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Ultraviolet-Sensitive Mutator Strain of *Escherichia coli* K-12

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An ultraviolet (UV)-sensitive mutator gene, *mutU*, was identified in *Escherichia coli* K-12. The mutation *mutU4* is very close to *uvrD*, between *metE* and *ilv*, on the *E. coli* chromosome. It was recessive as a mutator and as a UV-sensitive mutation. The frequency of reversion of *trpA46* on an F episome was increased by *mutU4* on the chromosome. The mutator gene did not increase mutation frequencies in virulent phages or in lytically grown phage λ. The *mutU4* mutation predominantly induced transitional base changes. Mutator strains were normal for recombination and host-cell reactivation of UV-irradiated phage T1. They were normally resistant to methyl methanesulfonate and were slightly more sensitive to gamma irradiation than Mut$^+$ strains. UV irradiation induced mutations in a *mutU4* strain, and phage λ was UV-inducible. Double mutants containing *mutU4* and *recA*, *B*, or *C* were extremely sensitive to UV irradiation; a *mutU4 uvrA6* double mutant was only slightly more sensitive than a *uvrA6* strain. The *mutU4 uvrA6* and *mutU4 recA*, *B*, or *C* double mutants had mutation rates similar to that of a *mutU4* strain. Two UV-sensitive mutators, *mut-9* and *mut-10*, isolated by Liberfarb and Bryson in *E. coli* B/UV, were found to be co-transducible with *ilv* in the same general region as *mutU4*.

Mutator genes are mutant genes that produce a generalized increase in mutation rates. Mutators have been found in many species of bacteria and in bacteriophage T4 (listed in 31). Only in the case of phage T4 has the protein product of a mutator gene been identified. The T4 mutators resulted from temperature-sensitive mutations of gene 43 which codes for deoxyribonucleic acid (DNA) polymerase (46). Other temperature-sensitive mutations in gene 43 had an antimutator effect (19). In bacteria, Treffers' mutator (*mutT1*) of *Escherichia coli* has been most extensively studied. Yanofsky, Cox, and Horn (56) and Cox and Yanofsky (13) determined that *mutT1* exclusively induced the transversional base change adenine-thymine → cytosine-guanine. Cox (12) showed that *mutT1* could mutate phage λ only if the phage genome had undergone replication. Siegel and Bryson (41), Breckenridge and Gorini (8), and E. C. Cox (*personal communication*) have found that a second *E. coli* mutator, *mutS3* (referred to as *mutS1* in earlier publications), induced transitions. Additional *E. coli* mutators exist but have not yet been characterized (23, 24, 31).

The wild-type allele of what is recognized as a mutator may function in DNA replication or repair, or both. An involvement in repair has been suggested for ultraviolet (UV)-sensitive mutators found in *Proteus mirabilis* (6), *Neisseria meningitidis* (28), and *E. coli* (31, 43). In *N. meningitidis*, it was shown that UV sensitivity and mutator were due to a single mutation. This report describes the identification of a UV-sensitive mutator in *E. coli*, its genetic analysis, and its characterization as a UV-sensitive mutation and as a mutator.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. Table 1 lists the bacterial strains used and their sources. In Table 1 and in the text, the most important characteristics of a strain are often given in parentheses after the strain designation. Table 2 lists phages and their sources.

Media. Minimal medium A was that of Davis and Mingioli (16) plus 1 μg of thiamine hydrochloride and 0.5 μg of ferrous sulfate per ml. When necessary, amino acids, thymine, and adenine were used at a final concentration of 50 μg/ml. Nutrient broth contained 0.8% (w/v) nutrient broth powder (Difco), 0.1% yeast extract (Difco), and 0.5% sodium chloride. Nutrient and minimal agar were made by adding 20 g of agar (Difco) per liter to the liquid media. L broth and agar (30) were adjusted to *p*H 7.0. L medium contained 2.5×10^{-3} M $CaCl_2$ when Ca^{2+} was

TABLE 1. *Bacterial strains*[a]

Strain	Sex	Description[b]	Source and/or[c] reference, comments
Derivatives of *E. coli* K-12			
CR63	?	λ^-	(3), Used to assay λ *h* mutants
K-12S(λ)	?	λ^+ *ton trp*	F. Bautz, used to assay *rII*$^+$ revertants
K37	Hfr	*supD*	D. Pratt (38), used to assay *am* mutants of M13
K38	Hfr	*supD*$^+$	D. Pratt (38), used to assay *am*$^+$ revertants of M13
N145	F$^-$	*trp gal metE11 strA uvrD3*	H. Ogawa (36)
N14-4	F$^-$	*trp gal strA uvrD3*	H. Ogawa (36)
JC12	Hfr	*thi met purC1* λ ; direction of transfer, O . . . *argG* . . . *str*	A. J. Clark
JC355	F$^-$	*argG6 his-1 leu-6 metB1 gal-6 malA1 lac sup-48? tonA* λ^-	J. G. Flaks
JC5088	Hfr	*thr-300 ilv-318 spc-300 recA56* λ^-; direction of transfer, O . . . *lysG* . . . *his*	A. J. Clark
JC5426	Hfr	*thr-300 ilv-318 spc-300 recC22* λ^-; direction of transfer, O . . . *lysG* . . . *his*	A. J. Clark
AT715	Hfr (H type)	*rbs-1 thi-1* λ^-	A. L. Taylor
AT2427	Hfr (H type)	*thi-12 cysC39* λ^-	A. L. Taylor
AB1203	F$^-$	*lac thi-1 ilvC7 argE3 xyl-5*	B. J. Bachmann
AB1206	F′	*thi-1 str-8 his-4 galK2 lacY1 proA2*; F14 carries and transfers O . . . *argE*$^+$ . . . *argH*$^+$ . . . *metE*$^+$ . . . *ilv*$^+$	B. J. Bachmann
AB2070	F$^-$	*thi ilvE12 metE46 his-4 trp-3 proA2 str sup-38*	B. J. Bachmann
AB2470	F$^-$	*recB21 proA2 leu-8 thr-4 thi argE3 his-4 str*	P. Howard-Flanders
AB2500	F$^-$	*uvrA6 thy*, otherwise as AB2470 but *recB*$^+$	P. Howard-Flanders
AB2533	F$^-$	*ilvD188 gal-6 purC1 lac thy-3 sup-19*	B. J. Bachmann
AB2545	Hfr (H type)	*metE46 thi-12* λ^-	B. J. Bachmann
AB3505	F$^-$	*ilvD188 metE46 argH1 his-4 trp-3 proA2*	B. J. Bachmann
P3478	F$^-$	*polA1 thy*	J. Cairns (17)
236	F′	F-*cysB*$^+$ *trpA46/trpA46 his pro cysB*	R. L. Somerville
240	F$^-$	*tonB-trpA, B* Δ	R. L. Somerville
RV F′Lac	F′	F-*lac*$^+$/lacΔX74	M. Malamy
ES108	F$^-$	As JC355 but *mutU4*	NTG induced
ES230	F$^-$	As ES108 but UV^r (*mut*$^+$)	Spont. revertant
ES232	F$^-$	As AB3505 but Met$^+$ *mutU4*	ES108 $\times$ AB3505 transd.
ES233	F$^-$	As JC355 but Leu$^+$ Ton$^+$ (*mutU*$^+$)	AT2427 $\times$ JC355 conj.
ES234	F$^-$	As ES108 but Leu$^+$ Ton$^+$ (*mutU4*)	AT2427 $\times$ ES108 conj.
ES242	Hfr	As AB2545 but Met$^+$ (*mutU*$^+$)	ES232 $\times$ AB2545 transd.
ES243	Hfr	As AB2545 but Met$^+$ *mutU4*	ES232 $\times$ AB2545 transd.
ES244	Hfr	As AT715 but Rbs$^+$ *ilvD188* (*mutU*$^+$)	ES232 $\times$ AT715 transd.
ES245	Hfr	As AT715 but Rbs$^+$ *ilvD188 mutU4*	ES232 $\times$ AT715 transd.

[a] Symbols: +, wild type or, in case of phage λ, lysogenic; −, in case of λ, in case of λ, nonlysogenic; Δ, deletion; Δ, O, origin of transfer; ^{s}, sensitivity; ^{r}, resistance; UV, ultraviolet irradiation; T2, phage T2; T7, phage T7. All other symbols as given by Taylor (49).

[b] Genotypic descriptions are not complete. In some cases fermentation markers and phage sensitivities were omitted. For certain strains the most pertinent characteristics are in parentheses.

[c] Abbreviations: conj., conjugation; spont., spontaneous; transd., transduction; EMS, ethyl methanesulfonate; NTG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. Donor strain listed first in crosses.

[d] The allele number *mutS3* has been assigned by the Coli Genetic Stock Center to the lesion previously designated *mutS1*.

