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Dominant Mutations (*lex*) in *Escherichia coli* K-12 Which Affect Radiation Sensitivity and Frequency of Ultraviolet Light-Induced Mutations

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Three mutations, denoted *lex-1*, *-2* and *-3*, which increase the sensitivity of *Escherichia coli* K-12 to ultraviolet light (UV) and ionizing radiation, have been found by three-factor transduction crosses to be closely linked to *uvrA* on the *E. coli* K-12 linkage map. Strains bearing these mutations do not appear to be defective in genetic recombination although in some conjugational crosses they may fail to produce a normal yield of genetic recombinants depending upon the time of mating and the marker selected. The mutagenic activity of UV is decreased in the mutant strains. After irradiation with UV, cultures of the strains degrade their deoxyribonucleic acid at a high rate, similar to *recA*$^-$ mutant strains. Stable *lex*$^+$/*lec*$^-$ heterozygotes are found to have the mutant radiation-sensitive phenotype of haploid *lex*$^-$ strains.

Howard-Flanders and Boyce (14) have described a mutation (*lex-1*) located near *malB* on the *Escherichia coli* K-12 linkage map (25) which increases sensitivity to ultraviolet light (UV) and ionizing radiation. Mutations denoted *exr* which have a similar effect on the radiation sensitivity of *E. coli* B have been located in a corresponding position on the *E. coli* B linkage map (11-13, 19). Strains carrying an *exr* or a *lex* mutation characteristically show very low rates of mutation induction by UV compared to normal *E. coli* strains (29, 31). The *lex*$^+$ function is also required for the induction of mutations in bacteriophage lambda by UV (9, 20). Witkin (31, 32) has reported that Lex$^-$ and Exr$^-$ mutants are defective in genetic recombination and has concluded that genetic recombination may be associated with UV mutagenesis. Evidence in support of this conclusion comes from the observation that various recombination-deficient (Rec$^-$) mutants also exhibit very low rates of UV-induced mutagenesis (20, 30).

The objective of this paper is to describe the properties of three Lex$^-$ mutants including the one described by Howard-Flanders and Boyce (14) and two newly isolated mutants. The three *lex* mutations have been mapped by three-factor transduction crosses and are closely linked to *uvrA* on the linkage map of *E. coli* K-12 (25). The mutations confer an increase in sensitivity to radiation and a decrease in the frequency of UV-induced mutations, but they do not appear to have a significant effect on genetic recombination. The three *lex* mutations are dominant, as demonstrated by the mutant radiation-sensitive phenotype of *lex*$^+$/*lex*$^-$ heterozygous strains.

MATERIALS AND METHODS

Media. Complex medium was either LB broth (18) or N broth containing 8 g of tryptone (Difco), 5 g of NaCl, 2 g of glucose, 2.5 g of Casamino Acids (Difco), and 20 μg of thiamine per liter. Minimal medium was either that described by Davis and Mingioli (8) or medium 56/2 described by Adelberg and Burns (1). Growth factors were added to minimal media to these concentrations: glucose, 0.2%; amino acids, 100 μg/ml; and thiamine, 0.1 μg/ml.

Transduction and mating procedures. Those procedures described by Clark and Margulies (7) and

Willetts et al. (27) were used. Matings were interrupted by the vibratory blending device of Low and Wood (17).

Bacterial strains. The properties and sources of the bacterial strains used are shown in Table 1.

Strain DM452 was prepared by several transductions with phage P1-*vira* (27). A phage lysate grown on donor JC5805 Met$^+$ Mal$^-$ was used to transduce AB1932 Arg$^-$ Met$^-$, and both an Arg$^-$ Met$^+$ Mal$^-$ transductant (DM441) and an Arg$^+$ Met$^-$ Mal$^+$ transductant (DM442) were obtained. DM452 was then prepared by a second cross between donor DM442 and recipient DM441, with an Arg$^+$ Met$^-$ Mal$^-$ transductant being selected.

The mutant strains DM48 *lex-2* and DM49 *lex-3* were isolated as X-ray sensitive mutants of AB1157 after mutagenesis by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine as described by Willetts and Mount (28). AB2474 *lex-1 uvrA6* was isolated by Howard Flanders (*personal communication*) as an X-ray sensitive isolate of strain AB1886 *uvrA6* (15) after mutagenic treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. The *lex-1* and *urvA6* mutations were subsequently separated by a conjugation cross between AB2474 and AB2383 (HfrP4X6 *metB1 thyA2* B$^-_1$) in which Arg$^+$ (Thy$^+$ Str^R) recombinants were selected.

