	4 ± 1	5 ± 2
UV-radiation (10 J m^{-2}) [c]	Wild-type	394 ± 65	45 ± 10	54 ± 9
	ruvA200	46 ± 12	9 ± 3	7 ± 3
	ruvB201	73 ± 28	12 ± 10	19 ± 5
	umuC	6 ± 0	−3 ± 3	0 ± 1

[a] Mutant frequencies are the means ± 1 SD of data from 3 or more experiments. See Methods for the calculation of mutant frequencies. Oc = ochre, Rif^r = forward mutation to rifampicin resistance.
[b] Wild-type, SR2209; *ruvB201*, SR2207; *ruvA200*, SR2210; *umuC*, SR2214.
[c] 9 J m^{-2} for Arg reversion.

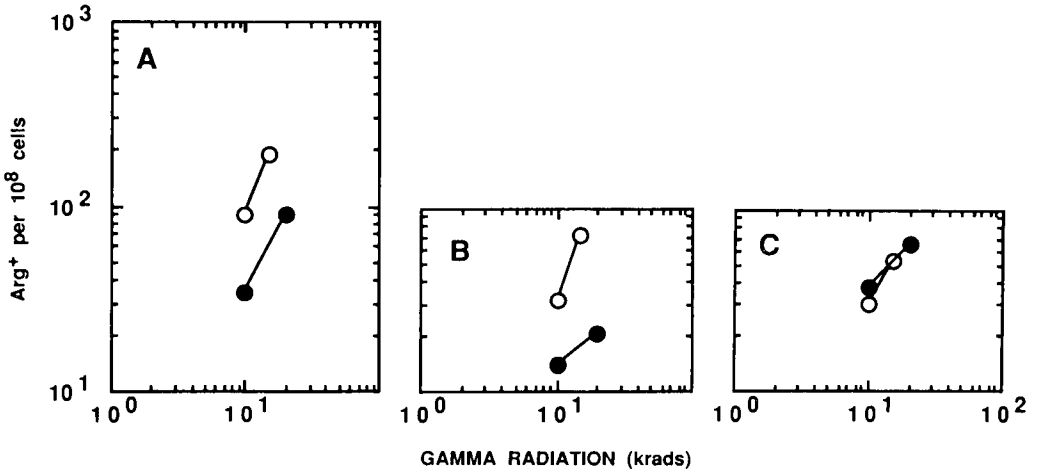

Fig. 3. Effect of the presence of oxygen on γ-radiation mutagenesis [*argE3*(Oc) → Arg$^+$] in *E. coli ruv* and *umuC* strains. Cells were grown in YENB in log phase, γ-irradiated in the presence of oxygen (○), or in its absence (●), and plated on Arg-1.5 to assay the radiation-induced mutant frequency. Data points are averaged from 3 Expts. per strain. Panels: A, wild-type (SR2209); B, *ruvA200* (SR2210); C, *umuC* (SR2214).

(Rif^s → Rif^r, Table 5). The *umuC* strain showed normal mutagenesis with both chemical mutagens (Table 5).

Since the *ruvB* strain generally gave the same mutagenesis results as the *ruvA* strain, only the *ruvA* strain was compared with the wild-type and *umuC* strains for the types of base changes that they could produce. Whether γ-irradiated in air or N_2, the *ruvA* strain showed less mutagenesis than the wild-type strain at each of the 6 chromosomal sites, but the relative amounts of the 6 types of mutants were about the same as in the wild-type (parenthetical data, Table 6). However, the *umuC* strain showed a disproportionately low level of mutagenesis at the *supC* and *supL* sites when irradiated in air. When irradiated in N_2, the *umuC*