TABLE 1—*Continued*

Strain	Sex	Description[b]	Source and/or[c] reference, comments
ES246	F⁻	As AB2070 but Ilv⁺ (*mutU*⁺)	ES108 × AB2070 transd.
ES247	F⁻	As AB2070 but Ilv⁺ *mutU4*	ES108 × AB2070 transd.
ES250	F⁻	*recA56 thi ilvE12 metE46 trp-3 proA2*	JC5088 × AB2070 conj.
ES258	Hfr	As ES244 but λ⁺ (*mutU*⁺)	λ from K-12S(λ)
ES271	F⁻	As AB2533 but Ilv⁺ *mutU4* (*mutU4 thy-3*)	ES108 × AB2533 transd.
ES272	F⁻	As AB2533 but Ilv⁺ (*mutU*⁺ *thy-3*)	ES108 × AB2533 transd.
ES275	F⁻	*metE46 mutU4 thi* Str^r *ton*	AT2427 × ES247 conj.
ES278	Hfr	As ES245 but UV^r (Mut⁺)	Spont. revertant
ES282	F⁻	As ES271 but UV^r (Mut⁺)	Spont. revertant
ES283	Hfr	As ES245 but λ⁺ (*mutU4*)	λ from K-12S(λ)
ES286	F⁻	As ES282 but Thy⁺ *recA56* (Mut⁺ *recA56*)	JC5088 × ES282 conj.
ES287	F⁻	As ES271 but Thy⁺ *recA56* (*mutU4 recA56*)	JC5088 × ES271 conj.
ES296	F⁻	As ES275 but *trp* (*mutU4*)	Spont.
ES298	F⁻	As ES271 but Thy⁺ *recB21* (*recB21 mutU4*)	AB2470 × ES271 transd.
ES299	F⁻	As ES271 but Thy⁺ (*mutU4 recB*⁺)	AB2470 × ES271 transd.
ES300	F⁻	As ES271 but Thy⁺ *recC22* (*recC22 mutU4*)	JC5426 × ES271 transd.
ES338	F⁻	As ES282 but Thy⁺ *recB21* (*Mut*⁺ *recB21*)	AB2470 × ES282 transd.
ES343	F⁻	*metE46 mutU4* Ton⁺ *azi*	AT2427 Azi^r × ES296 conj.
ES345	Hfr	As ES243 but *malB* (*mutU4*)	*malB* induced with NTG
ES351	Hfr	As ES345 but Mal⁺ *uvrA6* (*uvrA6 mutU4*)	AB2500 × ES345 transd.
ES352	F⁻	As ES345 but Mal⁺ (*mutU4 uvrA*⁺)	AB2500 × ES345 transd.
ES374	F⁻	As ES234 but *strA130* (*mutU4*)	Spont. mutation
ES381	F′	As ES374 but F-*lac*⁺/*lac* (*mutU4*)	RV F′ Lac × ES374 conj.
ES382	F′	As ES381 but UV^r (Mut⁺)	Spont. revertant
ES411	Hfr	Mut⁺ λ *cI857*⁺	Lysogenization of ES242
ES412	Hfr	*mutU4* λ *cI857*⁺	Lysogenization of ES243
ES430	F⁻	As ES345 but UV^r (Mut⁺)	Spont. reverant
ES434	F⁻	As ES430 but Mal⁺ *uvrA6* (Mut⁺)	AB2500 × ES430 transd.
ES440	F⁻	As AB3505 but Met⁺ *mutU4*	ES108 × AB3505 transd.
ES441	F⁻	His⁺ Trp⁺ *mutU4 recA56 proA2 metE46*	JC5088 × ES247 conj.
ES474	F⁻	As AB1203 but *tonB-trp* Δ	Spont. mutation to phage T1^r
ES475	F′	F-*cysB*⁺ *trpA46/tonB-trp*Δ *argE3 ilvC7*	236 × ES474 conj.
ES480	F′	As ES475 but Ilv⁺ (*trpA46/tonB-trp*Δ *mutU*⁺)	ES108 × ES475 transd.
ES481	F′	As ES475 but Ilv⁺ *mutU4* (*trpA46/tonB-trp*Δ *mutU4*)	ES108 × ES475 transd.
Derivatives of *E. coli* B			
E. coli B	F⁻	*malB* UV^s	E. Witkin
E. coli B/r	F⁻	*malB* UV^r	E. Witkin
L44	F⁻	*argF40 argR11 leu-2*	L. Gorini (8)
L44-6	F⁻	As L44 but *strA60*	L. Gorini (8)
L44-4	F⁻	As L44 but *strA40*	L. Gorini (8)
L44-2	F⁻	As L44 but *strA2*	L. Gorini (8)
L44-1	F⁻	As L44 but *strA1*	L. Gorini (8)
EMS107	F⁻	*mut-9 malB*	R. Liberfarb (31)
EMS195	F⁻	*mut-10 malB uvr*?	R. Liberfarb (31)
ES186	F⁻	As *E. coli* B but T2^r	Spont. mutation
ES217	F⁻	*tonB-trp*Δ *mutS3*[d]	Spont. mutation to T1^r
ES372	F⁻	As *E. coli* B but T7^r	Spont. mutation

TABLE 1—*Continued*

Strain	Sex	Description[b]	Source and/or[c] reference, comments
ES377	F⁻	As L44 but *ilv-291*	*ilv* induced with EMS
ES379	F⁻	As L44 but Ilv⁺ *mutU4*	ES108 × ES377 transd.
ES393	F⁻	As EMS107 but *ilv-292*	*ilv* induced with EMS
ES400	F⁻	*mut-10* Mal⁺	JC12 × EMS195 conj.
Derivatives of *E. coli* C			
E. coli C	F⁻	*rbs*	R. Sinsheimer
C1412	F⁻	*metE4 ilv-4 his rha rbs*	R. Calendar (10)
C1415	F⁻	*rha his rep-3 rbs*	R. Calendar (10)
ES266	F⁻	Rbs⁺ *mutU4*	ES108 × *E. coli* C transd.
ES273	F⁻	As C1412 but Ilv⁺ *mutU4*	ES108 × C1412 transd.
ES274	F⁻	As *E. coli* C but *strA131*	Spont. mutation
ES337	F⁻	As C1412 but Ilv⁺ *uvrD3*	N145 × C1412 transd.
ES354	F⁻	As C1412 but Ilv⁺ *rep-3*	C1415 × C1412 transd.
ES375	F⁻	As C1412 but Met⁺ *uvrD3*	N14-4 × C1412 transd.

TABLE 2. *Bacteriophages used*

Phage	Source
T1	—[a]
T1 *am11*	H. Drexler
T2L	—
T4 *rII* AP156	F. Bautz
T4 *rII* AP72	F. Bautz
T4 *ac41*[b]	E. Goldberg
T6	—
T7	—
T7 gene 12 *am3*	F. Studier
T7 gene 5 *am28*	F. Studier
T7 gene 19 *am10*	F. Studier
T7 gene 6 *am147*	F. Studier
φX174	R. Sinsheimer
M13 *am3-H4*	D. Pratt
M13 *am5-H3*	D. Pratt
M13 *ts1-N1*	D. Pratt
R17	M. Malamy
λ vir	M. Malamy
λ *cI857*	M. Malamy
λ *b2 red-7 cI857*	M. Malamy
λ[c]	Induced from K-12S(λ)
λ *cI857 spi-2*	R. Calendar
P1bt	R. Liberfarb
P1kc	—

[a] Where no source is given, the phage was from the collection of this laboratory.

[b] Used as wild-type phage T4.

[c] Used as wild-type phage λ.

required for phage adsorption. Antibiotic medium no. 3 (Difco), called Penassay (PA) broth, was also used.

Minimal medium A without glucose, thiamine, or ferrous sulfate was used as a diluent for cells. Phage were diluted in L broth or in the cell diluent containing 5% (v/v) L broth.

Culture conditions. Unless otherwise noted, all incubations were at 37 C, and cultures were aerated by shaking. Stationary-phase cultures were overnight cultures, usually grown for 17 hr.

Mutation rates. Mutation rates were determined by a fluctuation test as described by Siegel and Bryson (41).

UV irradiation, UV survival, and UV-induced reversion. The UV source was two General Electric 15-w germicidal lamps with all but 13 cm of their length blocked off. The dose rate was approximately 4 ergs per mm² per sec as determined by a UV dose meter similar to that described by Jagger (27). UV irradiation and subsequent manipulations were done in dim yellow light. For UV-survival determinations, cultures were grown overnight in nutrient broth; they were then diluted 1,000-fold in diluent, and a 5-ml sample was irradiated with constant stirring. Diluted samples were plated on nutrient agar and incubated overnight. The UV sensitivity of recombinants was determined by a rapid UV test; the cells to be tested were grown overnight in 2 ml of PA broth and streaked on nutrient agar. The plate was irradiated for 38.5 sec, incubated for 3 hr, and re-irradiated for 77 sec. After overnight incubation, resistant cultures gave a confluent streak and sensitive cultures showed a few surviving colonies. To distinguish *rec mutU4* double mutants, a similar test with single doses of 5, 10, 25, and 75 sec was used. The double mutant gave isolated colonies instead of a confluent streak at 5 and 10 sec.

