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Multiple, Independent Components of Ultraviolet Radiation Mutagenesis in *Escherichia coli* K-12 *uvrB5*

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Reversion systems involving the *lacZ53*(amber) and *leuB19*(missense) mutations were developed to study the mutant frequency response of *Escherichia coli* K-12 *uvrB5* (SR250) to ultraviolet radiation (254 nm). A one-hit mutant frequency response was discernible at ultraviolet radiation fluences below ~0.5 J m^{-2}. At higher fluences the overall mutant frequency response could be resolved into one-hit and two-hit components. A new interpretation of the published data on *E. coli* K-12 indicates that SR250 is not unique in this respect. In addition, the Lac reversion system showed enhanced mutagenesis after ultraviolet radiation fluences of ~1 to 3 J m^{-2}, whereas the Leu reversion system did not. We conclude that the complex ultraviolet radiation mutant frequency response curves for *E. coli* K-12 *uvrB5* were the result of three independent mutagenic processes for Lac reversion and two for Leu reversion.

The nonlinear nature of the mutant frequency response (MFR) to UV radiation was noted in the early radiation mutagenesis studies on bacteria (15). Witkin (39) proposed that this nonlinearity resulted from a two-hit process. When the logarithm of the mutant frequency (MF) was plotted against the logarithm of the UV radiation fluence, a straight line with a slope of 2 was obtained (1) [the slope is equal to the exponent of the fluence parameter in an equation for MF, i.e., MF = (fluence)^n]. This treatment of the data seemed to substantiate the two-hit nature of UV radiation mutagenesis. Much discussion has occurred concerning the nature of these two hits (reviewed in reference 43).

Although log-log plots are valuable in illustrating some concepts (41), other approaches to the treatment of mutagenesis data are also useful (17, 19, 21, 37). Doudney (17, 21) noted that, when the MFR to UV radiation in *Escherichia coli* B/r was plotted with linear coordinates, a distinct linear (one-hit) region was evident, although the adjacent MFR at higher or lower fluences was nonlinear. Using this plotting technique, he (19, 20) has identified three operationally different regions of a UV radiation-induced MFR curve for *E. coli* B/r *uvrA*. At the lowest fluences (<2 J m^{-2}; fluences were corrected by the method of Doudney [20]), the MFR curve (F^2 region) was nonlinear and was probably proportional to the square of the fluence. This suggested a two-hit mechanism, one being a lesion blocking the synthesis of DNA (17, 18, 21) and inducing SOS (error-prone) repair (14, 33), and the other hit was assumed to be the premutational lesion. At intermediate fluences (2 to 3 J m^{-2}), a linear (one-dimer [1DM]) MFR in relation to fluence was observed (19). This suggested that all of the cells in the population had received sufficient radiation for the full induction of error-prone repair, and thus premutational lesions produced mutations with one-hit kinetics. Other experiments supported this conclusion. For example, if the physiology of the cells was altered by giving an inducing UV radiation fluence as a pretreatment (19) or by incubating a *tif-1* strain at 42°C (41, 42), then the UV radiation-induced MFR was linear even in the usual F^2 region. Beyond the 1DM region was a so-called two-dimer (2DM) region that again conformed to a two-hit process (19). However, in this region the UV radiation-induced MFR was not altered by preinducing the cells (19, 43). This region of two-hit kinetics was thought to reflect the increasing importance of two interacting lesions (19), such as overlapping daughter strand gaps (16, 34-36).

In the work reported here, a new one-hit MFR region was noted at low UV radiation fluences (<0.5 J m^{-2}). This one-hit error-prone mechanism provided an explanation for the less than two-hit UV radiation mutant frequency responses commonly found for *E. coli* K-12 strains. Based upon our data, we suggest that UV radiation mutagenesis in *E. coli* K-12 *uvrB5* involves multiple mechanisms.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* K-12 F^- strains used in this study were SR250 (formerly called DY145;

reference 44) *uvrB5 leuB19 metE70 thyA36 deo(C2?) rha-5 lacZ53 rpsL151* λ^- and SR735 (also known as RFS817) *thi-1 relA1 rpsL220* Δ(*ara-leu*)*498* Δ*lac-3* λ^-. This latter strain was obtained from B. Bachmann (CGSC no. 5735). The genotype symbols are those used by Bachmann et al. (2). The allele designations were suggested by B. Bachmann (personal communication). The *lacZ53* marker is the amber mutation from *E. coli* K-12 Hfr300 YA14 and has been known as lac_{Y14} (5). The *leuB19* marker has also been called *leuB1* (26, 27) and originated in *E. coli* B (25).

Media. Cells were cultured in 0.4% glucose-salts medium (23) supplemented with thiamine·HCl at 0.5 μg/ml, D-biotin at 1 μg/ml, thymine at 10 μg/ml, 1 mM L-leucine, and 1 mM L-methionine. Survival and reversion to Lac^+ were assayed on modified glucose-salts plates (27 ml) solidified with Noble agar (Difco) (38) at 1.6%, containing lactose at 0.4% and glucose at 300 or 1,200 μg/ml. These plates are referred to as Δglu-300 or Δglu-1200 plates, respectively. Plates lacking any glucose (Δglu-0) were used to quantitate spontaneous Lac^+ mutants present when the culture was harvested. The small amount of glucose in the Δglu plates was necessary to allow the expression of induced mutants and also to allow relatively small numbers of auxotrophs to grow into colonies for the estimation of viability. Survival and reversion to Leu^+ were assayed on analogous plating media. Thus, Δleu-1 represents L-leucine at 1.0 μg/ml. LB medium (30) was supplemented with thymine at 10 μg/ml (LBt).

Transduction procedure. Donor strains were lysogenized by bacteriophage P1::Tn*9*(Cm^r)cts (kindly provided by M. Casadaban). Lysogenizations and transductions were performed generally as described by Miller (30).