To work with a set of strains which was isogenic except for the *lex* region, strains DM572 *lex*$^+$, DM573 *lex-2*, DM574 *lex-3*, and DM575 *lex-1* were made by transducing the respective *lex* alleles into DM452 and obtaining Mal$^+$ Met$^-$ Lex$^{+\text{ or }-}$ transductants. This set of strains is lysogenic for phage λ. A corresponding set of nonlysogenic strains (DM800 *lex*$^+$, DM801 *lex-2*, DM802 *lex-3*, and DM803 *lex-1*) was made. DM800 and DM801 were obtained from DM572 and DM573 by superinfection curing employing the heteroimmune phage λ b_2i^{21} (gift of Dale Kaiser). Two of the strains, DM574 and DM575, could not be cured in this manner. Their nonlysogenic derivatives were prepared by transducing DM800 with P1*vira* grown on JC5805 and isolating a Met$^+$ Mal$^-$ strain (DM837). DM837 was then transduced to obtain DM802 *lex-3* and DM803 *lex-1* with P1*vira* grown on DM574 and DM575, respectively, choosing Mal$^+$ Met$^-$ Lex$^-$ transductants.

Strain DM842 *uvrA6 lex-1* was prepared by transducing DM837 Mal$^-$ with P1*vira* grown on AB2474 *uvr*$^-$ *lex*$^-$ and choosing a Mal$^+$ transductant that was sensitive to methylmethane sulfonate. This property demonstrated the presence of the *lex-1* allele. Proof that the *uvrA6* allele is present is provided by the mapping data in Table 4 which show

TABLE 1. *Properties of bacterial strains*

Strain no.	Genotype[a]	Source
AB1157	F$^-$, *thr-1, leu-6, proA2, his-4, thi-1, argE3, lacY1, galK2, ara-14, xyl-5, mtl-1, tsx-33, strA31, sup-37*, λ$^-$	E. A. Adelberg
AB2474	as AB1157, also *lex-1, uvrA6*	P. Howard-Flanders
AB2494	as AB1157, also *lex-1, metB1*, argE$^+$, λ$^+$	P. Howard-Flanders
DM48	as AB1157, also *lex-2*	This paper
DM49	as AB1157, also *lex-3*	This paper
KL132	as AB1157, also *argE*$^+$, *mtl*$^+$, *thyA25, pyrB31, recA1, malA1*	This paper
F118/KL132	F', *pyrB*$^+$/KL132	This paper
JC1553	F$^-$, *leu-6, his-1, argG6, metB1, gal-6, xyl-7, mtl-2, lacY1* or *Z4, malA1, strA1, recA1*, λ$^-$, λ^R	A. J. Clark
F134/JC1553	F', *metB*$^+$ *leu*$^+$/JC1553	This paper
F134-1/JC1553	F', *metB*$^+$/JC1553	This paper
AB1932	F$^-$, *argE3* or *H1, metA28, lacY1* or *Z4*, λ$^+$, *thi-1, xyl-5* or *-7, galK2, tsx-6*	P. Howard-Flanders
DM498	as AB1932, also *str*^{R}	This paper
JC5805	F$^+$, *hisA323, malB32*	M. Schwarz
DM452	F$^-$, *metA28, malB32, lacY1* or *Z4*, λ$^+$, *thi-1, xyl-5* or *-7, galK2, tsx-6*	This paper
DM800	as DM452, also *mal*$^+$, λ$^-$	This paper
DM801	as DM800, also *lex-2*	This paper
DM802	as DM800, also *lex-3*	This paper
DM803	as DM800, also *lex-1*	This paper
DM837	as DM800, also *met*$^+$, *malB32*	This paper
DM842	as DM837, also *malB*$^+$, *uvrA6, lex-1*	This paper
AB259	HfrH, *thi-1, rel-1*, λ$^-$	E. A. Adelberg
KL84	Hfr, *thi-1, rel-1*, λ$^-$	This paper
KL983	Hfr, *xyl-7, lacY1* or *Z4, mglP1*, λ$^-$	This paper
Ra-2	Hfr, *mal-28, supE42*, λ^R, λ$^-$	This paper

[a] The nomenclature used is that of Demerec et al. (10), and Taylor (25). All markers are wild type except those listed. The origin and direction of transfer of the Hfr strains are given in Fig. 1.

that the *uvrA6* allele may be crossed out of DM842.