UV-induced reversion of *ilvD188* was determined in strains ES244 (*mutU*⁺) and ES245 (*mutU4*). They were grown overnight in 2 ml of PA broth, 8 ml of diluent was added to the culture, and after centrifugation the cells were resuspended in 10 ml of diluent. A 5-ml amount of this suspension was irradiated as described above. Survival was determined by plating samples on nutrient agar, and Ilv⁺ revertants were selected by plating 0.1-ml undiluted samples on minimal agar containing 50 mg of nutrient broth powder per liter. The enriched minimal plates were incubated for 3 days.

Gamma irradiation. A cesium-137 source with a dose rate of 520 rads/min was used. An overnight nutrient broth culture that had been at 4 C for 5 hr was diluted 1,000-fold in diluent, and 1.3 ml was placed in a flint glass vial (13 by 45 mm) with a plastic

top. Irradiation was done at 27 C, and samples were plated on nutrient agar.

MMS survival. Cultures were grown in PA broth to ca. 2×10^8 cells/ml. The culture was diluted fourfold in diluent, and 0.1 ml was added to 2.5 ml of a solution of 0.06 ml of methyl methanesulfonate (MMS; Eastman Organic Chemicals, Rochester, N.Y.) plus 10.9 ml of diluent. Incubation was at 37 C without shaking. Samples were taken into diluent and plated on nutrient agar.

Large numbers of recombinants were tested for MMS sensitivity by streaking a loopful of a stationary-phase PA broth culture on nutrient agar containing 0.05% MMS. The plates were used within 18 hr of being made. Streaked plates were incubated for 18 hr at 37 C and an additional 24 hr at 25 C.

Thymine starvation. The procedure of Cummings and Mondale (15) was used.

Phage procedures. The methods of growing and assaying phage were those described by Adams (1). Wild-type T phages were grown and assayed on *E. coli* B. When T phages were assayed for host-range mutants, strains ES186, ES217, and ES372 were used. Phage T1 *am11* was assayed on the permissive strain ES232, and am^+ revertants were assayed on *E. coli* B. Phage T4 *rII* mutants were assayed on *E. coli* B and revertants were detected on strain K-12 S(λ) as described by Benzer (5). Amber mutants of phage T7 were assayed on strain ES233 and revertants were assayed on *E. coli* B. Either L medium or nutrient agar with a soft (0.7%) nutrient agar overlay was used to assay the T phages.

Phage φX174 was grown on *E. coli* C according to Sinsheimer (42) except that L medium plus $CaCl_2$ was used. The *rep-3* marker (inability to support growth of φX174; 10) was detected by streaking ca. 10^5 plaque-forming units (PFU) of φX174 in a line on L agar and then cross-streaking an overnight PA broth culture of the strain to be tested.

M13 amber mutants were grown at 37 C in L broth cultures. Assays were done on strains K37 and K38 at 31 C in L medium. The M13 temperature-sensitive mutant *ts1-N1* was grown at 31 C and assayed on strain K38 at 31 and 42 C.

P1kc and P1bt were grown, assayed, and used for transduction as described (41). A multiplicity of infection of 0.5 was generally used in transduction. P2 was grown and assayed with the methods used for P1.

Phage λ was assayed for total PFU on *E. coli* C or its derivative ES274. Host-range, *h*, mutants were assayed on strains CR63 (3). Incubation of λ vir and wild-type λ assays was at 30 C and of λ *cI857* assays at 42 C. The λ lysogens were induced with UV irradiation by growing them to a titer of ca. 7×10^7 cells/ml in minimal medium plus Difco Casamino Acids (2.5 mg/ml). The 9-ml cultures were then irradiated with UV and incubation was continued. A 25-sec dose was used to induce the mutator strain and a 50-sec dose was used for the nonmutator strain. The absorbance was followed, and samples were taken into phage diluent plus chloroform to determine PFU per milliliter. The same procedure was used for mitomycin C induction except that mitomycin C (Sigma Chemical Co., St. Louis, Mo.) was added to give a concentration of 4 μg/ml when the cell density was ca. 7×10^7 cells/ml.

Host-cell reactivation (Hcr) of phage was determined by diluting phage T1 or λ vir in phage diluent and UV-irradiating the suspension. For T1, irradiated samples were assayed by plating with 0.1 ml of a log-phase nutrient broth culture. Irradiated λ vir was assayed on cultures that had been grown in L broth plus 0.3% maltose to ca. 4×10^8 cells/ml, centrifuged, and resuspended in one-half volume 0.01 M $MgSO_4$ and shaken for 1 hr. A rapid Hcr test was devised to distinguish *uvrD3* (Hcr^-) from *mutU4* (Hcr^+) strains. Phage T1 was diluted to ca. 2×10^9 PFU/ml and irradiated for 400 sec. About 10^{-3} ml of the irradiated phage was streaked on nutrient agar and then cross-streaked with ca. 10^{-3} ml of an overnight PA broth culture. After 18 hr of incubation, Hcr^+ streaks showed a clear zone of inhibition where the phage streak crossed; Hcr^- streaks were completely confluent or showed only a slight indication of phage growth. Known Hcr^+ and Hcr^- strains were used as standards.

Phenotypic suppression by antibiotics. As shown by Breckenridge and Gorini (8), the patterns of suppression of auxotrophic amber mutations in streptomycin-resistant mutants can indicate the types of base changes that resulted in the mutations to streptomycin resistance. This method was used to determine whether *mutU4* induced transitions or transversions. Strain ES379 (*mutU4*) colonies (ES379 is derived from strain L44, the original strain used by Breckenridge and Gorini) were transferred onto nutrient agar plates which were incubated for 32 hr. These plates were then replica-plated onto nutrient agar plus streptomycin (500 μg/ml) to obtain independent, streptomycin-resistant mutants. After 2 and 5 days of incubation, streptomycin-resistant spots of growth (never more than one per original colony) were picked and purified on nutrient agar plus streptomycin. The purified colonies were transferred to nutrient agar (no streptomycin) and, after incubation, were replica-plated onto nutrient agar and nutrient agar plus streptomycin to distinguish streptomycin resistance from streptomycin dependence. Streptomycin-dependent colonies were able to grow when transferred to nutrient agar from nutrient agar plus streptomycin but not when replica-plated from streptomycin-free medium onto streptomycin-free medium. All streptomycin-dependent colonies were discarded. The patterns of phenotypic suppression of the mutations *argF40* and *leu-2* in each streptomycin-resistant mutant were determined by use of cultures that had been grown for 18 hr in 2 ml of PA broth. A 5-ml amount of diluent was added to the cultures, which were then centrifuged at $3,000 \times g$ for 10 min and resuspended in 5 ml of diluent. A loopful of each washed culture was streaked onto minimal media, selective for Leu^+ or Arg^+ growth, and containing bluensomycin, streptomycin, or no antibiotic. The concentrations of amino acids and antibiotics were those used by Breckenridge and Gorini (8). For each set of determinations, cultures of strains L44-1, L44-2, L44-4, and L44-6, representatives of the four patterns of suppression, were used as standards. The plates were scored after 18 hr at 37 C and an additional 24 hr at 25 C. If a mutation did not fit into

one of the four patterns, it was backcrossed into strain L44 by P1 transduction and retested.

Isolation of mutator strains. A nutrient broth, late log-phase culture of strain JC355 was treated with 100 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) per ml (Aldrich Chemical Co., Milwaukee, Wis.) for 20 min by the procedure of Adelberg, Mandel, and Chen (2). Survivors were plated on nutrient agar and incubated overnight. These plates were then replica-plated onto nutrient agar plus streptomycin (200 μg/ml). The streptomycin plates were incubated for 3 days. Those colonies on the master plates which had given some growth on streptomycin were purified and grown overnight in nutrient broth; the mutation frequency of streptomycin resistance was then determined.

Genetic procedures. The methods of transduction and uninterrupted matings were previously described (41). All recombinants and all mutants or revertants were purified by single colony isolation. Nutritional and fermentation markers were scored by replica-plating onto the appropriate media.

Recombinants to be tested for the mutator phenotype (Mut$^-$) were grown overnight in PA broth, and 0.1 ml was plated on nutrient agar plus 200 μg of streptomycin or 50 μg of spectinomycin per ml. The plates were incubated for 2 days. Mutator cultures yielded about 10 to 60 resistant colonies per plate; nonmutators, about 1 colony per five plates.

RESULTS

Isolation of the UV-sensitive mutator strain. *E. coli* strain JC355 was treated with the mutagen NTG and the survivors were tested for the mutator phenotype. Of ca. 5,000 colonies tested, 4 were Mut$^-$. One of the four, strain ES108, was UV-sensitive by the rapid UV test. Twenty independent UV-resistant revertants of strain ES108 and, in later experiments, of UV-sensitive mutator strains with different genetic backgrounds were selected. All were Mut$^+$ as determined by the frequency of mutation to streptomycin resistance. This suggested that UV sensitivity and mutator were due to one mutation. The genotypic symbol *mutU4* will be used for this mutator. Figure 1 gives the UV survival curves of strain ES108 and its parent strain JC355. Some representative mutation frequencies for *mutU4* and Mut$^+$ strains are given in Table 3.

The *mutU4* derivatives were not temperature-sensitive. They grew as well as Mut$^+$ strains at 30, 37, and 42 C, as judged by the increase in absorbance, and they formed colonies with equal efficiency at these temperatures on nutrient agar. Mutation frequencies for *mutU4* strains were similar at these three temperatures (data not given).

