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umuC-dependent and *umuC*-independent γ - and UV-radiation mutagenesis in *Escherichia coli*

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Summary

The effects of the *umuC36* and *umuC122::Tn5* mutations on γ - and UV radiation mutagenesis (nonsense, missense, and frameshift mutation assays) in *Escherichia coli* K12 were studied. Although both mutations reduced radiation mutagenesis, the *umuC36* mutation appeared to be leaky since considerably more UV radiation mutagenesis could be detected in the *umuC36* strain than in the *umuC122::Tn5* strain. In general, the *umuC* strains showed much larger deficiencies in UV radiation mutagenesis than they did for γ -radiation mutagenesis. The mutability of the *umuC122::Tn5* strain varied depending on the radiation dose, and the mutation assay used. For γ -radiation mutagenesis, the deficiency varied from no deficiency to a 50-fold deficiency; for UV radiation mutagenesis, the deficiency varied from 100-fold to at least 5000-fold. We concluded that both *umuC*-dependent and *umuC*-independent modes function for γ -radiation mutagenesis, while UV radiation mutagenesis seems to depend almost exclusively on the *umuC*-dependent mode.

The concept that DNA repair enzymes play essential roles in radiation mutagenesis arose from the demonstration that a *lexA* mutant was not mutagenized by UV radiation (Witkin, 1967). Similarly, the *lexA* gene is required for all but perhaps 5% of ionizing radiation mutagenesis (IRM) (Bridges et al., 1968). The *recA* gene was subsequently also shown to be required for IRM (Kondo, 1968; Kondo et al., 1970; Ishii and Kondo, 1972) and for UV radiation mutagenesis (UVRM) (Witkin, 1969). Unlike the *recA* and *lexA* mutants, *umuC* mutants were originally selected for nonmutability to UV radiation (Kato and Shinoura, 1977). Subsequently, Kato and Nakano (1981) and Kato et al. (1982) reported that this gene was also required for IRM.

The notion that the *umuC* mutation eliminates UVRM and IRM is inconsistent with work performed in our laboratory showing that such mutagenesis could readily be detected in a *umuC36* strain (Sargentini, 1979). Also, Steinborn (1978, 1979) has described 3 *uvm* mutations that make cells unable to show UVRM, while showing almost normal IRM. Shinagawa et al. (1983) has concluded that these *uvm* mutations occur at the *umuC* and/or *umuD* loci, based on complementation studies using plasmids carrying the cloned *umuC* and *umuD* loci in a 3-kb insert. The question, of whether the *umuC36* and *uvm* mutations are leaky or whether, in some mutation assays *umuC*-independent modes can function in radiation mutagenesis, remained unanswered until recently, when a *umuC*-insertion mutant became available (courtesy of Dr. G.C. Walker). The

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umuC122::Tn5 mutant carries a 5.7-kb insertion sequence (Tn5) near the carboxy terminus of its *umuC* gene (Elledge and Walker, 1983), and is assumed to show the 'null' phenotype.

We report here that *umuC122::Tn5* strains, although grossly deficient in UVRM, generally show a much smaller deficiency in IRM, which depends on the DNA sites relevant to the mutation assay.

Materials and methods

Bacteria

Strains used are listed in Table 1. Bacteriophage P1 transductions were performed generally as described by Miller (1972).

Media

MM was a 0.4% glucose-salts medium (Ganesan and Smith, 1968), supplemented with thiamine · HCl at 0.5 µg/ml. SMM1 was MM supplemented with L-leucine and L-methionine at 1 mM, D-biotin at 1 µg/ml, and thymine at 10 µg/ml. SMM2 was MM supplemented with L-arginine, L-histidine, L-leucine, L-proline, and L-threonine; all at 1 mM. Media were solidified with Noble agar (Difco) at 1.6% to make plates (27 ml). YENB was 0.75% yeast extract (Difco) and 0.8% nutrient broth (Difco). YENB agar was yeast extract at 0.75% and nutrient agar (Difco) at 2.3%. Glu-0 was SMM1 agar with lactose in place of glucose. Glu-300 and Glu-1200 were Glu-0 medium with glu-