UV radiation mutagenesis procedure. Cells in the logarithmic phase of growth (~2.5×10^8 colony-forming units per ml) were centrifuged ($6,000 \times g$ for 6 min), washed (1×), and resuspended in 67 mM phosphate buffer, pH 7.0 (12) at an optical density at 650 nm of 0.2 in a Zeiss PMQII spectrophotometer. These cells were UV irradiated as required and plated to assay for mutants or viability. Mutant colonies were counted when their number no longer increased (after 3 days at 37°C). Data were compiled from at least four experiments per UV radiation fluence with four mutant selection and three viability plates per fluence. These tabulated data may be obtained from us.

Irradiation. An 8-W General Electric germicidal lamp, emitting primarily at 254 nm, was situated at a distance of 47 cm from the cell sample. Cell suspensions (10 ml in a 9.0-cm-inside-diameter glass petri dish bottom) were irradiated on a rotating platform. The UV radiation fluence rate was ~0.2 or ~0.04 J m^{-2} s^{-1} (after the fluence rate was reduced with metal screens) and was checked before each experiment with a germicidal photometer (International Light, Inc., no. IL-254) whose calibration was verified by uranyl oxalate actinometry (modified from Bowen [4]). All manipulations were carried out under General Electric "gold" fluorescent lights to prevent photoreactivation. The stated UV radiation fluences have been corrected for cell-masking effects (e.g., the measured incident fluence × 0.89, for optical density at 650 nm = 0.20, gives the corrected average incident fluence) according to an empirically derived relationship based on the survival of a *uvrB5 recA56* strain irradiated at various cell concentrations (45).

Quantitation of mutagenesis. The UV radiation-induced MF was calculated per average mutant selection plate by a modified version of the formula of Bridges (6): $MF = \{[M_t - M_{po} + M_o(1 - SF_c)](1 \times 10^8)\}/[(S_c)\ (\text{volume plated})]$ where M_t is the number of mutant colonies arising from irradiated cells on Δnutrient plates (i.e., Δglu or Δleu), M_{po} is the number of mutant colonies arising from nonirradiated cells on Δnutrient plates, M_o is the number of mutant colonies arising from nonirradiated cells on plates lacking the growth-limiting nutrient (i.e., glucose or leucine), SF_c is the surviving fraction of irradiated cells as influenced by the concentration technique used, 1×10^8 is the factor included to normalize mutant frequencies to the number of mutants per 1×10^8 survivors, S_c is the colony-forming units per milliliter in the irradiated cell suspension plated in the mutant assays, and volume plated is the volume of cell suspension plated (e.g., 0.2 ml) in the mutant assays.

Chemical mutagenesis. Mutagenesis by 2-aminopurine nitrate (Calbiochem) or sodium bisulfite was performed by the method of Miller (30). An overnight LBt culture of strain SR250 was diluted 10^{-6} in phosphate buffer, and 0.6 ml was added to 30 ml of LBt ± 2-aminopurine nitrate at 600 μg/ml. For bisulfite mutagenesis, the overnight culture was pelleted, washed, and resuspended in 0.2 M acetate buffer (pH 5.2) ± 1 M sodium bisulfite. After shaking for 30 min at 37°C, these latter cells were pelleted, washed, and resuspended in LBt at a 10-fold dilution. For both mutagenesis techniques, the seeded LBt was divided into 12 2.5-ml cultures ± mutagen and incubated with aeration for 24 h at 37°C. Culture volumes of 0.2 ml were spread upon two Δleu-0 plates per culture. Cultures were also assayed for colony-forming units per milliliter on Δglu-300 plates.

RESULTS

Characterization of the *leuB19* mutation. The *leuB19* deficiency does not appear to be a nonsense mutation because of the inability of Leu^+ revertants to support the growth of various amber or ocher bacteriophage T4 mutants (R. Bockrath, personal communication). However, a more conclusive test was performed. A total of 107 independent Leu^+ revertants of strain SR250 were induced by UV radiation fluences of either 0.25, 2.5, or 5.0 J m^{-2}. Transducing lysates were prepared from these revertants, and all were able to transduce strain SR735 Δ(*ara-leu*) to Leu^+. A transducing lysate prepared from SR250 *leuB19* did not transduce this strain to Leu^+. Since no known suppressor loci are cotransducible (i.e., less than 2 min away) with the *leuB* locus (2), this is good evidence that all of the Leu^+ revertants of SR250 were due to mutations within the *leuB* locus.

2-Aminopurine nitrate, a mutagen that induces AT $\rightleftarrows$ GC transition mutations (22), was

tested for its ability to revert strain SR250 to Leu^+. The resultant mutant frequencies from two replicate experiments, using 12 cultures each, were 42 ± 12 and 59 ± 9 Leu^+ per 10^8 cells from 2-aminopurine nitrate-treated cultures or 2.5 ± 2.2 and 1.6 ± 0.9 Leu^+ per 10^8 cells, respectively, from nontreated cultures. In two experiments with sodium bisulfite, a mutagen that specifically induces GC → AT transition mutations (30), the resultant average mutant frequencies were 1.3 ± 1.1 and 1.3 ± 1.0 Leu^+ per 10^8 cells compared with 1.9 ± 1.2 and 2.4 ± 1.7 Leu^+ per 10^8 cells for the respective nontreated control sets of cultures. These data are consistent with the *leuB19* mutation being the result of a GC → AT transition and revertible by an AT → GC transition.