Genetic location of mutU4. Genetic analysis of *mutU4* not only should give its position on the *E. coli* chromosome but also should give additional evidence that UV sensitivity and the mutator phenotype are due to one mutation and are not separable by recombination. The Hfr strain JC12 was mated with strain ES108 for 90 min, and Arg$^+$ Pur$^+$ recombinants were selected. Of the recombinants, 54% were nonmutator. All nonmutator recombinants were UV-resistant; mutator and UV sensitivity were not separable. This cross placed *mutU4* in the region represented by 70 to 80 min on the map of Taylor (49). P1kc-mediated transduction was used to determine a more precise location. The donor was strain ES108 and the recipients strains were AB1203 (*ilvC7*) and AB2545 (*metE46*). Among the recombinants, 34% of the Ilv$^+$ and 63% of the MetE$^+$ recombinants were of the Mut$^-$ phenotype. This indicated that *mutU4* was located near *ilv* and *metE*, closer to *metE*. A total of 130 recombinants from these two transductions were tested for the mutator phenotype and UV sensitivity. The two traits were not separable in any of the transductants. This has been confirmed for several hundred additional transductants in various crosses. In most of the subsequent work with *mutU4*, the marker was scored by its UV sensitivity.

The order of *mutU4*, *ilv*, and *metE* was determined by means of a three-point transduction with strain ES108 as the donor and strain AB3505 (*ilvD188metE46*) as the recipient. The results, given in Table 4, indicated that the order was *ilv mutU4 metE* on the basis of the phenotype of the least frequent or quadruple crossover class. The location of *mutU4* between *metE* and *ilv* was confirmed in the accompanying paper (40) by transducing *mutU4* into an *ilv metE* recipient and selecting Ilv$^+$ Met$^+$ transductants; 87% had received *mutU4*. Also located between *ilv* and *metE* are the loci *uvrD*, affecting UV repair (35, 36), and *rep-3*, which inhibits the replication of phage φX174 and has a small effect on UV repair (10). An attempt was made to determine the order of *rep-3*, *uvrD*, and *mutU4*.

By the rapid UV test, *uvrD3* and *mutU4* strains were equally UV-sensitive. The two mutations could be distinguished by the mutator test, by the rapid test for Hcr (*uvrD3* is Hcr$^-$ [36]), and by the increased sensitivity of a *uvrD3* strain to MMS. Data on the wild-type level of MMS resistance and the Hcr$^+$ character of *mutU4* will be given later. The order of *uvrD3*, *mutU4*, and *metE46* was determined by a three-point transduction with strain N14-4 (*uvrD3*) as the donor and strain ES343 (*mutU4 metE46*) as the recipient (Table 5). All but three

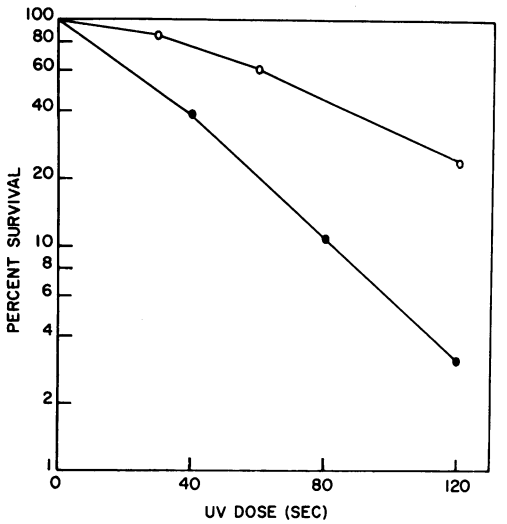

FIG. 1. *UV survival of strain ES108 (mutU4)* (●) *and its Mut⁺ parent strain JC355* (○).

TABLE 3. *Comparison of mutation frequencies in mutU4 and Mut⁺ strains*

Mutation	Strain[a]	Frequency (mutants/cell)	Mutator/nonmutator
Phage T7[s] → T7[r]	ES108 (Mut⁻)	7.3×10^{-5}	24
	ES230 (Mut⁺)	3.0×10^{-6}	
Leu⁻ → Leu⁺	ES108 (Mut⁻)	1.1×10^{-7}	14
	JC355 (Mut⁺)	7.7×10^{-9}	
Str[s] → Str[r]	ES108 (Mut⁻)	9.5×10^{-8}	>12
	JC355 (Mut⁺)	$< 7.7 \times 10^{-9}$	
Ilv⁻ → Ilv⁺	ES245 (Mut⁻)	5.4×10^{-6}	160
	ES244 (Mut⁺)	3.4×10^{-8}	
Phage T4[s] → T4[r]	ES440 (Mut⁻)	3.5×10^{-5}	32
	AB3505 (Mut⁺)	1.1×10^{-6}	

[a] Phenotype with respect to mutator in parentheses.

transductants were either MMS-sensitive, Hcr⁻, and nonmutator (*uvrD3 mutU⁺*), or MMS-resistant, Hcr⁺, and mutator (*uvrD⁺ mutU4*). The three aberrant transductants were MMS-resistant, Hcr⁻, and mutator. The "Hcr⁻" phenotype resulted from the lysogenization of these three transductants by transducing phage P1kc. A P1kc lysogen will restrict phage T1 (29) and thus mimic an Hcr⁻ reaction. These three transductants were *uvrD⁺ mutU4*. In additional transductions with the same donor and recipient, 280 transductants were tested (data not shown); all were UV-sensitive, as was found for the cross in Table 5. A second transduction utilized the Met⁺ *mutU4* donor ES266 and a *metE4 uvrD3* recipient, ES337. Met⁺ transductants were selected. Phage T1 grown on a P1kc lysogen was used for the Hcr test to prevent restriction. The results were similar to those of Table 5; no UV-resistant transductants or transductants having the combined phenotypes of *mutU4* and *uvrD3* (Mut⁻, Hcr⁻, MMS-sensitive, and UV-sensitive) were found among the 78 transductants tested. Among the transductants, 44 were *mutU4 uvrD⁺* and the remaining 34 were *mutU⁺ uvrD3*. The absence of any UV-resistant transductants in this series of crosses indicated that *mutU4* and *uvrD3* were very closely linked. The failure to find the double mutant class (*uvrD3 mutU4*) will be commented on in the Discussion.

TABLE 4. *Analysis of the three-point transduction involving the ilvD188, mutU4, and metE46 markers*

Donor: ES108 (*metE⁺ mutU4 ilvD⁺*). Recipient: AB3505 (*ilvD188 mutU⁺ metE46*). Met⁺ transductants selected.

Marker[a]			No. of transductants[b]
ilvD188	*mutU4*	*metE46*	
1	1	1	41
0	1	1	24
0	0	1	25
1	0	1	9

[a] Symbols: 1, donor; 0, recipient phenotype.
[b] The results indicated that the gene order was *ilvD188 mutU4 metE46*.

TABLE 5. *Analysis of the three-point transduction involving mutU4, uvrD3, and metE*

Donor: N14-4(*uvrD3 metE⁺*). Recipient: ES343 (*mutU4 metE46*). *Met⁺* transductants selected.

Marker[a]			No. of transductants
mutU4	*uvrD3*	*metE46*	
1	1	1	40
0	1	1	0[b]
0	0	1	31
1	0	1	0

[a] All transductants were tested with the rapid UV test. The *uvrD3* mutation was distinguished from *mutU4* by the Hcr test, frequency of Str[r] mutants, and sensitivity to methyl methanesulfonate (MMS). Symbols: 1, donor; 0, recipient phenotype.
[b] Phenotype: Hcr⁻ Mut⁻ UV[s] MMS[s].

The *rep-3* marker was easily distinguished from *mutU4* and *uvrD3*. It was UV-resistant by the rapid UV test and did not yield plaques with ϕX174 or P2 phages (10). Both *uvrD3* and *mutU4* strains yielded plaques with ϕX174 and P2 phages with the same efficiency as wild-type strains. As *E. coli* K-12 does not adsorb ϕX174, the *uvrD3 E. coli* C strain ES337 was used to determine the order of *rep-3* and *uvrD3*. A three-point transduction was done with strain C1415 (*rep-3*) as donor and strain ES337 (*uvrD3 metE4*) as recipient. The results (Table 6) favored the order *rep-3 uvrD3 metE4*. However, the quadruple crossover class was not found. This class was the double mutant *rep-3 uvrD3*. Its absence could reflect the closeness of the two loci or inviability of the *rep-3 uvrD3* combination. The three-point transduction was repeated in such a way as to make the quadruple crossover class wild type (Rep$^+$ UvrD$^+$); the donor was strain C1415 (*rep-3*) and the recipient was strain ES375 (*ilv-4 uvrD3*). Ilv$^+$ transductants were selected and scored for *rep-3* and *uvrD3*. Of 86 transductants tested, none was in the quadruple crossover class (Rep$^+$ UvrD$^+$). This confirmed the order *rep-3 uvrD3 metE4*. Although *rep-3 uvrD3* recombinants were found in this last cross, they grew slowly and Rep$^+$ revertants formed at high frequencies. The *rep-3 uvrD3* double mutant appeared to have reduced viability.