TABLE 1
STRAINS OF *E. coli* K12 USED

Stanford Radiology number		Genotype ^a	Source or derivation ^b
SR192	<i>lexA101</i>	<i>thyA36 deo(C2?) lacZ53 rha-5 rpsL151</i>	Sargentini and Smith, 1981
SR250	<i>uvrB5</i>	<i>leuB19 metE70</i> , other genotype same as SR192	Sargentini and Smith, 1981
SR251	<i>uvrB5 lexA101</i>	Other genotype same as SR250	Sargentini and Smith, 1981
SR741	<i>uvrB5</i>	Other genotype same as SR250	Sargentini and Smith, 1981
SR742	<i>uvrB5 umuC36</i>	Other genotype same as SR250	Sargentini and Smith, 1981
SR749	+	<i>argE3 his-4 leuB6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-1 thi-1 tsx-33 rpsL31 supE44</i>	AB1157, B.J. Bachmann
SR1018	<i>umuC122::Tn5</i>	Other genotype same as SR749	GW2100, G.C. Walker
SR1034	<i>uvrB5 umuC122::Tn5</i>	Other genotype same as SR250	SR250 × P1::Tn9cts · SR1018, select Kn ^r
SR1119	+	<i>deoC araD139 Δ (lac)U169 malE7::Tn5 f16B relA rpsL</i>	T5M7, T. Silhavy
SR1120	+	<i>malE7::T5</i> , other genotype same as SR749	SR749 × P1vir · SR1119, select Kn ^r
SR1165	<i>umuC122::Tn5</i>	Other genotype same as SR749	SR749 × P1::Tn9cts · SR1018, select Kn ^r
SR1181	+	<i>pyrF1189::Tn1 rpsL</i>	TH1161, S. Harayama
SR1265	+	<i>pyrF1189::Tn1</i> , other genotype same as SR749	SR749 × P1vira · SR1181, select Ap ^r
SR1276	+	<i>trpE9777</i>	W3110 <i>trpE9777</i> , C. Yanofsky
SR1279	<i>lexA101</i>	Other genotype same as SR749	SR1120 × Plkc · SR192, select Mal ⁺
SR1285	+	<i>trpE9777</i> , other genotype same as SR749	SR1265 × P1vira · SR1276, select Pyr ⁺
SR1314	<i>umuC122::Tn5</i>	Other genotype same as SR1285	SR1285 × P1::Tn9cts · SR1018, select Kn ^r
SR1319	<i>lexA101</i>	Other genotype same as SR1265	SR1279 × P1vira · SR1181, select Ap ^r
SR1320	<i>lexA101</i>	Other genotype same as SR1285	SR1319 × P1vira · SR1276, select Pyr ⁺

^a Genotype nomenclature is that used by Bachmann and Low (1980). All strains are F⁻ and λ⁻.

^b All transductants were tested for bacteriophage lysogeny. Kn^r and Ap^r indicate resistance to kanamycin (30 µg/ml) or ampicillin (20 µg/ml), respectively. Pyr⁺ indicates nonrequirement for uracil. Mal⁺ indicates ability to use maltose as a carbon source.

cose at 300 or 1200 $\mu\text{g}/\text{ml}$, respectively. Arg-0 was SMM2 agar without arginine. His-0 was SMM2 agar without histidine. Trp-0 was SMM2 agar. Arg-1.5 was Arg-0 medium with YENB at 1.5% (v/v). Trp-1 was Trp-0 medium with YENB at 1% (v/v). Rif medium was YENB agar containing rifampicin (Sigma) at 100 $\mu\text{g}/\text{ml}$ (dissolved first in dimethyl sulfoxide at 50 mg/ml). Spc medium was YENB agar containing spectinomycin dihydrochloride (Sigma) at 100 $\mu\text{g}/\text{ml}$. PB was Na_2HPO_4 at 5.83 g/l and KH_2PO_4 at 3.53 g/l, pH 7.0.

Preparation and irradiation of cells

Logarithmic-phase cells were prepared by diluting an overnight culture, 1:50 (for SMM1-grown cells) or 1:500 (for YENB-grown cells), into homologous medium and shaking the cultures at 37°C until an optical density at 650 nm (OD_{650} , Zeiss PMQ II spectrophotometer) of 0.5 (SMM1) or 0.4 (YENB) was obtained. Cultures were pelleted by centrifugation, washed twice, and resuspended in PB at $\text{OD}_{650} = 0.2$ (for UV irradiation) or $\text{OD}_{650} = 5$ (for γ -irradiation). At $\text{OD}_{650} = 5$, cell suspensions contained 4×10^9 (SMM1-grown cells) or 1.3×10^9 (YENB-grown cells) colony-forming units (CFU) per ml. UV (254 nm) and γ (^{137}Cs , oxic) irradiation procedures have been described (Sargentini and Smith, 1983).