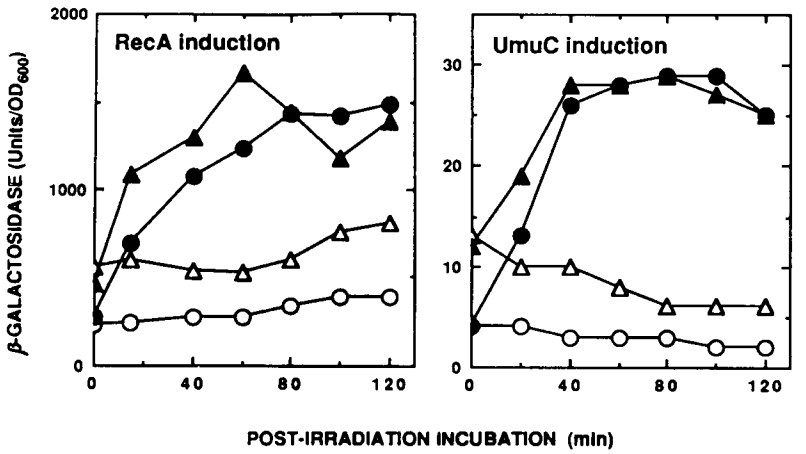

Fig. 4. Effect of *ruvA200* on spontaneous and UV-radiation induction of *recA* and *umuC* genes. Wild-type (○, ●) and *ruvA200* (△, ▲) co-transductant λd(*recA* :: *lacZ*) *cI ind* (SR2404 and SR2405) or *umuC121* :: Mud(Ap,*lac*) (SR2408 and SR2409) strains were UV-irradiated (0 J m^{-2}, open symbols; 10 J m^{-2}, closed symbols) and incubated in MM-1.5 under conditions that were selected to mimic those of the mutation assays. Cell samples were taken during the post-UV-irradiation incubation, and assayed for β-galactosidase as a measure of *recA* or *umuC* induction (see Methods). Data are from 1 of 3 Expts. with similar results.

TABLE 5

EFFECT OF *ruvA*, *ruvB* AND *umuC* MUTATIONS ON CHEMICAL MUTAGENESIS

Strain [a]	EMS [b] ($Arg^+/10^8$)	Streptozotocin [b] ($Rif^r/10^8$)
Wild-type	6200 ± 1000	3000 ± 500
ruvA200	6600 ± 400	1200 ± 300
ruvB201	6600 ± 1200	1300 ± 600
umuC	7800 ± 700	3700 ± 600

[a] Wild-type, SR2209; *ruvA200*, SR2210; *ruvB201*, SR2207; *umuC*, SR2214.

[b] Mutant frequencies are the means ± 1 SD of data from 3 Expts. See Methods for the calculation of mutant frequencies. Arg^+ cells are ochre revertants; Rif^r cells are rifampicin-resistant forward mutants. EMS treatment was 1 μl/ml for 60 min. Streptozotocin treatment was 10^{-5} M for 30 min. With both treatments, cell survival was greater than 50%.

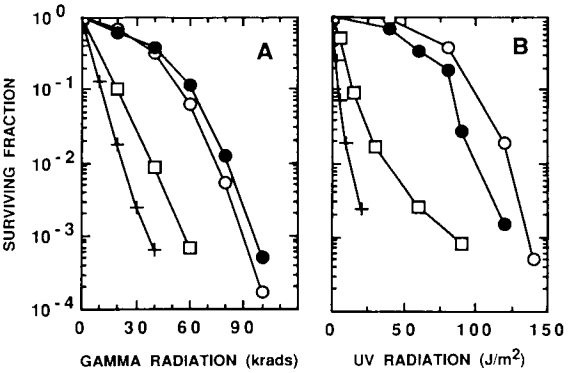

Fig. 5. UV- and γ-radiation survival of *E. coli ruvA200* and *umuC* strains. Cells were grown in YENB in log phase, γ- (A) or UV- (B) irradiated (oxic), and plated on Arg-1.5 to assay radiation survival. Data points are averaged from 3–4 Expts. per strain. Symbols: ○, wild-type (SR2209); ●, *umuC* (SR2214); □, *ruvA200* (SR2210); +, *umuC ruvA200* (SR2215).

strain still showed the inability to produce *supC* mutations, but its ability to induce *supX* mutations was greater than the wild-type level.

Since the *ruvA* strain showed a general depression in radiation mutagenesis, the effect of the *ruvA200* mutation on the UV-radiation induction of the *recA* and *umuC* genes was determined using λd(*recA* :: *lacZ*) and *umuC121* :: Mud(Ap,*lac*) fusion systems (in separate $recA^+$ strains), and measuring the UV-radiation induction of β-galactosidase activity (see Methods). The *ruvA200* strain consistently showed about twice the basal levels of *recA* and *umuC* induction as the wild-type strain, but after UV-irradiation the levels of *recA* and *umuC* induction were about the same as for the wild-type strain (after considering the difference in basal levels of induction; Fig. 4).