Strain ES266 (*mutU4* Met$^+$) was crossed to strain ES354 (*rep-3 metE4*), and Met$^+$ transductants were selected to determine the order of *rep-3*, *mutU4*, and *metE*. The double mutant transductants (*rep-3 mutU4*) were even more unstable than the *rep-3 uvrD3* transductants had been. They grew slowly and reverted to either Rep$^+$ or Mut$^+$. The results of this transduction (Table 7) did not give a clearly distinguishable quadruple crossover class, possibly because of the reversion of the double mutants. The order assumed in Table 7 was based on the proximity of *mutU4* to *uvrD3* and the latter's position in respect to *rep-3* and *metE*.

TABLE 6. *Analysis of the three-point transduction involving rep-3, vurD3, and metE4*

Donor: C1415 (*rep-3 metE*$^+$). Recipient: ES337 (*uvrD3 metE4*). Met$^+$ transductants selected.

Marker[a]			No. of transductants[b]
rep-3	*uvrD3*	*metE4*	
1	1	1	124
0	1	1	35
0	0	1	46
1	0	1	0

[a] The *rep-3* mutation was scored by cross-streaking against ϕX174; *uvrD3* was scored by the rapid UV test. Symbols: 1, donor; 0, recipient phenotype.

[b] The results indicated that the gene order was *rep-3 uvrD3 metE4*.

TABLE 7. *Analysis of the three-point transduction involving rep-3, mutU4, and metE4*

Donor: ES366 (*mutU4 metE*$^+$). Recipient: ES354 (*rep-3 metE4*). Met$^+$ transductants selected.

Markers[a]			No. of transductants
rep-3	*mutU4*	*metE4*	
1	1	1	58
0	1	1	12
0	0	1	111
1	0	1	11

[a] The *rep-3* mutation was scored as in Table 6; *mutU4* was scored by the rapid UV test. Symbols: 1, donor; 0, recipient phenotype.

Knowledge of the genetic location of *mutU4* made it possible to determine whether the UV-resistant revertants of *mutU4* were due to external suppressors. Phage Plkc was grown on three independent UV-resistant revertants of strain ES108 and one revertant of strain ES271, designated ES282, and was used to transduce strain AB2545 to MetE$^+$. One hundred transductants from each of the four crosses were tested for UV sensitivity. None was UV-sensitive, indicating that the reversion to UV resistance occurred within the *mutU* cistron or in a very closely linked suppressor. One UV-resistant revertant, strain ES278, was compared in respect to UV survival with the isogenic wild-type strain ES244. The survival curves were similar (data not shown).

Characterization of mutU4 as a repair-deficient mutation. The genetic analyses previously discussed demonstrated that *mutU4* strains were obviously not recombination-deficient (Rec$^-$), since they could act as recipients in conjugation and transduction. For quantitative comparison, frequencies of recombination were determined with the Hfr strain AT2427 as a donor and isogenic Mut$^+$ and Mut$^-$ strains as recipients. An uninterrupted mating of 1 hr was made. With strains ES108 (*mutU4*) and ES230 (Mut$^+$) as the recipients, 3.0 and 3.7 Leu$^+$ Cys$^+$ recombinants per 10^2 males were found. With strains ES247 (*mutU4*) and ES246 (*mutU*$^+$), 7.0 and 9.7 Pro$^+$ Cys$^+$ recombinants per 10^2 males were found. The results showed no significant differences between mutator and nonmutator recipients.

The capacity of the host cell to reactivate UV-irradiated phage is an important criterion in characterizing a UV-sensitive mutant (20, 26,

36). Host-cell reactivation of UV-irradiated phages T1 and λ vir by *mutU4* and isogenic *mutU*$^+$ strains, and also by strain N145(Hcr$^-$), are shown in Fig. 2 and 3. The T1 survival curves were very similar for *mutU4* and *mutU*$^+$ strains (Fig. 2); *mutU4* was clearly Hcr$^+$ in respect to T1. With λ vir, increased survival was found on the *mutU*$^+$ strain, although the *mutU4* strain had much more Hcr capacity than the Hcr$^-$ strain N145 (Fig. 3).

Gamma irradiation survival curves for *mutU4* and *mutU*$^+$ strains (Fig. 4) showed that the *mutU4* strain was slightly more sensitive to gamma irradiation than was the *mutU*$^+$ strain. MMS alkylates DNA and also results in single-strand breaks (45, 48), as does ionizing irradiation (33). MMS-survival curves for a mutator and nonmutator pair and for a *polA1* strain, known to be very MMS-sensitive (17, 45), are shown in Fig. 5. The sensitivity of the mutator was identical to that of the nonmutator.

Prophage λ is not UV-inducible in *recA* strains (9), and is induced at a low frequency and after a long delay in *exrA* strains (18). An attempt was made to UV-induce the λ lysogenic strains ES259 (*mutU*$^+$) and ES283 (*mutU4*).

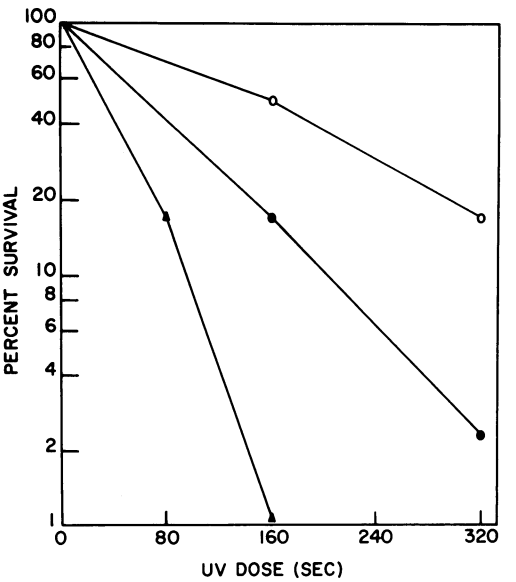

FIG. 3. *Host-cell reactivation of UV-irradiated phage λ vir plated with strains ES244 (mutU*$^+$*) (○), ES245 (mutU4) (●), and the Hcr$^-$ strain N145 (uvrD3) (▲).*

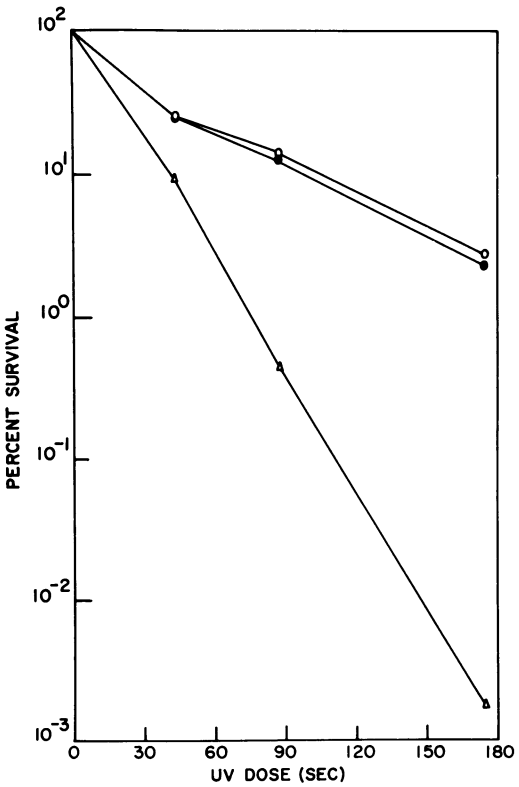

FIG. 2. *Host-cell reactivation of UV-irradiated phage T1 plated with strains ES233 (mutU*$^+$*) (○), ES234 (mutU4) (●), and the Hcr$^-$ strain N145 (uvrD3) (△).*

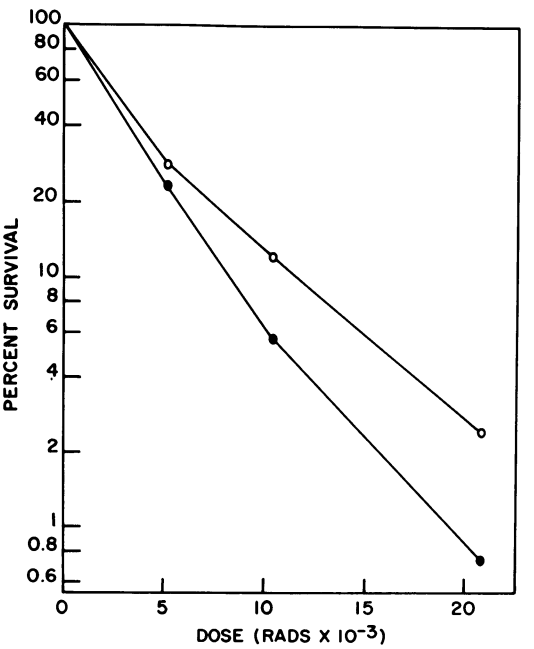

FIG. 4. *Gamma irradiation survival of strains ES246 (mutU*$^+$*) (○) and ES247 (mutU4) (●).*