Mutation assays

The calculation of the radiation-induced mutant frequency has been described (Sargentini and Smith, 1980). In general, the listed mutant frequency (e.g., Lac^+ per 10^8 cells) is the frequency of radiation-induced mutants corrected for spontaneous 'plate mutants', and the death of pre-existing spontaneous mutants. The frequency of pre-existing spontaneous mutants was determined by plating nonirradiated cells on Glu-0, Arg-0, or Trp-0 plates as appropriate. Radiation survival was determined on the mutant-selection plates, except that YENB plates were used in the Rif^r and Spc^r mutant assays. When radiation mutagenesis was not detected, 'theoretical upper limits' for the radiation-induced mutant frequency were calculated on the supposition that one mutant clone (above the background level) was detected on the mutant-selection plates scored (e.g., 0.25 radiation-induced mutants per plate if 4 plates

were scored). Unless otherwise specified, the plating conditions were: (1) for Lac^+ UVRM, SMM1-grown cells ($\text{OD}_{650} = 0.2$) were concentrated 10-fold before spreading (0.2 ml) on Glu-1200 plates in triplicate; (2) for Lac^+ IRM, SMM1-grown cells ($\text{OD}_{650} = 5$) were spread (0.2 ml) on Glu-300 plates in quadruplicate; (3) for Arg⁺ UVRM, YENB-grown cells ($\text{OD}_{650} = 0.2$) were concentrated 10-fold before spreading (0.2 ml) on Arg-1.5 plates in quadruplicate; (4) for Arg⁺ IRM, YENB-grown cells ($\text{OD}_{650} = 5$) were spread (0.2 ml) on Arg-1.5 plates in quadruplicate; (5) for Trp⁺ UVRM, YENB-grown cells ($\text{OD}_{650} = 0.2$) were spread (0.2 ml) on Trp-1 plates in quadruplicate; (6) for Trp⁺ IRM, YENB-grown cells ($\text{OD}_{650} = 5$) were spread (0.2 ml) on Trp-1 plates in quadruplicate; (7) for Rif^r and Spc^r UVRM, YENB-grown cells were inoculated into YENB at 2×10^6 CFU/ml, shaken 16 h at 37°C, then spread (0.2 ml) on Rif and Spc plates in duplicate; (8) for Rif^r and Spc^r IRM, conditions were as for UVRM except that 0.1 ml was spread on the Rif plates. Mutant-selection plates were incubated 3 days at 37°C (Rif plates, 1 day).

Mutant differentiation

Since both the *argE3* and *his-4* markers in derivatives of strain AB1157 are ochre nonsense mutations (Kato and Shinoura, 1977), one can easily identify Arg⁺ mutants as being back or suppressor mutants. 100 Arg⁺ mutants per dose were patched onto His-0 and Arg-0 plates. Suppressor mutants were identified as those that were able to demonstrate growth on both plates in 3 days. Back mutants were those that could grow only on the Arg-0 plates. The fractional values for back and suppressor mutants in each sample of 100 Arg⁺ mutants were applied to the total Arg⁺ mutants to calculate total values for back and suppressor mutants at each dose. These data were then used to calculate back and suppressor mutant frequencies, as described above.

Results

Two mutant alleles of the *umuC* locus were compared for their effect on UV- and γ -radiation-induced Lac reversion (*lacZ53* amber \rightarrow Lac⁺, Sargentini and Smith, 1979) in *E. coli* K12 KH21