Determination of optimum conditions for the quantitation of UV radiation mutagenesis. Experiments were performed to determine a nutrient concentration that would allow maximal mutation (Lac^+ or Leu^+) expression. Cells were UV irradiated and spread on mutant selection plates containing various concentrations of glucose or leucine. On the basis of these data, glucose concentrations of 300 or 1,200 μg/ml were chosen for experiments in which nonconcentrated cells or cells concentrated 10-fold, respectively, would be plated for Lac^+ mutants. A leucine concentration of 1 μg/ml was chosen for experiments in which fivefold-concentrated cells would be plated for Leu^+ mutants. The amount of limiting nutrient (especially in the case of glucose) used in these experiments represents an amount sufficient for maximal mutation yield without production of an inordinately large number of spontaneous mutant colonies per plate.

Variability in the number of viable cells plated in the UV radiation mutagenesis experiments was not considered to be important because in an experiment in which different numbers of nonirradiated cells were plated, the value for spontaneous mutants arising on the mutant selection plates (corrected for preexisting mutants) was largely unaffected by the number of viable cells plated (data not shown).

Reversion to Lac^+ or Leu^+ after low UV radiation fluences. The initial data obtained by plating 10-fold-concentrated cells on Δglu-1200 plates suggested a linear increase in the Lac^+ MFR with fluence up to 0.4 J m^{-2}. This plating procedure, while yielding substantial numbers of radiation-induced mutants, is complicated by the large background of spontaneous Lac^+ mutants and can only be of value for low UV radiation fluences. To look at the MFR over the fluence range 0.0 to 1.2 J m^{-2}, nonconcentrated cells were spread on Δglu-300 plates. Both sets of data are presented in Fig. 1 and indicate linearity up to ~0.5 J m^{-2}. The pooled data points are defined by the relation: Lac^+ mutants per 10^8 cells = 414 × fluence, obtained by fitting a straight line through the data points for 0.0 to 0.5 J m^{-2} for strain SR250.

The UV radiation-induced MFR (*leuB19* → Leu^+) also appeared to be linear up to ~0.5 J m^{-2} (Fig. 2). A straight line was fitted through the data points for 0.0 to 0.35 J m^{-2} to yield the relation for strain SR250: Leu^+ mutants per 10^8 cells = 50.6 × fluence.

Reversion to Lac^+ or Leu^+ after higher UV radiation fluences and plotted with linear coordinates. To determine whether these linear MFR curves correspond to the 1DM region described by Doudney (19) (suggesting that *E. coli* K-12 is always induced for error-prone repair) or to a heretofore unnoticed type of linear MFR, the MFR (*lacZ53* → Lac^+) was measured over the UV radiation fluence range 0.0 to 9.0 J m^{-2} for strain SR250. Figure 3 shows that at UV radiation fluences of >0.5 J m^{-2} the F^2, 1DM, and 2DM regions of the MFR described by Doudney (19) can be seen in our data. Thus, two linear MFR regions are observed for Lac reversion, a linear MFR below ~0.5 J m^{-2} and a 1DM region for ~1 to 3 J m^{-2}. When our *leuB19* → Leu^+ data for UV radiation fluences of 0.0 to 6.5 J m^{-2} were plotted with linear coordinates (Fig. 4), a 1DM region could not be discerned.

UV radiation-induced reversion to Lac^+ or Leu^+ plotted with logarithmic coordi-

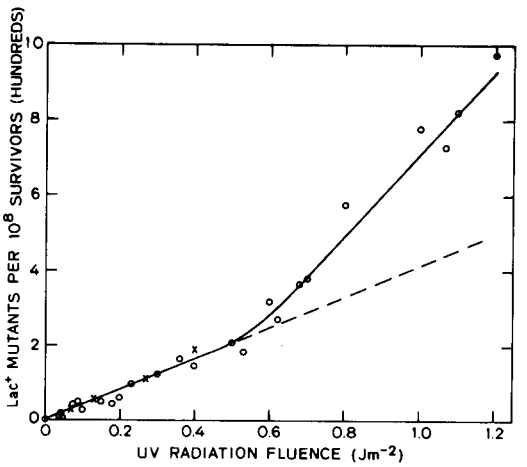

FIG. 1. *UV radiation mutagenesis to Lac^+ of E. coli K-12 uvrB5 (SR250). Symbols: ○, 0.2 ml of nonconcentrated cells spread upon Δglu-300 plates; ×, 0.2 ml of 10-fold-concentrated cells spread upon Δglu-1200 plates. A straight line has been visually fitted to the data for 0.0 to 0.5 J m^{-2}.*

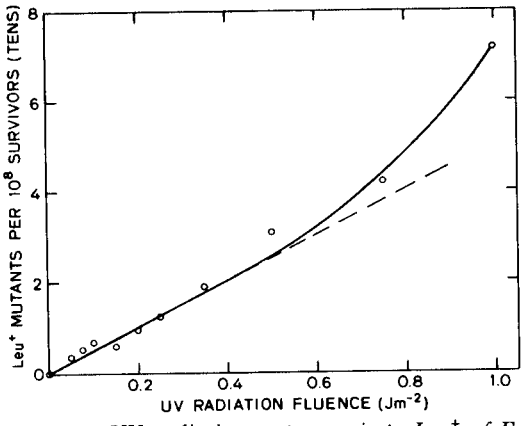

FIG. 2. *UV radiation mutagenesis to Leu$^+$ of E. coli K-12 uvrB5 (SR250). A straight line has been visually fitted to the data for 0.0 to 0.35 J m^{-2}.*