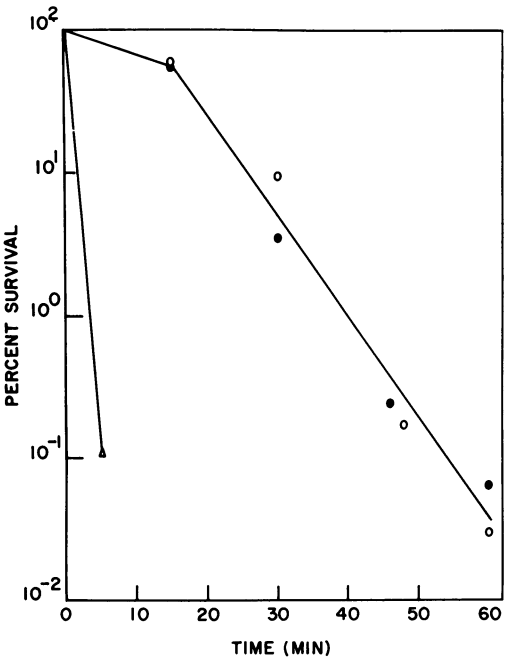

FIG. 5. *Survival in methyl methanesulfonate of strains ES242 (mutU+) (O), ES243 (mutU4) (●), and P3478 (polA1) (Δ).*

Both cultures lysed and the number of phage λ PFU per milliliter had increased more than 1,000-fold at 2.5 hr after irradiation. Similar results were found with mitomycin C induction.

Another characteristic shared by *exrA* and *recA* mutants is that such strains are not mutated by UV irradiation (34, 53, 54). On the other hand, mutants defective in dimer excision are more UV-mutable than a wild-type strain (22, 52). UV-induced reversion of *ilvD188* was studied in strains ES244 (*mutU+*) and ES245 (*mutU4*). The results (Fig. 6) showed that the *mutU4* strain was UV-mutable, mutants increasing with dose at a somewhat greater rate than in the *mutU+* strain. Results (not shown) similar to those for the reversion of *ilvD188* were found for the forward mutation, T6 resistance.

If a thymine-requiring strain of *E. coli* is starved for thymine, it will undergo "thymineless" death (11). Thymineless death was followed in strain ES271 (*mutU4 thy-3*) and a UV-resistant revertant, strain ES282 (Fig. 7). The mutator died more rapidly than the nonmutator. Similar results (not shown) were found when strain ES271 (*mutU4*) was compared with strain ES272 (Mut+).

Interaction of mutU4 with other UV-sensitive mutations. By means of conjugation or transduction, double mutants were made

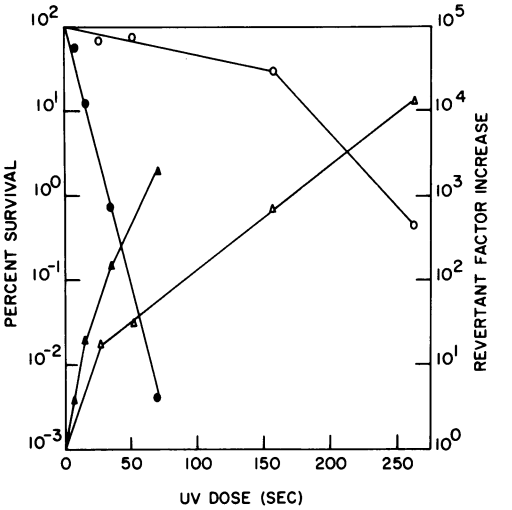

FIG. 6. *UV survival and reversion of ilvD188 in strains ES244 (mutU+) (open symbols) and ES245 (mutU4) (closed symbols). Circles represent survival and triangles factor increase in revertants per viable cell.*

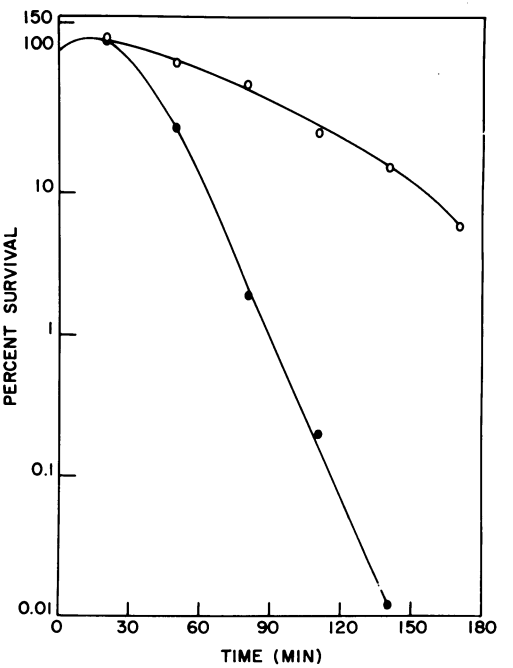

FIG. 7. *Survival after transfer to thymine-free medium of strains ES282 (Mut+) (O) and ES271 (mutU4) (●).*

containing *mutU4* and *uvrA*, *recA*, *recB*, or *recC*. For each of these combinations UV-survival curves were done and mutation rates were determined. Table 8 gives mutation rates for these combinations. All rates were within the

mutator range. The rates for mutation to T6 resistance were higher in the *mutU4 recB21* and *mutU4 recC22* strains than in the isogenic *mutU4* strain. As rates were not increased for streptomycin resistance, the increased rate for T6 resistance might have resulted from a selective effect. The results demonstrated that a functional recombination or excision repair pathway was not necessary for the expression of the mutator phenotype of *mutU4*.

Figure 8 gives the UV-survival curves for combinations of *recA* and *recB* with *mutU4*.

TABLE 8. *Mutation rates in mutU4 rec and mutU4 uvrA6 combinations*

Strain	Relevant genotype	Mutation rate[a]		
		$Str^s \rightarrow Str^r$	$T6^s \rightarrow T6^r$ (non-mucoid)	$T6^s \rightarrow T6^r$ (mucoid)
AB2533	Wild type	$<1 \times 10^{-9}$	5×10^{-8}	2×10^{-8}
ES282	Mut^+ revertant of *mutU4*	$<1 \times 10^{-9}$		
ES287	*recA56 mutU4*	8×10^{-9}		
ES298	*recB21 mutU4*	8×10^{-9}	3×10^{-5}	2×10^{-6}
ES299	*mutU4*	2×10^{-8}	4×10^{-6}	5×10^{-7}
ES300	*recC22 mutU4*	3×10^{-8}	3×10^{-5}	6×10^{-6}
ES352	*mutU4*	3×10^{-8}		
ES351	*mutU4 uvrA6*	2×10^{-8}		

[a] Mutants per cell per generation.

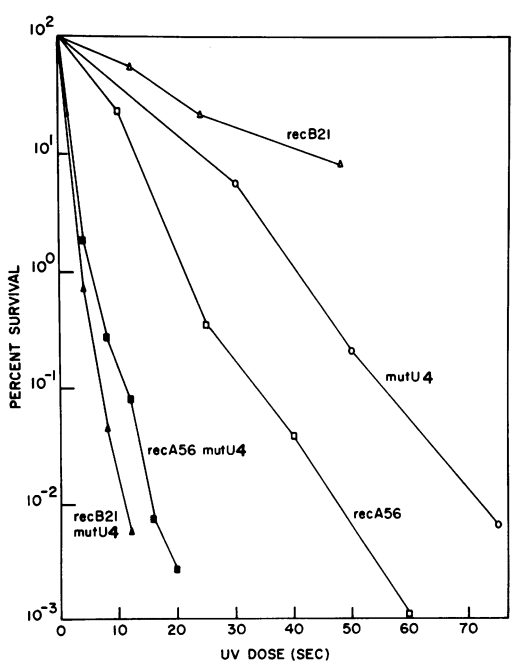

FIG. 8. *UV survival of mutU4 rec strains: ES338 (recB21) (△), ES299 (mutU4) (○), ES286 (recA56) (□), ES287 (mutU4 recA56) (■), and ES298 (mutU4 recB21) (▲).*

The result for the *recC* combination was identical to that with *recB* and is not shown. The great sensitivity of the *rec mutU4* combinations is similar to what has been found by others for the *uvrA6 recA13* combination (25) in which both systems of dark repair, excision and post-replication repair, were blocked. The survival curves of a *uvrA6 mutU4* double mutant and of strains containing *uvrA6* or *mutU4* alone in the same genetic background are shown in Fig. 9. The *mutU4 uvrA6* combination was only slightly more sensitive than *uvrA6* alone. These results, together with those for the *rec mutU4* double mutants, suggested that *mutU4* was defective in excision repair.

mutU4 and bacteriophage. As was obvious from the method of distinguishing the *rep-3* mutation from the mutator, *mutU4* strains supported growth of phage ϕX174 and also phage P2. A *mutU4* strain also yielded plaques with λ *b2 red-7 cI857* phage with wild-type efficiency and with λ *spi-2 cI857* phage at ca. 50% efficiency compared to a $mutU^+$ strain. The *red* mutation blocked growth of λ on a *polA* mutant strain (58) and the *spi* mutation blocked growth on *recA* $recB^+$ but not *recA recB* strains (32). Treffers' mutator *mutT1* could mutate phages λ and T7 (14). To see whether *mutU4* could mutate virulent phage, a series of phages were grown on *mutU4* and Mut^+ strains, and frequencies of phage mutations were determined. The temperate phage λ was grown vegetatively by plating the temperature-inducible λ *cI857* with the cells at 42 C,