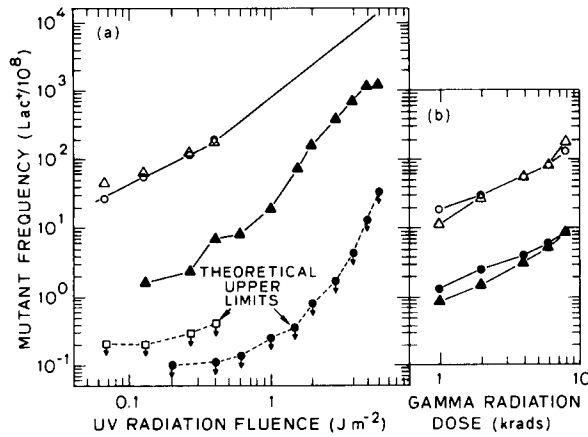


Fig. 1. Effect of *umuC* mutations on UV (a) and γ - (b) radiation mutagenesis (*lacZ53* amber \rightarrow Lac⁺) in *E. coli* K12 KH21 *wrB5* strains. The curve through the *wrB5* data points (\circ , Δ) in (a) is for cells ($OD_{650} = 0.05$) spread on Glu-300 plates, and has been published (Sargentini and Smith, 1979). Theoretical upper limit data (\bullet , \square) were calculated as described in Materials and methods. For γ -radiation, strains SR742 and SR1034 were concentrated 4-fold relative to strains SR741 and SR250 to improve the numbers of mutants detected. Data are the means from triplicate experiments. \circ , SR250 (*wrB5*); \bullet , SR1034 (*wrB5 umuC122::Tn5*); Δ , SR741 (*wrB5*); \blacktriangle , SR742 (*wrB5 umuC36*); \square , SR251 (*wrB5 lexA101*).

wrB5 strains. The *wrB5 umuC36* strain showed a 10–30-fold deficiency in UVRM, while the *wrB5 umuC122::Tn5* strain and a *wrB5 lexA101* strain (included as a nonmutable control) showed no detectable UVRM (Fig. 1a). Based on our calculation of the theoretical upper limits for UVRM (see Materials and methods), the *umuC122::Tn5* strain showed at least a 300–5000-fold deficiency in UVRM, depending on the dose (Fig. 1a). Both *wrB umuC* strains showed significant IRM, although it was about 15-fold lower than that detected in the *wrB* control strains (Fig. 1b).

After we had completed the Lac reversion studies in the KH21 strains, we found that strain KH21 is partially deficient in *recA*-dependent, inducible radiation resistance relative to strain AB1157 (data not shown). This radiation resistance phenomenon, called medium-dependent resistance, is maximized by growing cells to logarithmic phase in a rich medium such as YENB (Sargentini et al., 1983). Since we wanted to study the effect of a *umuC* mutation on additional assays for UVRM and IRM, further studies used YENB-grown *E. coli* K12 AB1157 (*wrB*⁺) cells.