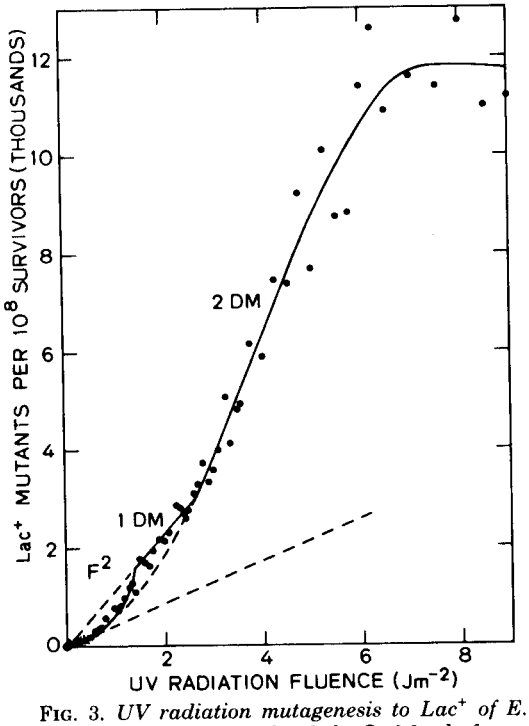

FIG. 3. *UV radiation mutagenesis to Lac$^+$ of E. coli K-12 uvrB5 (SR250). Symbols: ●, 0.2 ml of non-concentrated cells spread upon Δglu-300 plates; ×, 0.2 ml of 10-fold-concentrated cells spread upon Δglu-1200 plates. The terms F^2, 1DM, and 2DM are defined in the text. The lower dashed line is an extension of the line through the data for 0.0 to 0.5 J m^{-2} shown in Fig. 1. The upper dashed line fits the 1DM region. These curves have been visually fitted to the data.*

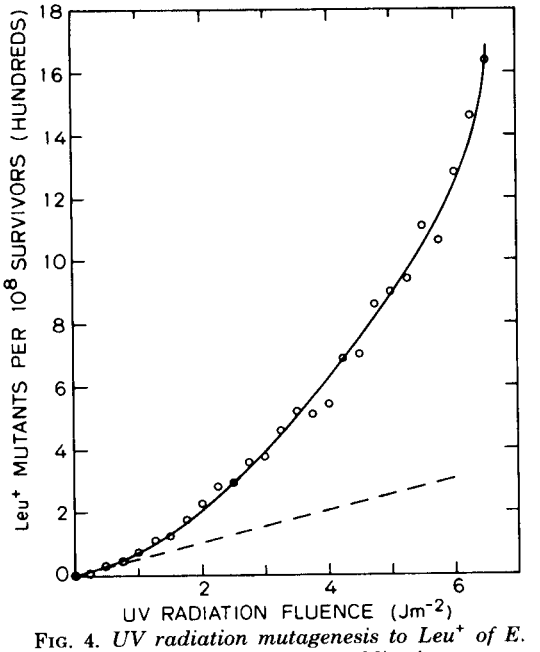

FIG. 4. *UV radiation mutagenesis to Leu$^+$ of E. coli K-12 uvrB5 (SR250). The dashed line is an extension of the line through the data for 0.0 to 0.35 J m^{-2} shown in Fig. 2. These curves have been visually fitted to the data.*

nates. When the Lac$^+$ MFR data (Fig. 3) were plotted with logarithmic coordinates (Fig. 5), the low-fluence linear region, but not the 1DM region, became clearly evident. We believe that these data points fit the relation MF = $aF + bF^2$ and thus should continually curve upward until a slope of 2.0 is reached. At fluences greater than 6 J m^{-2}, the reversion system no longer appears to respond to increasing amounts of radiation. A straight line was fitted through the data points, and its slope was calculated. The line through the Lac$^+$ UV radiation MFR data points for 0.5 to 6.0 J m^{-2} has a slope of 1.59. The line "Lac$^+$ mutants per 10^8 cells = 414 × fluence" (derived from Fig. 1) drawn through the data points for 0.038 to 0.5 J m^{-2} (Fig. 5) emphasizes the change in rate of UV radiation mutagenesis in the region of 0.5 J m^{-2}.

The slope value of 1.59 is markedly different from the value of 2.0 frequently associated with the UV radiation mutagenesis of *E. coli* B/r (1). If we make the assumption that the low-fluence linear MFR region (0 to 0.5 J m^{-2}) is the result of a linear process that operates at least up to 6.0 J m^{-2}, an extrapolated line between 0.5 and 6.0 J m^{-2} may be used to determine the contribution of this process to the total observed MFR. After subtracting the contribution of the linear process, the data points corresponding to the corrected MFR (Fig. 5) could be fitted with a line having a slope of 2.0. Also, the 1DM region

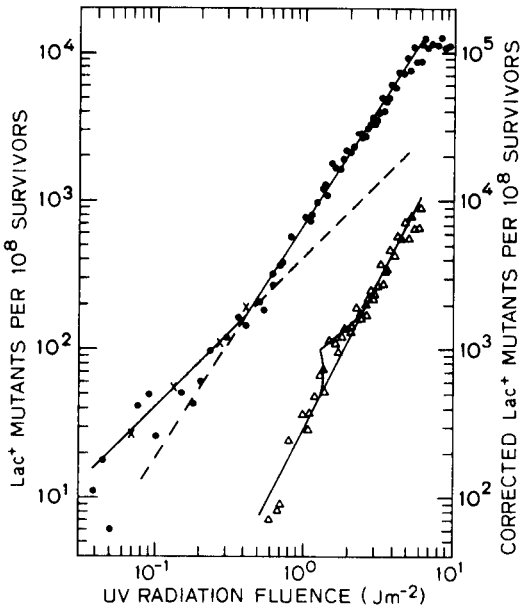

FIG. 5. *UV radiation mutagenesis to Lac$^+$ of E. coli K-12 uvrB5 (SR250). Symbols: ●, 0.2 ml of non-concentrated cells spread upon Δglu-300 plates; ×, 0.2 ml of 10-fold-concentrated cells spread upon Δglu-1200 plates; △, Lac$^+$ mutant data corrected as described in the text. These curves have been visually fitted to the data.*