Derivatives of *lex* mutant strains carrying F134, which carries their respective *lex*$^+$ alleles, could not be prepared evidently because of their inability to maintain this rather long episome (Fig. 1). An apparently shorter episome designated F134-1 (Fig. 1) was isolated by mating F134/JC1553 with DM49 *lex-3* and selecting for rare Arg$^+$ (*argG*$^+$ *argE*$^+$) colonies. One such colony was found to be a donor, and its episome was transferred by conjugation to JC1553 selecting for Met$^+$ (Pro$^+$ Thr$^+$) colonies. The resulting strain F134-1/JC1553 was used for storage of the episome. It was subsequently found that F134-1 is not a donor of either *leu*$^+$ or *pyrB*$^+$ and probably has had these markers deleted (Fig. 1).

Derivatives of DM800-803 bearing F134-1 were freshly prepared for each experiment as they are somewhat unstable. F134-1/JC1553 was mated with DM800-803 and Met$^+$ (Leu$^+$ Arg$^+$ His$^+$) colonies selected. These were purified by a single-colony isolation and grown overnight in liquid cultures on selective minimal medium at 37 C.

The presence of F134-1 in the majority (>90%) of the cells in test cultures was demonstrated by testing 200 colonies grown from cultures on Met$^+$ selective medium or on N agar for their ability to donate *metA*$^+$ to DM498, selecting for Met$^+$ (Str^R) colonies. The presence of the respective *lex*$^+$ alleles in the cells was demonstrated by growing P1*vira* on test cultures, transducing the corresponding Met$^-$ Lex$^-$ strain, and selecting for Met$^+$ colonies. The frequency of cotransduction of *metA*$^+$ and the respective unselected *lex*$^+$ alleles was always found to be approximately the same as that obtained by transducing the same recipients with P1*vira* grown on a *metA*$^+$ *lex*$^+$ strain (AB1157), i.e., between 6 and 15% for each of the three *lex* mutations.

F118 was introduced into DM800-803 by mating them with a 10-fold majority of F118/KL132 cells and plating without selection on LB agar. Colonies bearing the episome were identified by their ability to donate *pyrB*$^+$ to KL132. They were purified by single-colony isolation on LB agar and grown into liquid cultures in LB broth. It was shown that more than 52 of 100 cells from test cultures carried both F118 and the *lex*$^+$ allele. The presence of F118 was detected by mating cultures of colonies grown from test cultures with KL132, and the presence of the *lex*$^+$ alleles was shown by demonstrating that cultures of colonies grown from test cultures gave rise to UV-resistant segregants.

Sensitivity to radiations and methylmethanesulfonate (MMS). For measurement of the UV sensitivity of cultures and for plate tests of UV sensitivity, the techniques described by Willetts and Mount (28) were used. The dose rate from a 15-W germicidal lamp (General Electric) employed was reduced by enclosing the lamp in a light-tight box with a 44-mm slit. Irradiations were carried out at a distance of 40 cm from the lamp at a dose rate of 1.6 ergs per mm^2 per sec.

Sensitivity to ionizing radiation was measured by spreading chilled exponential cultures on LB plates and exposing the plates to the beam from a Varian 6 Mev clinical linear electron accelerator. The dose rate was 30 krad/min.

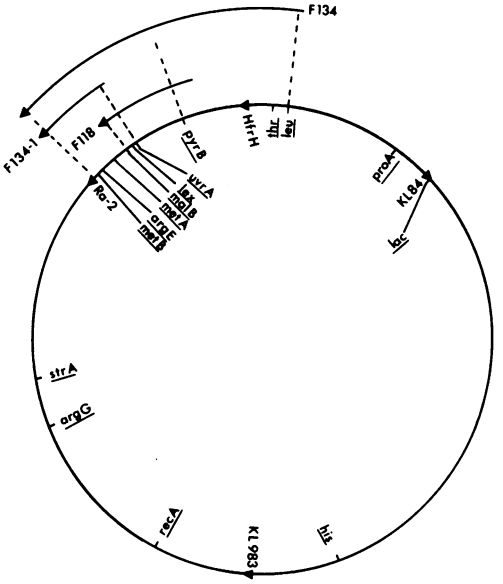

FIG. 1. *Partial linkage map of Escherichia coli K-12 (25) showing position of markers and origin and direction of transfer of donor strains.*