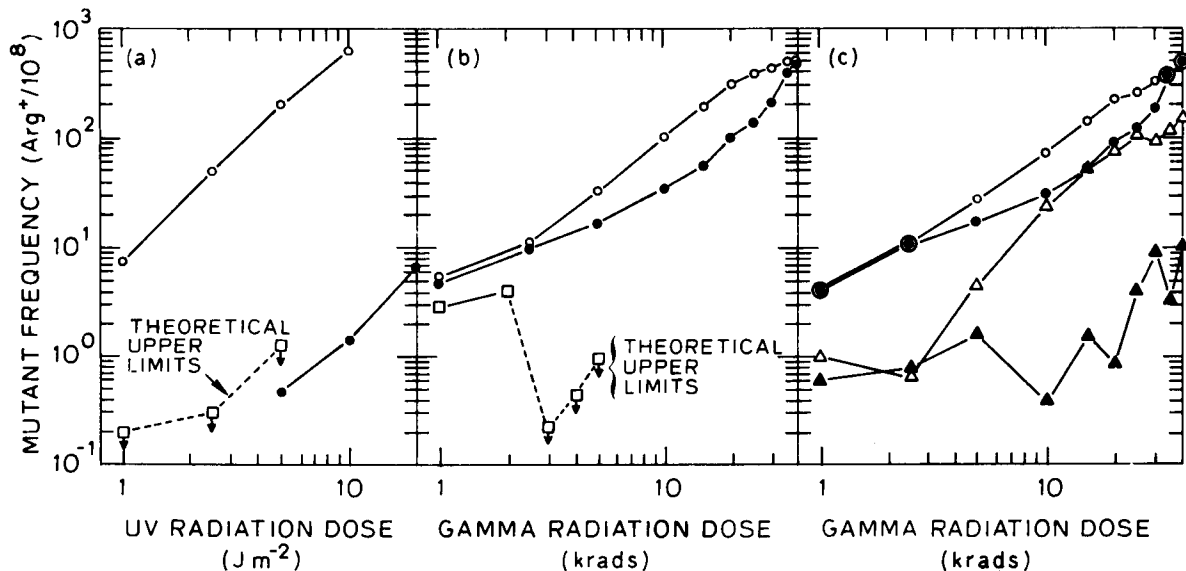


Fig. 2. Effect of the *umuC122::Tn5* mutation on UV (a) and γ - (b, c) radiation mutagenesis (*argE3* ochre \rightarrow Arg⁺) in *E. coli* K12 AB1157 strains. In (c) the γ -radiation-induced Arg⁺ mutants have been classified as suppressor (Δ , \blacktriangle) or back (\circ , \bullet) mutants. Theoretical upper limit data (\square) were calculated as described in Materials and methods. For UV radiation, strain SR1165 was concentrated 10-fold relative to strains SR749 and SR1279 before plating for mutants (because, without concentration, very few SR1165 mutants were detected). Data are the means from triplicate or more experiments. (a) and (b): \circ , SR749 (wild-type); \bullet , SR1165 (*umuC122::Tn5*); \square , \square , SR1279 (*lexA101*). (c) \circ , SR749 back mutants; \bullet , SR1165 back mutants; Δ , SR749 suppressor mutants; \blacktriangle , SR1165 suppressor mutants.

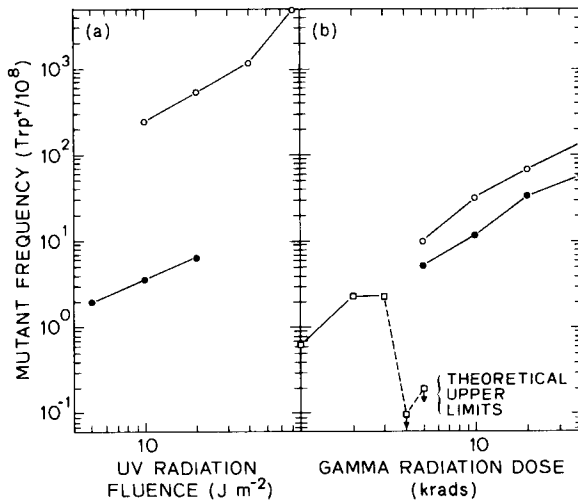


Fig. 3. Effect of the *umuC122::Tn5* mutation on UV (a) and γ - (b) radiation mutagenesis (*trpE9777* frameshift \rightarrow Trp⁺) in *E. coli* K12 AB1157 strains. Theoretical upper limit data (\square) were calculated as described in Materials and methods. After UV irradiation, strain SR1314 was concentrated 100-fold relative to strains SR1285 and SR1320 before plating (because, without concentration, very few SR1314 mutants were detected). Data are the means from triplicate or more experiments. \circ , SR1285 (wild-type); \bullet , SR1314 (*umuC122::Tn5*); \square , \square , SR1320 (*lex A101*).

These studies used only the *umuC122::Tn5* allele because it seemed to cause a greater deficiency in UVRM (Fig. 1a). The mutagenesis assays mea-