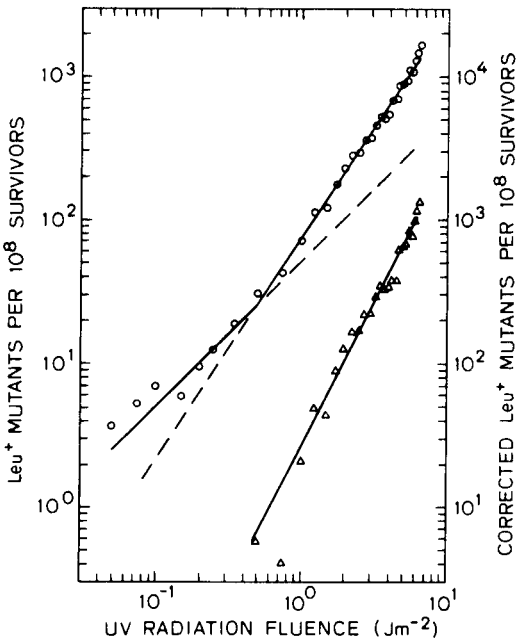

FIG. 6. *UV radiation mutagenesis to Leu$^+$ of E. coli K-12 uvrB5 (SR250). Symbols: ○, experimental data; △, Leu$^+$ mutant data corrected as described in the text. These curves have been visually fitted to the data.*

is more evident in the corrected data plot than in the uncorrected data plot, since the linear process had effectively obscured the 1DM region.

Since the corrected Lac$^+$ data points (Fig. 5) fit the relation "Lac$^+$ mutants per 10^8 cells = bF^2," the fluence coefficient, b, can be determined by finding the corrected mutant frequency at 1.0 J m^{-2}; it is 300 (Lac$^+$ mutants·10^{-8} cells·J^{-2}·m^4). Thus, combining the relation for the linear process with that for the calculated two-hit process, we obtained a quadratic formula for UV radiation-induced reversion to Lac$^+$: Lac$^+$ mutants per 10^8 cells = 414 × fluence + 300 × (fluence)^2. Similar calculations were performed with the Leu$^+$ data (Fig. 6), which had a slope of 1.54 for 0.5 to 6 J m^{-2}, and yielded the relation: Leu$^+$ mutants per 10^8 cells = 50.6 × fluence + 26 × (fluence)^2. The curves defined by these relations were virtually congruent with the experimental MFR curves (data not shown).

DISCUSSION

New linear MFR observed in *E. coli* K-12. UV radiation mutagenesis data obtained for Lac (Fig. 1) and Leu (Fig. 2) reversion in *E. coli* K-12 *uvrB5* (SR250) indicated a previously undetected linear or one-hit mutant frequency response region. This linear region supplements the F^2, 1DM, and 2DM regions previously described by Doudney (19) for *E. coli* B/r. The presence of this linear region allowed us to explain why our UV radiation MFR curves had slopes of 1.59 (Lac$^+$) or 1.54 (Leu$^+$) for fluences of 0.5 to 6.0 J m^{-2} when plotted with log-log coordinates. Data for *E. coli* B/r on a similar plot fit a line with a slope of ~2.0, i.e., a two-hit process (39). We assumed that the linear process, although evident only at fluences less than ~0.5 J m^{-2} (Fig. 1 and 2), actually operated at the same rate up to ~6 J m^{-2}. When the effect of the linear process was subtracted from the total UV radiation mutagenesis data, the remaining data had a slope of 2.0 (Δ in Fig. 5 and 6) on a log-log plot. This suggested that, whereas UV radiation mutagenesis in *E. coli* B/r was generally a two-hit process, in strain SR250 both one-hit and two-hit processes were important to UV radiation mutagenesis.

To determine whether *E. coli* K-12 (SR250) was unique in possessing the linear process, we surveyed the published UV radiation mutagenesis data. Since data obtained at UV radiation fluences less than 0.5 J m^{-2} were not generally available, we chose to determine the slopes of

available data plotted with log-log coordinates. These slopes (Table 1) were expected to indicate the presence of both one-hit and two-hit processes in the same fashion as that for strain SR250 (Fig. 5 and 6). The UV radiation-induced MFR slopes for the most commonly tested *E. coli* B/r strains, WP2 and $WP2_s$, were consistently found to be near 2.0. In contrast, the *E. coli* K-12 strains, when plated on defined-selective media as we had done, consistently had slopes that were less than 2.0. This analysis suggests that *E. coli* K-12 strains, regardless of excision-repair proficiency, generally employ both one-hit and two-hit mutagenic processes. In some systems, broth supplementation was correlated with MFR slopes of approximately three (Table 1).

New interpretation of the 1DM region of mutagenesis. Doudney (19), using a Trp nonsense reversion system, suggested that UV radiation mutagenesis in *E. coli* B/r *uvrA* ($WP2_s$) consisted of two processes. One process was nonlinear (F^2 region) until it was fully induced (1DM region), whereupon it continued to operate as a linear process up to 6 J m^{-2}. The second process

TABLE 1. *Slopes calculated for straight lines fitted to UV radiation MFR data plotted with log-log coordinates*