For measurement of sensitivity of cultures to MMS, a log-phase culture grown in N broth at 37 C was diluted 100-fold into 4.9 ml of phosphate-buffered saline (0.1 M phosphate, 0.125 M NaCl, *p*H 7.0). A 0.1-ml amount of 1 M MMS was added and the mixture was incubated at 37 C. The reaction was stopped by diluting 100-fold into ice-cold N broth. After allowing 15 min for equilibrium in the stopping mixture, the cells were diluted further and plated on N agar plates.

Measurement of radiation-induced mutations. For measurement of mutation induction by UV, log-phase cultures were centrifuged, and the cells were suspended in one-fourth volume phosphate-buffered saline. They were irradiated in shallow layers in glass petri dishes with agitation, diluted fourfold into fresh N broth, and then shaken at 37 C for 4 to 6 hr to allow expression of streptomycin resistance (29). During this interval, the viable cell yield increased from 10 to 30 times. Streptomycin was then added to a final concentration of 100 μg/ml, and the culture was centrifuged and spread on N agar plates containing 100 μg of streptomycin per ml. Streptomycin-resistant colonies were scored after 48 hr of incubation at 37 C.

DNA degradation. The method used to label deoxyribonucleic acid (DNA) with ^{3}H-thymidine and measure the release of cold trichloroacetic acid-soluble radioactivity after UV irradiation was that described by Clark et al. (6). Labeled cells were routinely exposed to a dose of 100 ergs/mm^2.

RESULTS

Radiation sensitivity of the Lex$^-$ strains. The mutations designated *lex-1*, *lex-2*, and *lex-3* confer greater sensitivity to ionizing radiation, UV and MMS, than *lex*$^+$ (Fig. 2). The

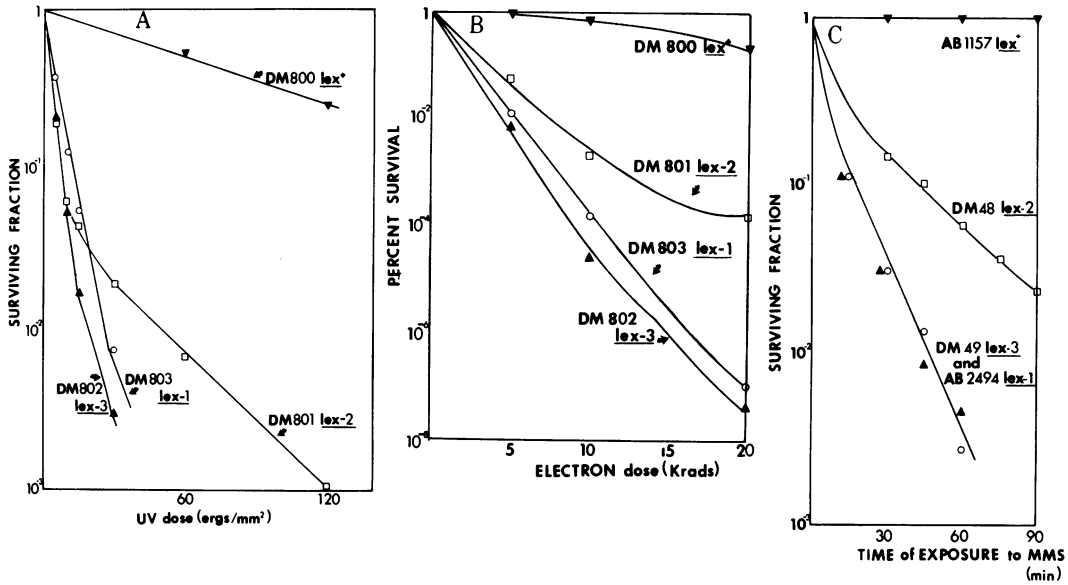

FIG. 2. *A, UV survival curves of Lex⁺ and Lex⁻ strains. Symbols: DM800 lex⁺, ▼; DM801 lex-2, □; DM803 lex-1, ○; DM802 lex-3, ▲. B, X-ray survival curves of Lex⁺ and Lex⁻ strains. Legend, same as A. C, MMS survival curves of Lex⁺ and Lex⁻ strains. Symbols: AB1157 lex⁺, ▼; AB2494 lex-1, ○; DM48 lex-2, □; DM49 lex-3, ▲.*

degree of sensitivity to these agents conferred by *lex-2* is somewhat less than that conferred by *lex-1* and *lex-3*.