Finally, the *ruvA200* mutation made cells very sensitive to the killing effects of γ- and UV-radia-

TABLE 6

EFFECTS OF THE *ruvA* AND *umuC* MUTATIONS ON OXIC AND ANOXIC γ-RADIATION MUTAGENESIS IN *E. coli*

Irradiation gassing condition	Relevant genotype [a]	Arg^+ mutants/10^8 cells induced by 10 krad [b]						
		argE$^+$	*supC*	*supX*	*supB*	*supL*	*supE*	Total
Air	WT	17.1(28)	12.8(21)	10.9(18)	9.7(16)	7.1(12)	3.8 (6)	61.4
	ruvA	5.4(23)	5.2(23)	3.5(15)	4.4(19)	2.4(10)	2.1 (9)	23.0
	umuC	9.8(40)	0.9 (4)	5.6(23)	5.6(23)	0.2 (1)	2.4(10)	24.5
	*umuC** [c]	9.8(28)	7.0(20)	5.6(16)	5.6(16)	4.0(12)	2.4 (7)	34.4
N_2	WT	10.9(34)	6.9(22)	3.2(10)	7.1(22)	1.0 (3)	2.6 (8)	31.7
	ruvA	7.2(46)	1.9(12)	1.1 (7)	4.3(27)	0.3 (2)	0.9 (6)	15.7
	umuC	13.2(39)	0.2 (1)	8.9(26)	8.2(24)	1.2 (4)	2.4 (7)	34.1

[a] The wild-type (WT, SR2209), *ruvA200* (SR2210), and *umuC122* :: Tn*5* (SR 2214) strains were all derived from the K12 AB1157 strain.

[b] The types of Arg^+ mutants were determined as described in Methods. The numbers of each type of Arg^+ mutant were determined for samples from each of the M_o, M_{po} and M_t categories of Arg^+ mutants, and these are listed in Table 7. Fractional values for each type of mutant were calculated and applied to the 'mean total mutants per plate' (Table 7), to determine M_o, M_{po}, and M_t values for each type of mutant. These data, along with the values for the surviving fraction and cells per plate (Table 7), were used to calculate the mutant frequencies (see Methods) for each type of Arg^+ mutant. The associated values in parentheses are the percentages of each type of Arg^+ mutant relative to the total Arg^+ mutants for the specified strain.

[c] See Discussion.

TABLE 7

DATA USED TO CALCULATE THE INDUCED Arg^+ MUTANT FREQUENCIES PRESENTED IN TABLE 6

Strain [a]	Irradiation gassing condition	Data term [b]	Cell surviving fraction after 10 krad	Mean viable cells spread per plate	Mean total mutants per plate	Total mutants classified	Total Arg^+ mutants/class [c]					
							argE$^+$	*supC*	*supX*	*supB*	*supL*	*supE*
WT	Air	M_o	–	–	4.0	118	30	27	8	24	19	10
		M_{po}	–	–	11.4	307	129	54	13	67	25	19
		M_t	0.83	2.34×10^8	151.7	299	87	62	49	49	34	18
	N_2	M_o	–	–	12.6	116	11	19	7	18	54	7
		M_{po}	–	–	21.1	255	72	38	15	42	74	14
		M_t	1.06	3.16×10^8	120.8	300	101	59	28	64	26	22
ruvA	Air	M_o	–	–	1.6	50	9	6	2	23	2	8
		M_{po}	–	–	4.9	136	41	18	3	58	9	7
		M_t	0.64	8.00×10^7	22.6	200	50	42	25	47	20	16
	N_2	M_o	–	–	1.4	55	9	6	4	25	2	9
		M_{po}	–	–	5.9	231	82	24	8	86	19	12
		M_t	0.94	1.18×10^8	24.1	282	128	34	17	79	10	14
umuC	Air	M_o	–	–	3.6	58	36	0	5	13	3	1
		M_{po}	–	–	11.7	154	69	6	19	44	11	5
		M_t	0.84	1.97×10^8	59.0	200	82	7	42	47	4	18
	N_2	M_o	–	–	3.0	84	40	2	5	27	5	5
		M_{po}	–	–	13.2	250	111	11	26	78	14	10
		M_t	0.94	2.20×10^8	88.1	298	118	3	71	74	12	20

[a] As in Table 6.

[b] Data terms are defined in Methods.

[c] 'Total mutants' and 'mutants per class' are the sums from 2–3 Expts., and were classified by the indirect sequence determination procedure described in Methods.

tion, especially when the *umuC* mutation was also present (Fig. 5).

Discussion

Otsuji et al. (1974) first isolated *ruv* mutants by their large sensitivity to killing by γ- and UV-radiation and by mitomycin C. DNA damage also causes *ruv* cells to elongate without septation (i.e., they filament). However, Lloyd et al. (1984) concluded that the large radiation sensitivity of the *ruv* mutant is due to a deficiency in DNA repair because the UV-radiation-induced filamentation can be blocked by an *sfi* mutation without changing the cells' radiation sensitivity.