E. coli	Excision repair genotype	Reversion system[a]	Plating medium[b]	MFR slope[c]	Fluence range (J m^{-2})	References
B/r strains						
WP2	+	*trpE65*	Δtrp ± CAA	2.1 ± 0.1	1.5–220	1, 7, 9–11, 13, 31
WP2	+	*trpE65*	ΔNB	1.4 – 1.8	2–50	19, 31
$WP2_s$	*uvrA155*	*trpE65*	ΔNB, Δtrp ± CAA	2.0 ± 0.2	0.3–6	1, 7, 10, 13, 16, 19, 20, 31, 40
WU36-10	+	*tyr*	Δtyr	3.1	10–50	8
WU36-10	+	*leu*	Δleu	1.7	10–50	8
WU36-10-89	*uvrA*	*leu*	ΔNB	2.0 } [d] <1.0 }	0.053–0.37 0.66–4.5	32
K-12 strains						
AB1157	+	*his-4*	Δhis	1.3	15–120	24
AB1884	*uvrC34*	*his-4*	Δhis	1.5	2.5–15	24
AB1886	*uvrA6*	*his-4*	Δhis	1.7	1–15	24
PAM579	*uvrB*	*his-4*	Δhis	1.6	1–15	24
AB1885	*uvrB5*	*his-4*	Δhis	1.2	2.5–15	24
SR315	*uvrB5*	*leuB19*	Δleu	1.3 } [d] 2.7 }	1–4 4–8.5	3
SR312	*uvrA6*	*leuB19*	Δleu	1.4 } [d] 2.6 }	1–4 4–8.5	3
AB1157, JC3872	+	*his-4*	ΔNB	1.6 – 1.9	5–120	24, 28
JC3912, AB1886	*uvrA6*	*his-4*	ΔNB	1.4 – 1.7	0.25–15	24, 28
JC3890, TK603	*uvrB301*	*his-4*	ΔNB	1.7 ± 0.2	0.25–2	28, 29
AB1884	*uvrC34*	*his-4*	ΔNB	1.1 } [d] ~2 }	1–5 5–15	24
PAM579	*uvrB*	*his-4*	ΔNB	1.8	1–15	24
AB1885	*uvrB5*	*his-4*	ΔNB	2.0 } [d] <1 }	1–5 5–15	24
DY174	+	*leuB19*	ΔNB	2.0	3–80	37
DY178, SR315	*uvrB5*	*leuB19*	ΔNB	2.5–2.8	0.5–8	3, 37
SR312	*uvrA6*	*leuB19*	ΔNB	3.3	1–6.5	3
SR329	*uvrA155*	*leuB19*	ΔNB	2.9	1–8	3
SR314	*uvrA6 uvrB5*	*leuB19*	ΔNB	2.4	2–8	3

[a] *trpE65, tyr,* and *his-4* are ocher (UAA) nonsense mutations; *leu* is an amber (UAG) nonsense mutation; *leuB19* is a missense mutation.

[b] The symbol Δ signifies that the nutrient (e.g., tryptophan, Casamino Acids, nutrient broth, leucine, tyrosine, or histidine) was the growth-limiting factor in the mutant-selection medium, except for a fraction of the inoculum that had reverted to prototrophy for the phenotype being studied.

[c] Mutant frequency response data were replotted (if necessary) on log-log coordinates and the slope was determined for a line visually fitted to at least 3 points. Data for similar strains were pooled to get a range for two slopes or an average for three or more slopes.

[d] The brace indicates that the UV radiation MFR was clearly biphasic; thus, two slopes were derived.

was consistent with a two-hit mechanism that began to have an effect only above ~3 J m^{-2}. An analysis of both our own mutagenesis data and those of Doudney (19) led us to different conclusions.

The quadratic equations derived for the Lac^+ and Leu^+ data (see Results) define a close-fitting curve (data not shown) through all of each respective set of data (in the former case excluding the fluence range of ~1 to 3 J m^{-2}). Since a similar smooth curve can also be drawn through all of Doudney's data (19; excluding the 1DM region data points) (data not shown), we presume that the 1DM "bump" (Fig. 3) represents a third mutagenic process, and it operates only between UV radiation fluences of ~1 to 3 J m^{-2} in these *uvr* strains. The independence of this presumed third mechanism is further suggested by its presence in the Lac but absence in the Leu reversion system (compare Fig. 3 and 4), whereas the aforementioned one-hit and two-hit mechanisms both function in the Lac and Leu reversion systems of strain SR250.

The following analysis also suggests that, unlike our linear mechanism, the 1DM mechanism does not operate at UV radiation fluences greater than ~3 J m^{-2} in strains SR250 and $WP2_s$. Subtraction of the assumed contribution of the linear process from the total MFR (*lacZ53* → Lac^+) achieved the expected two-hit kinetics for UV radiation mutagenesis. It seemed reasonable that if our apparently linear 1DM region (Fig. 3) was also the result of a process that was linear between ~1 and 6 J m^{-2}, then the subtraction of its assumed contribution from the total MFR should yield data fitting a line with a slope of 2.0 even better than after subtracting the effect of the other linear process. This subtraction procedure was also performed with Doudney's data for *E. coli* B/r *uvrA* ($WP2_s$) (19). We calculated the resultant MFR data (for both strains) to fit a line with a slope of ~6.5 (data not shown). This result suggested to us that, rather than postulating a 6.5-hit error-prone mechanism, it is more reasonable to believe that a 1DM (?-hit) process does not operate at the same rate, if at all, at fluences above ~3 J m^{-2}. This conclusion was also reached by Witkin (43) to explain a very similar phenomenon, the fluence-range limitation for thermal enhancement of UV radiation mutagenesis in a *tif-1* strain.

Three mechanisms of UV radiation mutagenesis in *E. coli*. Since the three components of the UV radiation MFR appear to function independently, they can be resolved as shown in Fig. 7. The one-hit component was subtracted from the total UV radiation MFR curve (both taken from Fig. 3) to obtain coordinates for the two-hit component. A smooth curve was then drawn through these calculated points (excluding the data points for 1.5 to 3.0 J m^{-2}). The ?-hit component was then plotted by using data obtained by subtracting the sum of the contribution from both the one-hit and the two-hit components from the total UV radiation MFR. Our resolution of these three proposed components indicates that the nonlinear nature of the F^2 region (Fig. 3) appears to be largely a result of the contribution of the two-hit component.