Genetic mapping of the lex⁻ mutations. Howard-Flanders and Boyce (14) reported that *lex-1* is linked to *malB* and *metA* on the *E. coli* K-12 linkage map. We have found that *lex-1*, *lex-2*, and *lex-3* are 59, 71, and 70% cotransducible, respectively, with *malB⁺* in P1 transduction crosses with a *malB32 lex⁺* recipient (Table 2).

Several multifactor transductional crosses were carried out to locate the *lex* mutations with respect to other genes neighboring *malB*. A transductional cross between AB2494 *lex-1*, DM48 *lex-2*, and DM49 *lex-3* donors and recipient DM452 Met⁻ Mal⁻ is shown in Table 3. Met⁺ transductants were selected and analyzed for inheritance of the unselected *malB* and *lex* markers. Since in each cross nearly all recombinants that inherit the Lex⁻ marker also inherit *mal⁺* the most probable order is *metA-malB-lex*.

The *uvrA* locus is on the same side of *malB* and is cotransducible with *malB* at approximately the same frequency as the *lex* mutations (24). To establish the relative order of *lex-1* and *uvrA6*, a three-factor transduction cross was performed between a *malB⁺ uvrA6 lex-3* donor and a *malB32uvrA⁺ lex⁺* recipient, selecting for the donor *malB⁺* marker. The data in Table 4 show that the yield of Lex⁺ Uvr⁻ transductants was considerably smaller than the yield of Lex⁻ Uvr⁺ transductants. These results are in best agreement with the order *malB-lex-uvrA*.

TABLE 2. *Frequency of cotransduction of lex with malB*[a]

Donor strain	Unselected marker	Fraction of *malB⁺* transductants which inherit unselected marker
AB2494	*lex-1*	117/200 (59%)
DM48	*lex-2*	142/200 (71%)
DM49	*lex-3*	139/200 (70%)

[a] The recipient strain was JC5805 *malB⁻lex⁺*.

Dominance of lex⁻ mutations. The dominance characteristics of the *lex* mutations were measured by introducing *lex⁺*-carrying episomes into strains DM800–803 which are isogenic except in the *lex* region of the genetic map. The resulting *lex⁺/lex⁻* merodiploid strains were then tested for their sensitivity to UV and ionizing radiation. Construction of the merodiploids and the proof of their heterozygous nature are described above.

Survival curves for typical *lex⁺/lex⁺* and *lex⁺/lex⁻* merodiploid strains are shown in Fig. 3. Where F134-1 *lex⁺* is used as the exogenote (Fig. 3A), the merodiploid F134-1 *lex⁺*/DM801 *lex-2* is seen to be just as sensitive to UV at low doses as the parental haploid strain

TABLE 3. *Ordering of metA, malB, and lex*[a]

Donor	*lex* allele	No. of transductants scored	Unselected markers			
			malB$^+$ *lex*$^+$	*malB*$^+$ *lex*$^-$	*malB*$^-$ *lex*$^+$	*malB*$^-$ *lex*$^-$
AB2494	1	368	24	11	332	1
DM48	2	200	16	14	170	0
DM49	3	200	28	22	150	1

[a] The donor strains were *metA*$^+$ *lex*$^-$, the recipient strain was DM452 *metA32*$^-$ *malB*$^-$, and the selected marker was *metA*$^+$.