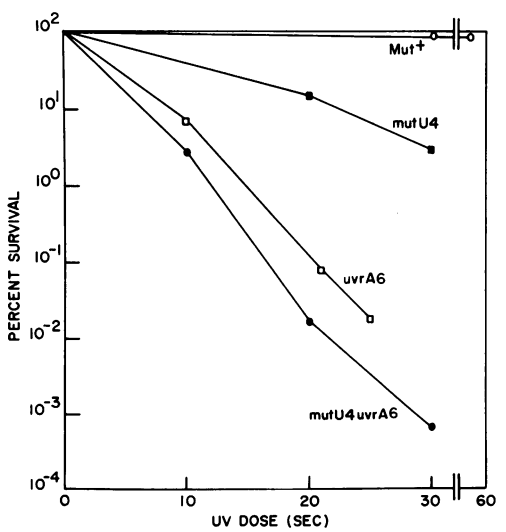

FIG. 9. *Interaction of mutU4 and uvrA6; UV survival of strains ES351 (mutU4 uvrA6), (●), ES352 (mutU4) (■), ES430 (Mut+ revertant) (○), and ES434 (uvrA6) (□).*

and was induced by transferring a λ *cI857* lysogen from 30 to 42 C. Phage λ vir was also used. For the virulent phages, no significant increases in mutation frequencies were found in phage grown on the mutator (Table 9). The same was true for vegetatively grown λ, when λ *vir* or λ *cI857* grown at 42 C was used. Phage λ vir, as noted by Appleyard, McGregor, and Baird (3), gave higher frequencies of *h* mutants as compared with λ *cI857*. When λ was induced, higher frequencies of *h* mutants were repeatedly found when the lysogen contained *mutU4*. This indicated that the integrated prophage was mutated along with the genes of the bacterial chromosome.

Recessiveness of mutU4. The *metE4 mutU4* strain ES273, a derivative of strain C1412, was crossed with strain AB1206. This latter strain carries the F14 episome, which includes the wild-type allele of *mutU4*, and contains a chromosomal deletion of the region corresponding to the episome (37). Of 25 Met^+ Pro^+ (Pro^+ was used for counterselection) exconjugants from this cross, 23 were UV-resistant, as determined by the rapid UV test. The average frequency of spectinomycin-resistant mutants in cultures of the UV-resistant exconjugants was 1.2×10^{-9} mutants/cell, considered nonmutator; the value for the Mut^+ AB1206 parent was less than 2.4×10^{-9} mutants/cell, and that for strain ES273 (*mutU4*) was 6.0×10^{-8} mutants/cell. Two UV-resistant exconjugants were grown in L broth to promote spontaneous loss of the F14 episome, which would demonstrate that they were acually heterogenotes. Met^- derivatives obtained from the L broth cultures were UV-sensitive and mutator, but they were still sensitive to the male-specific phage R17, indicating partial deletion of the episome rather than its complete loss. Deletion of the F14 episome was found previously by Yu, Vermeulen, and Atwood (57). When strain AB1206 was mated with strain ES441, a *recA46 mutU4* recipient, *mutU4* was again found to be recessive as a mutator. The heterogenotes had the UV sensitivity of *recA56* alone, indicating that *mutU4* was also recessive as a UV-sensitive mutation.

TABLE 9. *Mutation frequencies in phage grown on mutU4 and Mut⁺ strains*

Phage	Selected phenotype[a]	Host[b]	Revertants or mutants per PFU	Mut^-/Mut^+
T1	*h*	ES233 (Mut^+)	5.4×10^{-8}	1.9
		ES234 (Mut^-)	1.0×10^{-7}	
T1*am11*	*am*⁺	ES233 (Mut^+)	7.4×10^{-7}	0.27
		ES234 (Mut^-)	2.0×10^{-7}	
T2	*h*	ES244 (Mut^+)	4.3×10^{-8}	3.0
		ES245 (Mut^-)	1.3×10^{-7}	
T4*rII* AP156	*rII*⁺	ES244 (Mut^+)	1.6×10^{-8}	0.94
		ES245 (Mut^-)	1.5×10^{-8}	
T4*rII* AP72	*rII*⁺	ES244 (Mut^+)	2.5×10^{-7}	0.80
		ES245(Mut^-)	2.0×10^{-7}	
T7	*h*	ES233 (Mut^+)	1.8×10^{-9}	0.13
		ES234 (Mut^-)	2.3×10^{-10}	
T7 gene 12 *am3*	*am*⁺	ES233 (Mut^+)	2.5×10^{-6}	1.1
		ES234 (Mut^-)	2.8×10^{-6}	
T7 gene 5 *am28*	*am*⁺	ES233 (Mut^+)	1.4×10^{-6}	0.79
		ES234 (Mut^-)	1.1×10^{-6}	
T7 gene 19 *am10*	*am*⁺	ES233 (Mut^+)	3.1×10^{-6}	0.39
		ES234 (Mut^-)	1.2×10^{-6}	
T7 gene 6 *am147*	*am*⁺	ES233 (Mut^+)	1.1×10^{-6}	2.1
		ES234 (Mut^-)	2.3×10^{-6}	
M13 *am3-H4*	*am*⁺	ES382 (Mut^+)	6.8×10^{-6}	1.0
		ES381 (Mut^-)	6.8×10^{-6}	
M13 *am5-H3*	*am*⁺	ES382 (Mut^+)	5.3×10^{-6}	2.5
		ES381 (Mut^-)	1.3×10^{-5}	
M13 *ts1-N1*	*ts*⁺	ES244 (Mut^+)	1.2×10^{-5}	0.48
		ES245 (Mut^-)	5.7×10^{-6}	
λ vir	*h*	ES244 (Mut^+)	5.9×10^{-8}	1.3
		ES245 (Mut^-)	7.7×10^{-8}	
λ *cI857*[c]	*h*	ES242 (Mut^+)	$<3.1 \times 10^{-9}$	—[d]
		ES243(Mut^-)	$<4.2 \times 10^{-10}$	
λ *cI857*[e]	*h*	ES411 (Mut^+)	$<1.6 \times 10^{-9}$	>31
		ES412 (Mut^-)	5.0×10^{-8}	

[a] Symbols: *h*, extended host range; ⁺, reversion to wild type.
[b] Phenotype with respect to mutator in parentheses.
[c] Lytically grown at 42 C.
[d] Not calculated because no *h* mutants were found.
[e] Induced by transfer of a lysogen to 42 C.

mutU4 reversion of an episomal locus. Strain ES475 was prepared with a *tonB-trp* deletion on the chromosome and the mutation *trpA46* on an F episome (see Table 1 for the origin of this strain). The mutation *trpA46* can yield four classes of Trp^+ revertants, all of which are due to base changes within the *trpA* cistron (55). By transduction, isogenic *mutU*⁺ (ES480) and *mutU4* (ES481) derivatives of strain ES475 were prepared, and the frequency of reversion to Trp^+ was determined. The average frequency of Trp^+ revertants in strain ES480 (*mutU*⁺) was 5.1×10^{-10} mutants/cells and in strain ES481 (*mutU4*) the value was 2.4×10^{-7} mutants/cell. The low frequency of reversion of *trpA46* in the nonmutator strain was within the expected range (47), but the fact that strain ES480 carried a revertable mutation was verified by reverting it to Trp^+ with ethyl methanesulfonate (EMS), which increased the reversion frequency ca. 100-fold. The episomal location of the Trp^+ reversions of strain ES481 was demonstrated by transferring the Trp^+ phenotype with high frequency to strain 240, bearing a *tonB-trpA,B* deletion, and to strain ES250, a *trp recA56* strain. The results showed that *mutU4* exerted its effect in the *trans*

configuration.

Base change induced by mutU4. Breckenridge and Gorini (8), in a study of the *strA* locus of *E. coli*, identified four, and only four, classes of streptomycin-resistant *strA* alleles on the basis of the phenotypic suppression of nonsense mutations by the antibiotics bluensomycin and streptomycin. Two classes, represented by mutations *strA60* and *strA40*, arose by transitions, and the remaining two classes, represented by mutations *strA1* and *strA2*, arose by transversions. Mutations of the *strA1* and *strA2* type were induced by *mutT1*, and *mutS3* (formerly designated *mutS1*) induced *strA40*- and *strA60*-type mutations (data of Breckenridge and Gorini in Table 10). Independent streptomycin-resistant mutants of strain ES379 (*mutU4*) were selected. Suppression tests gave the results shown in Table 10; data from Breckenridge and Gorini (8) are included for comparison. The mutations induced by *mutU4* were primarily of the *strA60* and *strA40* type, and were presumably transitions. When Breckenridge and Gorini found a mutant that did not fit into any of the four classes, they backcrossed the streptomycin-resistant mutation into strain L44. This backcross showed that all novel patterns of suppression were due to mutations external to the *strA* locus. This procedure was used for several *mutU4*-induced *strA* mutations that gave novel suppression patterns and allowed most of these mutations to be assigned to one of the four classes. However, seven of these mutants (7% of total mutants) would not support the growth of phages P1kc or P1bt and had to be placed in the unclassifiable category. The remaining two mutants in this category gave a novel suppression pattern even after being backcrossed into strain L44.