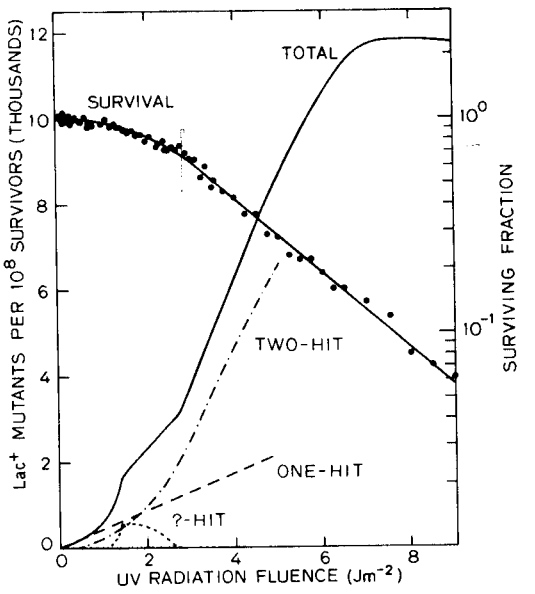

FIG. 7. *UV radiation survival and mutagenesis to Lac^+ of E. coli K-12 uvrB5 (SR250) with presumed UV radiation MFR components. The "total" curve was taken from Fig. 3. The method of resolution of the one-hit, two-hit, and ?-hit components of the total UV radiation MFR curve is explained in the text.*

We conclude that the UV radiation MFR in *E. coli* K-12 *uvrB5* (SR250), and perhaps other strains, is a result of three mechanisms, as depicted in Fig. 7: (i) a "one-hit" process that yields a previously undetected linear MFR that is readily observed at UV radiation fluences of <0.5 J m^{-2} (however, this process appears to operate at least up to 6 J m^{-2}); (ii) a "two-hit" process that yields a fluence-squared MFR at fluences greater than ~0.5 J m^{-2} (in part, approximating the 2DM region of Doudney [19]); (iii) a "?-hit" process that yields a MFR that does not appear to result from a one-hit or a two-hit process, but was initially designated by Doudney (19) as the 1DM region. This process is unusual in that it appears to initiate at ~1 J m^{-2} and terminate at

~ 3 J m^{-2} and is specific for nonsense reversion. This process may only occur in cells that have undergone a specific physiological change, e.g., involving the *tif* locus (41) or perhaps the blockage of DNA synthesis (19).

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LITERATURE CITED

1. **Ashwood-Smith, M. J., and B. A. Bridges.** 1966. Ultraviolet mutagenesis in *Escherichia coli* at low temperatures. Mutat. Res. **3**:135-144.
2. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116-167.
3. **Barfknecht, T. R., and K. C. Smith.** 1977. Ultraviolet radiation-induced mutability of isogenic *uvrA* and *uvrB* strains of *Escherichia coli* K-12 W3110. Photochem. Photobiol. **26**:643-645.
4. **Bowen, E. J.** 1946. The chemical aspects of light. Clarenden Press, Oxford.
5. **Brenner, S., and J. R. Beckwith.** 1965. Ochre mutants, a new class of suppressible nonsense mutants. J. Mol. Biol. **13**:629-637.
6. **Bridges, B. A.** 1972. Simple bacterial systems for detecting mutagenic agents. Lab. Pract. **21**:413-419.
7. **Bridges, B. A., R. E. Dennis, and R. J. Munson.** 1967. Differential induction and repair of ultraviolet damage leading to true reversions and external suppressor mutations of an ochre codon in *Escherichia coli* B/r WP2. Genetics **57**:897-908.
8. **Bridges, B. A., R. E. Dennis, and R. J. Munson.** 1970. Mutagenesis in *Escherichia coli*. V. Attempted interconversion of ochre and amber suppressors and mutational instability due to an ochre suppressor. Mol. Gen. Genet. **107**:351-360.
9. **Bridges, B. A., and R. P. Mottershead.** 1978. Mutagenic DNA repair in *Escherichia coli*. VII. Constitutive and inducible manifestations. Mutat. Res. **52**:151-159.
10. **Bridges, B. A., R. P. Mottershead, and S. G. Sedgwick.** 1976. Mutagenic DNA repair in *Escherichia coli*. III. Requirement for a function of DNA polymerase III in ultraviolet-light mutagenesis. Mol. Gen. Genet. **144**: 53-58.
11. **Bridges, B. A., R. J. Munson, C. F. Arlett, and D. R. Davies.** 1967. Interaction between ultraviolet light and γ-radiation damage in the induction of mutants of *Escherichia coli*: the effect of some modifying treatments. J. Gen. Microbiol. **46**:339-346.
12. **Castellani, A., J. Jagger, and R. B. Setlow.** 1964. Overlap of photoreactivation and liquid holding recovery in *Escherichia coli* B. Science **143**:1170-1171.
13. **Davies, D. R., C. F. Arlett, R. J. Munson, and B. A. Bridges.** 1967. Interaction between ultraviolet light and γ-radiation damage in the induction of mutants of *Escherichia coli*: the response in strains with normal and reduced ability to repair ultraviolet damage. J. Gen. Microbiol. **46**:329-338.
14. **Defais, M., P. Fauquet, M. Radman, and M. Errera.** 1971. Ultraviolet reactivation and ultraviolet mutagenesis of λ in different genetic systems. Virology **43**:495-503.
15. **Demerec, M., and R. Latarjet.** 1946. Mutations in bacteria induced by radiations. Cold Spring Harbor Symp. Quant. Biol. **11**:38-49.
16. **Doubleday, O. P., B. A. Bridges, and M. H. L. Green.** 1975. Mutagenic DNA repair in *Escherichia coli*. II. Factors affecting loss of photoreversibility of UV induced mutations. Mol. Gen. Genet. **140**:221-230.
17. **Doudney, C. O.** 1963. Ultraviolet light-induced mutation as an event in the physiology of the bacterial cell, p. 125-159. *In* F. H. Sobels (ed.), Repair from genetic radiation damage. Pergamon Press, Oxford
18. **Doudney, C. O.** 1966. Photoreversal of ultraviolet induced mutation, lethality and damage to nucleic acid formation in bacteria. Mutat. Res. **3**:280-297.
19. **Doudney, C. O.** 1976. Complexity of the ultraviolet mutation frequency response curve in *Escherichia coli* B/r: SOS induction, one-lesion and two-lesion mutagenesis. J. Bacteriol. **128**:815-826.
20. **Doudney, C. O.** 1977. Is DNA polymerase I necessary for most UV-induced mutation from tryptophan auxotrophy in *Escherichia coli* B/r WP2 ? Mutat. Res. **45**:147-152.
21. **Doudney, C. O., and C. S. Young.** 1962. Ultraviolet light induced mutation and deoxyribonucleic acid replication in bacteria. Genetics **47**:1125-1138.
22. **Freese, E.** 1959. The specific mutagenic effect of base analogues on phage T4. J. Mol. Biol. **1**:87-105.
23. **Ganesan, A. K., and K. C. Smith.** 1968. Dark recovery processes in *Escherichia coli* irradiated with ultraviolet light. I. Effect of *rec*$^-$ mutations on liquid holding recovery. J. Bacteriol. **96**:365-373.
24. **Green, M. H. L., A. Rothwell, and B. A. Bridges.** 1972. Mutation to prototrophy in *Escherichia coli* K-12: effect of broth on UV-induced mutation in strain AB1157 and four excision-deficient mutants. Mutat. Res. **16**: 225-234.
25. **Helling, R. B.** 1967. The effect of arabinose-specific enzyme synthesis on recombination in the arabinose genes of *Escherichia coli*. Genetics **57**:665-675.
26. **Helling, R. B.** 1968. Selection of a mutant of *Escherichia coli* which has high mutation rates. J. Bacteriol. **96**: 975-980.
27. **Helling, R. B.** 1973. Ultraviolet light-induced recombination. Biochem. Biophys. Res. Commun. **55**:752-757.
28. **Kato, T., R. H. Rothman, and A. J. Clark.** 1977. Analysis of the role of recombination and repair in mutagenesis of *Escherichia coli* by UV irradiation. Genetics **87**: 1-18.
29. **Kato, T., and Y. Shinoura.** 1977. Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutations by ultraviolet light. Mol. Gen. Genet. **156**:121-131.
30. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
31. **Munson, R. J., and B. A. Bridges.** 1966. Non-photoreactivating repair of mutational lesions induced by ultraviolet and ionizing radiations in *Escherichia coli*. Mutat. Res. **3**:461-469.
32. **Pollard, E. C., S. Person, M. Rader, and D. J. Fluke.** 1977. Relation of ultraviolet light mutagenesis to a radiation-damage inducible system in *Escherichia coli*. Radiat. Res. **72**:519-532.
33. **Radman, M.** 1974. Phenomenology of an inducible mutagenic DNA repair pathway in *Escherichia coli*: SOS repair hypothesis, p. 128-142. *In* L. Prakash, F. Sherman, M. Miller, C. Lawrence, and H. W. Tabor (ed.), Molecular and environmental aspects of mutagenesis. Charles C Thomas, Publisher, Springfield, Ill.
34. **Sedgwick, S. G.** 1975. Genetic and kinetic evidence for different types of postreplication repair in *Escherichia coli* B. J. Bacteriol. **123**:154-161.
35. **Sedgwick, S. G.** 1975. Inducible error-prone repair in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **72**:2753-2757.