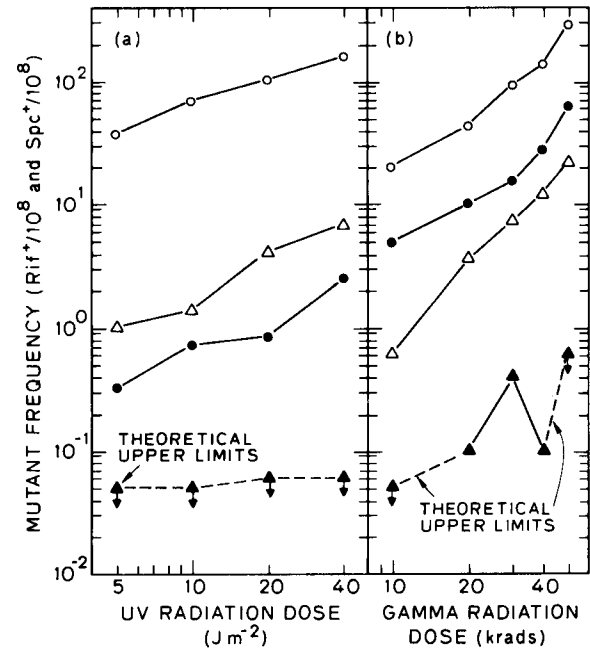


Fig. 4. Effect of the *umuC122::Tn5* mutation on UV (a) and γ - (b) radiation mutagenesis (*Spc*^s \rightarrow *Spc*^r missense; *Rif*^s \rightarrow *Rif*^r missense) in *E. coli* K12 AB1157 strains. Theoretical upper limit data (\blacktriangle) were calculated as described in Materials and methods. Data are the mutant frequencies of the 16-h cultures inoculated with irradiated cells minus the mutant frequency of the 16-h culture inoculated with nonirradiated cells, and are the means from triplicate or more experiments. Symbols for SR749 (wild-type): \circ , *Rif*^r; \triangle , *Spc*^r. Symbols for SR1165 (*umuC122::Tn5*): \bullet , *Rif*^r; \blacktriangle , \blacktriangle , *Spc*^r.

TABLE 2

EFFECT OF THE *umuC122::Tn5* MUTATION ON THE RADIATION MUTABILITY OF *E. coli*

Mutation assay	Relative radiation mutagenesis in <i>umuC</i> strain ^a	
	γ (10 krad)	UV (10 J/m ²)
<i>argE3</i> (ochre) \rightarrow Arg ⁺		
Total mutants	0.34	0.002
Back mutants	0.41	NT ^c
Suppressor mutants	0.02	NT ^c
<i>trpE9777</i> (frameshift) \rightarrow Trp ⁺	0.37	0.01
<i>Rif</i> ^s \rightarrow <i>Rif</i> ^r (putative missense) ^b	0.25	0.01
<i>Spc</i> ^s \rightarrow <i>Spc</i> ^r (putative missense) ^b	< 0.08	< 0.04

^a Values represent the mutant frequency in the *umuC122::Tn5* strain (SR1165) divided by that for the wild-type strain (SR749) irradiated with the same dose. The doses for the two radiations give about the same surviving fraction (60–90%, from Fig. 5) in both strains. Data are derived from the results in Figs. 2–4.

^b Rif, rifampicin; Spc, spectinomycin; superscripts: s, sensitive, r, resistant.

^c NT, not tested.

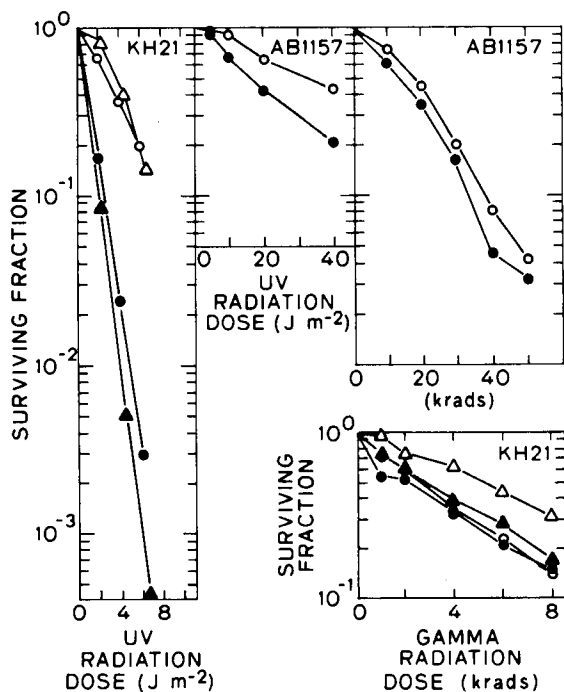


Fig. 5. Effect of *umuC* mutations on radiation survival of *E. coli* K12 strains. Survival data for representative strains were gathered as part of the mutagenesis assays. KH21 strains were grown in SMM1 and plated on Glu-1200 (UV) or Glu-300 (γ) plates (Lac⁺ assay). AB1157 strains were grown in YENB and plated on YENB plates (Rif^r, Spc^r assay). Symbols for KH21 strains: ○, SR250 (*wvrB5*); ●, SR1034 (*wvrB5 umuC122::Tn5*); △, SR741 (*wvrB5*); ▲, SR742 (*wvrB5 umuC36*). Symbols for AB1157 strains: ○, SR749 (wild-type); ●, SR1165 (*umuC122::Tn5*).