TABLE 4. *Ordering of malB, uvrA, and lex*[a]

Total no. of Mal$^+$ transductants scored[b]	Unselected markers			
	uvrA$^+$*lex*$^+$	*uvrA*$^-$*lex*$^-$	*uvrA*$^-$*lex*$^+$	*uvrA*$^+$*lex*$^-$
150	46	54	5	34

[a] The donor strain was DM842 *malB*$^+$ *uvrA6 lex-3*, and the recipient was DM837 *malB*$^-$ *uvrA*$^+$ *lex*$^+$, and Mal$^+$ transductants were selected. The four classes of transductants *uvrA*$^+$ *lex*$^+$, *uvrA*$^+$ *lex*$^-$, *uvrA*$^-$ *lex*$^+$, and *uvrA*$^-$ *lex*$^-$ were distinguished by their sensitivities to UV and MMS. *uvrA*$^+$ *lex*$^+$ transductants are UV and MMS resistant, and *uvrA*$^-$ *lex*$^+$ transductants are UV sensitive and MMS resistant, *uvrA*$^+$ *lex*$^-$ and *uvrA*$^-$ *lex*$^-$ transductants are both UV and MMS sensitive but may be distinguished by their degree of UV sensitivity. *uvrA*$^-$ *lex*$^-$ strains are much more sensitive to UV than *uvrA*$^+$ *lex*$^-$ strains (D. Mount, *unpublished observation*).

[b] Of 150 transductants, 11 were unstable and have not been assigned a genotype.

DM801 *lex-2*. A small fraction (5%) of the cells appear to be UV resistant as seen from the shallower slope of the survival curve at high doses. The latter may be either *lex*$^+$/*lex*$^+$ homozygous segregants or haploid *lex*$^+$ segregants. The presence of the episome appears to have little, if any, effect on the UV-sensitivity of the parental *lex*$^+$ strain, DM800. UV survival curves of *lex*$^+$/*lex-1* and *lex*$^+$/*lex-3* heterozygotes are very similar to those shown in Fig. 3A for *lex*$^+$/*lex-2*.

The Lex$^-$ phenotype was also observed in *lex*$^+$/*lex*$^-$ merodiploid strains irradiated with ionizing radiation (Fig. 3B). The results for the merodiploid strain F118 *lex*$^+$/DM803 *lex-1* are shown, and similar results were obtained with *lex*$^+$/*lex-2* and *lex*$^+$/*lex-3* merodiploids.

Recombinant production of Lex$^-$ strains. AB2494 *lex-1* and the corresponding Lex$^+$ strain AB1157 were mated with several Hfr strains to measure their ability to form genetic recombinants. The data given in Table 5 show that, for short matings of 20 min at 37 C with selection for an early marker, the Lex$^-$ strain