36. **Sedgwick, S. G.** 1976. Misrepair of overlapping daughter-strand gaps as a possible mechanism for UV induced mutagenesis in *uvr* strains of *Escherichia coli*: a general model for induced mutagenesis by misrepair (SOS repair) of closely spaced DNA lesions. Mutat. Res. **41:** 185–200.

37. **Smith, K. C.** 1976. Ultraviolet radiation-induced mutability of *uvrD3* strains of *Escherichia coli* B/r and K-12: a problem in analyzing mutagenesis data. Photochem. Photobiol. **24:**433–437.

38. **Van der Schueren, E., D. A. Youngs, and K. C. Smith.** 1974. Sensitization of ultraviolet-irradiated *Escherichia coli* K-12 by different agars: inhibition of a *rec* and *exr* gene-dependent branch of the *uvr* gene-dependent excision-repair process. Photochem. Photobiol. **20:**9–13.

39. **Witkin, E. M.** 1959. Post-irradiation metabolism and the timing of ultraviolet-induced mutations in bacteria, p. 280–299. *In* Proceedings of the X international congress on genetics, vol. 1. University of Toronto Press, Toronto.

40. **Witkin, E. M.** 1966. Radiation-induced mutations and their repair. Science. **152:**1345–1353.

41. **Witkin, E. M.** 1974. Thermal enhancement of ultraviolet mutability in a *tif-1 uvrA* derivative of *Escherichia coli* B/r: evidence that ultraviolet mutagenesis depends upon an inducible function. Proc. Natl. Acad. Sci. U.S.A. **71:**1930–1934.

42. **Witkin, E. M.** 1975. Persistence and decay of thermoinducible error-prone repair activity in nonfilamentous derivatives of *tif-1 Escherichia coli* B/r: the timing of some critical events in ultraviolet mutagenesis. Mol. Gen. Genet. **142:**87–103.

43. **Witkin, E. M.** 1976. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. Bacteriol. Rev. **40:** 869–907.

44. **Youngs, D. A., and K. C. Smith.** 1973. Evidence for the control by *exrA* and *polA* genes of two branches of the *uvr* gene-dependent excision repair pathway in *Escherichia coli* K-12. J. Bacteriol. **116:**175–182.

45. **Youngs, D. A., and K. C. Smith.** 1976. Genetic control of multiple pathways of post-replicational repair in *uvrB* strains of *Escherichia coli* K-12. J. Bacteriol. **125:** 102–110.