The *ruv* mutant's radiation sensitivity and its concomitant tendency to filament are potentiated by plating treated cells on rich medium rather than on minimal medium (Otsuji et al., 1974). Thus, it is particularly relevant to note that the small amount of rich medium (YENB at 1.5% v/v) that was part of our mutant-selection media for rich medium-grown cells, did not enhance the radiation-induced killing of *ruvA200* cells (strain SR2210) vs. that seen for cells grown and plated on minimal medium; in fact, the UV-radiation survival was almost 2-fold higher for these cells (at 5 J m^{-2}; data not shown).

DNA sequencing of the *ruv* region shows the presence of two genes (*ruvA* and *ruvB*) that constitute a *recA–lexA* regulated operon (Benson et al., 1988; Shinagawa et al., 1988), which is DNA-damage-inducible (Shurvinton and Lloyd, 1982). Although Otsuji et al. (1974) had described a *ruvA4* mutant, one can now deduce from the available information (Shurvinton et al., 1984;

Attfield et al., 1985; Benson et al., 1988) that all of the known *ruv* mutations, save one, actually map in the *ruvB* gene. The one previously known *ruvA* mutation, *ruvA59*::Tn*10* (Benson et al., 1988), seems to effect the expression of both the *ruvA* and *ruvB* genes because it was protected from radiation-induced killing only by plasmid pPVA101, which contains both the $ruvA^+$ and the $ruvB^+$ genes (Table 3, Fig. 1). Plasmids pPVA-101::Tn*1000*-4, pPVA101::Tn*1000*-12 and pFB502, which are all known to produce the RuvA protein (Attfield et al., 1985; Benson et al., 1988), did not protect the *ruvA59*::Tn*10* strain, while the *ruvA200* strain was fully protected by all of these plasmids (Table 3). This suggests that the *ruvA59*::Tn*10* mutation has a polar effect on the *ruvB* gene. In contrast, the plasmid complementation pattern of the *ruvA200* mutant (Table 3) suggests that this strain is deficient just in the *ruvA* gene product, and that it is presently the only known *ruvA* mutant that does not also have a defect in the *ruvB* gene.

We have compared our *ruvA200* and *ruvB201* strains for radiation survival under various growth conditions (log phase, stationary phase, minimal medium, rich medium, etc.), and have not found any marked difference between the responses of these two strains (data not shown). Both strains show the large degree of radiation sensitivity that is associated with the *ruv* phenotype (e.g., Fig. 5). Thus, both *ruvA* and *ruvB* genes are likely to be involved in the same mechanisms for the repair of radiation damage, and perhaps both gene products function as subunits in a single repair complex. However, what is notable from our radiation-survival studies is that the *umuC ruvA200* and the *umuC ruvB201* (not shown) double mutants both showed a greater than additive sensitization to killing by γ- and UV-irradiation [Fig. 5; synergism calculated according to Wang and Smith (1981)]. This was surprising since the *umuC* mutation has little effect on the γ- and UV-radiation-induced killing of wild-type cells (e.g., Fig. 5). This synergistic interaction of the *ruvA200* and *umuC* mutations suggests that these two genes function in alternate DNA repair pathways competing for the same class of DNA damage.

One phenotypic character of *ruv* mutants that we find to be different from what is published is the substantial deficiency in UV-radiation mutagenesis that we report here, which was not observed earlier for the *ruvB4* mutant (previously called *ruvA4*; Lloyd et al., 1984). While the *ruvB4* mutation may be leaky in terms of the mutagenesis defect, we are more troubled by the high UV-radiation doses that were used in that study; i.e., the surviving fractions were 0.1, 0.02 and 0.002. It is difficult to assess the mutagenesis data when such minor fractions of the population are being scored.

Our data suggest that the *ruvAB* operon plays a significant role in both UV and gamma radiation mutagenesis; in fact, the *ruvA* and *ruvB* mutants were relatively more deficient in UV-radiation mutagenesis than in γ-radiation mutagenesis (e.g., Fig. 2). Because all of UV-radiation mutagenesis in *E. coli* appears to be *umuC*-dependent (e.g., Kato and Shinoura, 1977; Sargentini and Smith, 1984), our data indicate that *umuC*-dependent UV-radiation mutagenesis can be divided into *ruvA*-dependent and *ruvA*-independent mechanisms, since the *ruvA200* mutation only blocks about 85% of UV radiation mutagenesis (Fig. 2B).

Although all of ionizing radiation mutagenesis is *recA*-dependent, it has been subdivided into *umuC*-dependent and *umuC*-independent processes (Sargentini and Smith, 1989). Since both the *umuC* and the *ruvA200* mutations each blocked about 70% of γ-radiation mutagenesis (Fig. 2A), one could conclude that these two genes might be part of the same mechanism for γ-radiation mutagenesis. However, the *ruvAB* operon appears to also function in the *umuC*-independent mechanism of γ-radiation mutagenesis, as suggested by the following information.