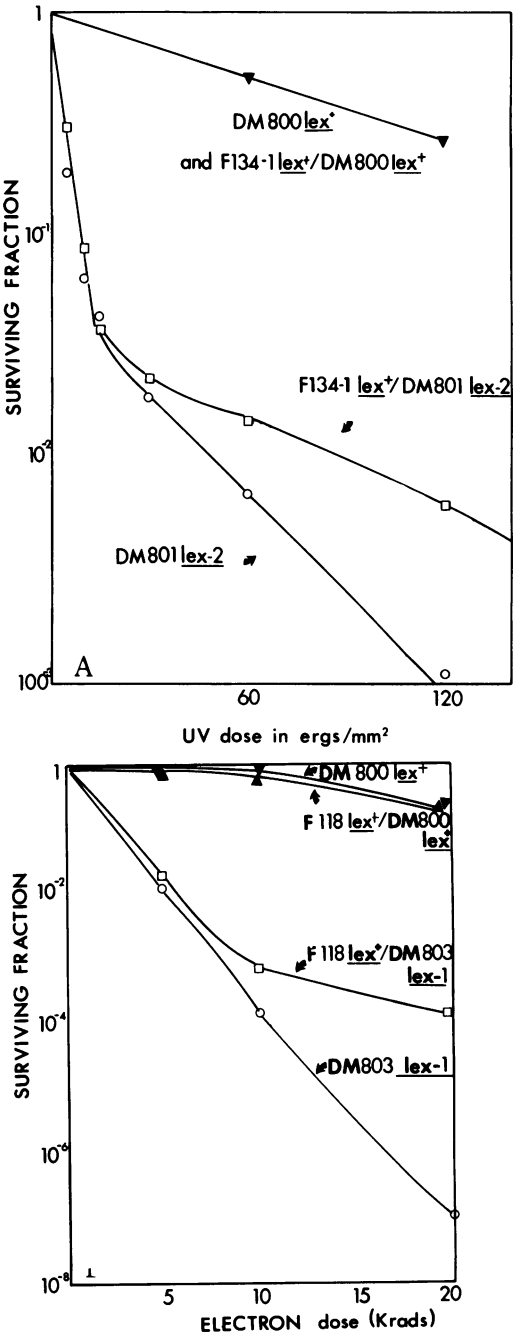

FIG. 3. *UV and ionizing radiation survival curves of lex*$^+$*/lex*$^-$ *heterozygous strains. A, UV survivals of DM800 lex*$^+$, ▼; *F134-1 lex*$^+$*/DM800 lex*$^+$, ▼; *DM801 lex-2*, ○; *F134-1 lex*$^+$*/DM801 lex-2*, □. *Cultures grown to log phase in N broth and plated on N agar. B, Ionizing radiation survivals of DM800 lex*$^+$, ▼; *F118 lex*$^+$*/DM800 lex*$^+$, ▲; *DM803 lex-1*, ○; *F118 lex*$^+$*/DM803 lex-1*, □. *Cultures were grown to log phase in LB broth and plated on LB agar.*

TABLE 5. *Conjugational crosses to measure genetic recombination in a Lex⁻ mutant*[a]

Donor strain	Mating time (min)	Rates of recombinant yield from Lex⁻ over yield from Lex⁺ strain		
		Thr⁺ (Str^R)	Pro⁺ (Str^R)	His⁺ (Str^R)
AB259	20	0.70	0.17	
AB259	50	0.31	0.15	
AB259	80	0.21	0.12	0.07
KL 84	20	0.56	0.58	
KL 84	50	0.40	0.26	
KL 84	80	0.26	0.15	
Ra-2	90	0.21	0.25	0.26
KL983	30			0.39
KL983	70			0.27

[a] Strains AB1157 *lex⁺* and AB2494 *lex-1* were grown in LB broth and mated with the Hfr strains with gentle shaking at 37 C. All samples were blended prior to plating.

produced 58 to 70% as many recombinants as the Lex⁺ strain. Increasing the time of mating or selection for a later marker tended to decrease the recombinant yield in the Lex⁻ strain relative to the Lex⁺ strain. Similar crosses were also carried out with DM48 *lex-2* and DM49 *lex-3*. These strains behaved similarly to AB2494 *lex-1* and produced 23 to 60% of the normal (Lex⁺) numbers of recombinants.

Induction of mutations by radiation. Cultures of the streptomycin-sensitive strains DM800–DM803 were irradiated with UV, and the frequency of mutation to streptomycin resistance was measured in each strain. The mutation frequencies are given in Table 6. Among the survivors of irradiated cultures of DM800 *lex⁺*, considerably more Str^R mutants are found compared to unirradiated cultures. In contrast, in irradiated cultures of any of the three Lex mutants, there is little significant increase, if any, in the frequency of streptomycin-resistant mutants compared to unirradiated cultures.

UV-induced DNA degradation. The rates of degradation of DNA in unirradiated and UV-irradiated cultures of DM49 *lex-3* are shown in Fig. 4. The dose of UV used (100 ergs/mm^2) which resulted in 15% survival of the Lex⁺ and 1% survival of the Lex⁻ strain was followed by a far more rapid degradation of DNA in the Lex⁻ strain than in the Lex⁺ strain. Similar results have been obtained with *lex-1* and *lex-2* strains.

DISCUSSION

Three mutant strains of *E. coli* K-12 which are sensitive to UV, ionizing radiation, and alkylating agents, and which do not appear to be defective in genetic recombination (*see below*), have been shown to carry mutations (denoted *lex-1*, *-2*, and *-3*) located close to *uvrA* on the *E. coli* K-12 linkage map (25). One of them, *lex-1*, had been reported to be in approximately the same region of the linkage map by Howard-Flanders and Boyce (14). The order *metA-malB-lex-uvrA* has been established by three-factor transduction crosses.