The relative frequencies of streptomycin-resistant mutants in strains L44 (ES377) and ES379 (*mutU4*) were determined by comparing the percentage of colonies that when replica-plated gave streptomycin-resistant growth (not dependent). This calculation gave the value of eightfold in Table 10. When determined by assaying cultures of strains ES377 and ES379 on nutrient agar and nutrient agar plus streptomycin, this relative frequency was 100-fold. The discrepancy between 8- and 100-fold occurred because many strain ES379 colonies gave more than one streptomycin-resistant clone per colony on replica-plating, but only one clone per colony was utilized for testing and therefore tabulated in the calculations giving the eightfold value. If approximately one-eighth of the mutants tested were of spontaneous origin, this could account for all mutations arising in strain ES379 that were of the *strA2* and *strA1* types.

TABLE 10. *Frequency of classes of strA mutations induced by mutU4*

Class of mutation	Mutations induced by			
	Spontaneous[a] (%)	*mutT1*[a] (%)	*mutS3*[a] (%)	*mutU4*[b] (%)
strA60	4	0	74	37
strA40	0	0	24	45
strA2	38	71	1	6
strA1	58	29	1	2
Unclassifiable	0	0	0	9
Relative frequency (induced/spontaneous)	—	1,000	100	8[c]

[a] All data in this column from Breckenridge and Gorini (8).

[b] Ninety-nine streptomycin-resistant mutants tested. The *mutU4* strain ES379 was used.

[c] See text for method of calculation.

Breckenridge and Gorini (8) found that streptomycin-dependent mutants were induced by *mutS3* but not by *mutT1*. Among the mutants capable of growth on streptomycin that were isolated from strain ES379 (*mutU4*), 62% were streptomycin-dependent.

Additional UV-sensitive mutators. After EMS mutagenesis, Liberfarb and Bryson (31) found two UV-sensitive mutator strains in *E. coli* B/UV, a derivative that is more UV-resistant than wild-type *E. coli* B (41). The mutator strains were designated strains EMS107 and EMS195, and their mutational sites, respectively, *mut-9* and *mut-10*. In this laboratory, two independently isolated UV-resistant revertants of strain EMS107 were selected and found to be nonmutator. This suggested that the mutator phenotype and UV sensitivity were due to one mutation in strain EMS107. Liberfarb and Bryson (31) were not able to map *mut-9* because the transducing phage P1 would not grow on strain EMS107. Therefore, an Ilv$^-$ mutant of strain EMS107 was selected (ES393), and it was transduced to Ilv$^+$ with P1bt grown on *E. coli* B/r. Of the 100 transductants, 33 were UV-resistant and Mut$^+$.

Strain EMS195 was more UV-sensitive than strain EMS107, and no UV-resistant revertants could be isolated from it. This suggested that strain EMS195 had two UV-sensitive mutations. Strain EMS195 was crossed to the Hfr strain JC12, and Mal$^+$ Pur$^+$ recombinants were

selected. Of 40 recombinants, 39 were UV-sensitive by the rapid UV test. However, all Mut$^-$ recombinants (as determined by the frequency of streptomycin resistance) gave 0 to 3 surviving colonies per streak in the rapid UV test, whereas the Mut$^+$ recombinants gave 5 to 30 colonies per streak; the single UV-resistant recombinant was Mut$^+$. The Mut$^+$ recombinants were thought to give more survivors after irradiation because they contained only one UV-sensitive mutation. From the facts that 43% of the recombinants from this cross were Mut$^+$ and only 2.5% (1 of 40) were UV-resistant, it was concluded that strain EMS195 had a UV-sensitive mutator near the origin of transfer of strain JC12 and a second UV-sensitive mutation at some distance from the origin. Liberfarb and Bryson (31) could not grow phage P1 on strain EMS195. However, several of the Mut$^-$ recombinants from the JC12 $\times$ EMS195 cross could support the growth of phage P1bt. The phage was grown on one such recombinant, strain ES400, and used to transduce strain AB1203 to Ilv$^+$. Five of the 70 transductants (7%) were UV-sensitive and mutator. The 7% linkage to *ilv* is lower than that found for *mutU4* or *mut-9*, but this may be attributed to decreased homology, to restriction in the transduction from a B strain, ES400, to a K-12 strain, AB1203, or to a combination of these phenomena. These results placed the UV-sensitive mutator lesions of strains EMS107 and EMS195 near *ilv* in the same region as *mutU4*.

DISCUSSION

The data support the hypothesis that *mutU4* is a single mutation resulting in both UV sensitivity and the mutator phenotype. However, the possibility that *mutU4* represents two very closely linked mutations has not been disproved. The *mutU4* mutation was induced with NTG, which can cause multiple, closely linked mutations (21). If *mutU4* is actually two sites, one site would determine UV sensitivity and the second would determine the mutator phenotype. However, the mutator phenotype would be dependent upon the first site; i.e., a reversion to UV resistance at the first site would suppress the mutator phenotype of the second site. This hypothesis is less probable than the single-site hypothesis, not only because it is more involved but also because UV-sensitive mutators mapping in the same general region as *mutU4* have been isolated by others (31, 43). Two of these, *mut-9* and *mut-10*, were induced with EMS, which is not known to induce multiple, closely linked mutations. Smirnov and Skavronskaya (44) located *uvr-502* between *metE* and *ilv*. Unlike *mutU4*, *uvr-502* is closer to *ilv* than to *metE*, but this may be due to strain differences. Recently, Smirnov, Filkova, and Skavronskaya (*personal communication*) proved that the mutation *uvr-502* determined both UV sensitivity and the mutator phenotype. Complementation tests are needed to determine whether *mut-9*, *mut-10*, *uvr-502*, and *mutU4* are in the same cistron.

The *mutU4* mutation is very close to *uvrD3*. The latter is phenotypically very different from *mutU4*; unlike *mutU4*, it is dominant, and a *uvrD3* strain is Hcr$^-$ and very sensitive to gamma irradiation (36). In the crosses between *mutU4* and *uvrD3* strains, no wild-type (UV-resistant) or double mutant (*uvrD3 mutU4*) recombinants were found. The absence of wild-type transductants indicated the close linkage between the two mutations. However, the absence of the *mutU4 uvrD3* class did not necessarily result from the close linkage. Possibly, the *uvrD3 mutU4* combination was not viable or its phenotype was not distinguishable from *mutU4* or *uvrD3*. Again, a complementation test will be needed to determine whether *mutU4* and *uvrD3* are in the same cistron or possibly in adjacent cistrons. The *rep-3* mutation is also close to *mutU4* between *metE* and *ilv* (10). Some functional relationship of *mutU4* and *uvrD3* with the *rep-3* mutation was indicated by the poor growth and instability of the *mutU4 rep-3* and *uvrD3 rep-3* double mutants.

In the repair of UV-damaged DNA, *mutU4* appeared to be involved in excision repair rather than postreplication repair. This was shown by the extreme UV sensitivity of the *mutU4 rec* double mutants. The *mutU4 uvrA6* double mutant was only slightly more UV-sensitive than a single *uvrA6* mutant. These combinations of *mutU4* with *recA*, *B*, or *C* mutations, or with the *uvrA6* mutation, were phenotypically mutator. Neither a functional excision-repair or postreplication repair system was needed for the expression of the mutator gene. Determination of the defective step in excision repair in a *mutU4* cell will require additional experiments, but from the present work it can be said that repair of single-strand breaks in DNA caused by MMS or gamma irradiation was normal or close to normal. A *mutU4* strain showed increased sensitivity to thymine starvation. Evidence is contradictory in respect to the accumulation of single-strand breaks in the bacterial chromosome under these conditions (4, 39, 50). If the only lesion involved in thymineless death were the single-strand break, a *mutU4* strain would not be expected to show

increased sensitivity. Possibly, another lesion is involved or the decreased survival is due to a secondary effect resulting from unbalanced growth (51).

As a mutator, *mutU4* did not simply result in an increase in the types of mutants that occur spontaneously. This can be seen from the frequencies of *strA* classes among spontaneous and *mutU4*-induced streptomycin-resistant mutations in Table 10. Spontaneous mutations were mostly transversions, whereas *mutU4*, like *mutS3*, induced predominantly transitions. The product of the wild-type *mutU* cistron may be involved in the correction of transitional errors occurring during DNA replication as well as functioning in UV repair. A second possibility is that some form of damage, such as a break or gap, might result during normal replication. The *mutU4* product would repair such damage inaccurately, making transitional errors, or it would have no capacity for such repair, and a second error-prone back-up system would repair the damage. This latter possibility is given support by the fact that *mutU4* is lethal with *polA*. This will be discussed in the accompanying paper (40).

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