sured the effect of UV and γ -radiation on Arg reversion (*argE3* ochre \rightarrow Arg⁺, Kato and Shinoura, 1977), the induction of Arg⁺ back and suppressor mutants, Trp reversion (*trpE9777* frameshift \rightarrow Trp⁺, Bronson and Yanofsky, 1974), and the induction of rifampicin- and spectinomycin-resistant mutants (these two classes of drug-resistant mutants can be assumed to be missense mutants; e.g., Austin et al. (1971) and Silengo et al. (1967), respectively). The *umuC122::Tn5* strains (SR1165, SR1314) were very poorly mutable by UV radiation (Figs. 2a, 3a and 4a), but showed substantially more mutability by γ -radiation (Figs. 2b, 3b and 4b), except with the Arg-suppressor mutant assay (Fig. 2c) and the spectinomycin-resistant mutant assay (Fig. 4b). A *lexA101* strain (SR1279 or SR1320) was included in some

of the mutation assays as a nonmutable control. The *lexA* strains showed some IRM at the lower doses tested, but showed no UVRM at any dose tested (Figs. 2a, 2b and 3b).

To quantitate the deficiency in radiation mutagenesis shown by the *umuC122::Tn5* strain, data from Figs. 2–4 were compared at approximately equal-killing doses (Table 2). To allow other comparisons of the mutant and survival data, the radiation survival of strains relevant to these studies is shown in Fig. 5.

Discussion

Based on the comparison of the *umuC36* and *umuC122::Tn5* mutant alleles for their effect on the UV-radiation-induced reversion of the *lacZ53* mutation (Fig. 1a), we concluded that the *umuC36* allele is leaky. Consistent with this conclusion is the paper of Shinoura et al. (1983), in which they detected 2–3% of the wild-type level of UVRM (His reversion) in their *umuC36* strain. Also, with Leu reversion (*leuB19* missense \rightarrow Leu⁺), 2–12% of the *wvrB5* level of UVRM has been detected in a *wvrB5 umuC36* strain (Sargentini, 1979).

In agreement with the work of Kato and Shinoura (1977), we found (using the *umuC122::Tn5* allele) that the *umuC* gene was very important to UVRM, both in *wvrB5* (Fig. 1a) and in *wvrB*⁺ (Figs. 2a, 3a and 4a) strains. In the *wvrB5* strain, no UVRM (*lacZ53* \rightarrow Lac⁺) could be detected in the *lexA101* or *umuC122::Tn5* derivatives (Fig. 1a). In the AB1157 strain background, a *wvrB*⁺ *umuC122::Tn5* strain also failed to show UVRM (data not shown). When these *umuC122::Tn5* cells were concentrated another 10-fold before plating for mutants, significant UVRM was detected, but it was still about 1000-fold less than that detected in the wild-type strain (Fig. 2a). When some of these Arg⁺ revertants were tested, some had become kanamycin-sensitive. When tested for UVRM (Rif^r assay), the Arg⁺ revertants that were still kanamycin-resistant showed the low level of UVRM characteristic of strain SR1165 (*umuC122::Tn5*), while the Arg⁺ kanamycin-sensitive isolates showed the whole range of UVRM from the *umuC122::Tn5* level to the wild-type level (data not shown). This result was presumably due to excisions of the transposon

Tn5 that produced deletions of various sizes (reviewed by Kleckner, 1977) at the *umuC* locus in a small proportion of the cells being tested for Arg reversion. Some deletion mutants presumably regained enough *umuC* activity to show considerable UVRM. This suggests that the very small amount of UVRM detected in the *umuC122::Tn5* strain with the Arg reversion (Fig. 2a), Trp reversion (Fig. 3a), and rifampicin-resistance mutagenesis assays (Fig. 4a) should not be described as *umuC*-independent UVRM. Therefore, we conclude that all UVRM in *E. coli* may depend on the presence of a functional *umuC* gene product.