First, the *umuC ruvA* double mutant is much less mutable by γ-irradiation than either single mutant (Fig. 2A). Second, the *ruvA* mutant shows an oxygen-enhancement effect for Arg reversion (Fig. 3B) that is somewhat larger than that seen in the wild-type strain (Fig. 3A), while the *umuC* strain shows no oxygen effect (Fig. 3C, and Sargentini and Smith, 1989). Third, the data for mutation production in the *umuC* strain has a slope (log–log plot) that is a linear function of dose (D) (i.e., a slope of one in Fig. 2A; also see data in Sargentini and Smith, 1989), while the

data for the wild-type and the *ruv* strains *approximate* a slope of two (i.e., D^2) (Fig. 2A). The simplest interpretation of a slope of two is that two radiation "hits" (e.g., two closely spaced lesions in the gene of interest) are required to produce one measured event (e.g., a mutation). It is of interest, therefore, that for this mutation assay (i.e., *argE3*(Oc) → Arg^+) at least the major portion of *umuC*-dependent mutagenesis for both UV- and γ-radiation requires 2 hits, while *umuC*-independent γ-radiation mutagenesis requires only 1 hit.

Consistent with the γ-radiation data, which suggest that a certain degree of independence exists for the *ruvAB*-dependent and *umuC*-dependent mutagenic mechanisms, the *umuC* mutant is normal for *recA*-dependent streptozotocin mutagenesis (Fram et al., 1986; Table 5, this work), while the *ruvA200* and *ruvB201* mutations block about half of streptozotocin mutagenesis (Table 5). Thus, we can conclude that the *ruvAB* mutagenic mechanism plays a role both in *umuC*-dependent mutagenesis and in *umuC*-independent mutagenesis, and that the *ruvAB* genes do not seem to play any role in *recA*-independent mutagenesis, e.g., EMS mutagenesis (Table 5).

More precise data on the nature of the *ruvAB*-dependent mechanism for γ-radiation mutagenesis can be obtained by comparing the mutational spectrum for *ruvA* cells with that for *umuC* and wild-type cells, because *umuC*-dependent and *umuC*-independent mutagenic mechanisms are known to produce different mutational spectra (Sargentini and Smith, 1989). In this analysis, the *ruvA* cells seem to show less mutagenesis at every site (Table 6), but it can be deduced that the wild-type and *ruvA* cells exhibit the same mutational spectrum [i.e., by comparing the percentage values (parenthetical data, Table 6) for each type of Arg^+ mutant]. On the other hand, the oxically-irradiated *umuC* cells show smaller percentages of *supC* and *supL* mutants, and larger percentages of the remaining types of Arg^+ mutants (vs. wild-type cells). These results can be explained if the *umuC* mutation blocks *supC* and *supL* mutagenesis, and also causes a general reduction in mutagenesis at all sites. To test this hypothesis, one can add 6.1 *supC* and 3.8 *supL* Arg^+ mutants per 10^8 survivors to the mutant frequencies produced in the *umuC* strain, to approximate the percentages for these mutants seen in the wild-type strain, i.e., 21 and 12%, respectively. When this is done, the mutational spectrum of this hypothetical *umuC* strain (*umuC**) closely resembles that of the wild-type strain in terms of percentages, although this *umuC** strain still only shows about half the wild-type level of mutagenesis (Table 6). This argues, for oxic irradiation, that the *ruvA* mutation causes a general depression in radiation mutagenesis, i.e., γ-radiation mutagenesis (oxic) is reduced to about 37% at each site, while the *umuC* mutation causes a general reduction in γ-radiation mutagenesis (oxic) to 56% of the wild-type level, and also causes a specific reduction in the γ-radiation induction of *supC* and *supL* mutants.

One explanation for the general depression in radiation mutagenesis seen in the *ruvAB* mutants is that the radiation-stimulated induction of the *recA* and *umuC* genes might be depressed in these strains. However, the induction of the *recA* and *umuC* genes by UV-irradiation was found to be normal in the *ruvA200* strain (Fig. 4). These data do not rule out the possibility that *ruvAB* mutations may interfere with the function of the RecA, UmuD and UmuC proteins. Alternatively, the general depression of radiation mutagenesis seen in the *ruv* strain may reflect a general change in DNA structure that reduces the efficiency by which the mutagenic mechanisms produce mutations.

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