In contrast to other workers (Kato and Nakano, 1981; Kato et al., 1982), we found that the *umuC* gene is generally not essential for IRM, although some dependence was detected (Figs. 1b, 2b, 3b and 4b). We used the spectinomycin-resistance assay to measure IRM because it had also been used by Kato and Nakano (1981). Our data, showing little or no induction of spectinomycin-resistant mutants by ionizing radiation in a *umuC* strain, are in agreement with those of Kato and Nakano (1981). The quantitation of all of our data for the AB1157 strains (Table 2) leads to the conclusion that the *umuC* gene product is very important to IRM at some sites, e.g., those sites involved in the induction of spectinomycin-resistant mutants and Arg⁺ suppressor mutants, but it is less important to IRM at other sites, e.g., sites relevant to the mutagenesis detected by the Lac⁺, Arg⁺ back mutant, Trp⁺, and Rif^r mutant assays. In fact, for some doses of γ -radiation (1, 2.5, and 40 krad), the *umuC122::Tn5* strain showed essentially normal levels of Arg reversion (Fig. 2b). Similarly, Steinborn (1978) reported that his *uvm* mutants, which are now known to be *umuC* and/or *umuD* mutants (Shinagawa et al., 1983), showed about 50% of the X-ray-induced (10 krad) Arg reversion (*argE3* \rightarrow Arg⁺) that was seen in his wild-type cells. The comparison of our data and those of Kato and Nakano (1981) suggests that by chance these authors used only mutation assays in which the *umuC* gene plays a major role. Our study shows that there are also other sites at which the *umuC* gene product is not essential for IRM (i.e., those sites that were mutated in our Lac⁺, Arg⁺ back mutant, Trp⁺, and Rif^r mutant assays).

Consistent with the work of Bridges et al. (1968),

our *lexA* mutant also showed some IRM at low doses, but this disappeared at higher doses (Figs. 2b and 3b). Knowing the role of the *lexA* product in regulating the *recA* and *umuC* genes (reviewed by Little and Mount, 1982), we propose that the basal level of *recA* (and/or *umuC* protein) is sufficient for IRM when the DNA contains only a small amount of damage; with larger amounts of DNA damage, the induction of *recA* (and/or *umuC* protein) is essential to IRM. [Recall that *recA* trains do not show radiation mutagenesis even when the *umuC* gene product has been fully induced, as in a *recA spr* strain (Bagg et al., 1981).] UV-radiation-induced damage may be much more efficient at using up the basal level of *recA* and/or *umuC* protein, since we were never able to detect UVRM in the *lexA* strains (Figs. 1a and 2a).

We conclude that there are *umuC*-dependent and *umuC*-independent modes of radiation mutagenesis, although both modes should function in the 'misrepair' category of mutagenesis discussed by Kato et al. (1982). The molecular basis of the two modes of mutagenesis is not readily apparent. It seems unlikely that their radiation specificity is related to the classes of mutations that are produced, since both UV and ionizing radiation appear to produce the same classes of mutations in fairly similar amounts (Hartman et al., 1971). Therefore, the chemical nature of the mutagenic lesion would appear to determine the role of the *umuC* gene in mutagenesis, however, the salient features of the mutagenic lesion are unclear. Todd and Schendel (1983) have substantiated an earlier hypothesis (Kato et al., 1982; Shinoura et al., 1983) that the bulkiness of a mutagenic DNA lesion can determine the role of the *umuC* gene in mutagenesis. Although the bulkiness of the mutagenic lesion seems relevant to the requirement of the *umuC* gene product for UV radiation mutagenesis, it seems to be of less relevance in explaining the role of the *umuC* gene in ionizing radiation mutagenesis, since Schaaper et al. (1982) have shown that the mutagenicity of apurinic sites, which are likely, nonbulky ionizing radiation-induced lesions, is also *umuC* dependent. At this point, we can only emphasize that, after ionizing irradiation, there must exist a class of site-specific DNA lesions that are substantially different in nature from those induced by UV radiation, and

whose mutagenicity is *umuC* independent.

That we can define two modes of radiation mutagenesis in terms of the *umuC* gene is in line with a growing body of data that support the existence of *multiple* mechanisms of radiation mutagenesis. Such data include the complex shape of the UV radiation mutant frequency response curve (Doudney, 1976; Sargentini and Smith, 1979), the correlation of the theoretical components of the UV radiation mutant frequency response curve with the induction of different classes of mutants (Sargentini et al., 1982), and also the finding that the *uvrD* and *recB* genes control different genetic pathways of UV radiation mutagenesis (Sargentini and Smith, 1980).

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