Strains which are *lex⁺/lex⁻* merodiploids have been constructed by appropriate conjugation crosses and have been found to retain the mutant radiation-sensitive phenotype of the parental Lex⁻ haploid strains. From the apparent dominance of the *lex-1*, *-2*, and *-3* alleles, it may be concluded that the Lex⁻ phenotype results from the synthesis of a diffus-

TABLE 6. *Frequency of mutation-induction by ultraviolet light*

Strain	Dose	Percent survival	Total no. of Str^R colonies scored	Str^R mutants per 10^{10} survivors
DM800 *lex⁺*	0	100	37	4
	25	100	496	43
	50	100	~2,000	~250
DM803 *lex-1*	0	100	19	~1
	25	11	14	~5
	50	3	10	~8
DM801 *lex-2*	0	100	27	~5
	25	45	6	~3
	50	23	11	~11
DM802 *lex-3*	0	100	1	~0.1
	25	4	21	10
	50	1	6	20

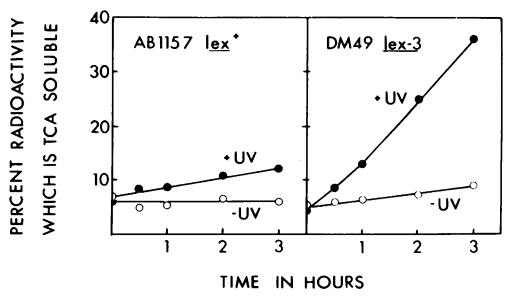

FIG. 4. *DNA breakdown in UV-irradiated cultures of Lex⁻ mutants.*

ible product which affects radiation sensitivity. The *lex* mutations may lie either in a structural or regulatory gene or in a regulating element (e.g., promoter, operator), i.e., act *trans* or *cis*.

Ogawa, Shimada, and Tomizawa (22) have found that another dominant mutation in *E. coli* K-12, denoted *uvrD3*, also affects radiation sensitivity. This mutation differs from the *lex* mutations in its location on the linkage map (*uvrD3* is linked to *metE*). Strains carrying *uvrD* and *lex* mutations are similar with respect to their UV and X-ray sensitivities, and UV-irradiated mutant cultures in both cases degrade their DNA at a high rate (22).

The recombinant-forming ability of Lex$^-$ strains has been measured. The large yield of recombinants obtained for short matings and selection for early markers (Table 2) indicates that the strains produce recombinants at a near normal frequency (58–70%). The recombinant yields relative to a Lex$^+$ strain decrease for longer mating times and more distant markers. This effect might be due in part to a defect in chromosome transfer to Lex$^-$ recipient strains. Thus, the transfer of late markers might be reduced compared to Lex$^+$ strains. There is also an apparent decrease in the recovery of a given class of recombinants in a Lex$^-$ strain when matings are allowed to proceed for longer times, e.g., Thr$^+$ (Str^R) recombinants in matings with AB259. Presumably, this decrease is associated with merozygotes which have received longer pieces of DNA from the Hfr. There is presently no simple explanation of this observation. When Lex$^-$ strains are used as recipients in transduction crosses, the yield of transductants is approximately the same as that obtained with Lex$^+$ recipients (D. Mount, *unpublished observation*). This supports the conclusion that they are probably not recombination deficient.

When cultures of streptomycin-sensitive Lex$^-$ mutant strains are irradiated with UV, few, if any, streptomycin-resistant mutations are induced compared to high yields of mutations in irradiated cultures of Lex$^+$ strains. These results confirm an earlier report of Witkin (32). Bridges et al. (3) have shown that the apparently similar *exr* mutations in *E. coli* B also reduce the mutagenic effects of X rays and thymine starvation of a thymine-requiring mutant.

Based upon her observations that genetic recombination and UV-induced mutagenesis are both reduced in Exr$^-$ and Lex$^-$ strains, Witkin (31) has concluded that genetic recombination is mutagenic in a strain bearing a *lex*$^+$ or *exr*$^+$ gene. Our results do not substantiate the claim that Lex$^-$ mutants are defective in genetic recombination.

Two more general explanations have been put forward to account for the induction of mutations in bacteria by UV. One of these states that mutations arise as errors when DNA containing thymine dimers is replicated (32). The other proposes that the mutations arise by an inaccurate excision-repair mechanism (4, 21). To our knowledge, there is little compelling evidence for or against either model. Since *lex* mutations largely abolish UV-induced mutations, they may be useful for deciphering which one, if either, of these explanations is the correct one.

The high rate of DNA degradation in irradiated cultures of a Lex$^-$ mutant has also been described by Howard-Flanders and Boyce (14). In the case of *recA*$^-$ mutants, the high rate of degradation is not observed if the *recA* mutant also bears an additional *recB* or *recC* mutation (26). The *recB* and *recC* mutations also abolish an adenosine triphosphate (ATP)-dependent exonuclease activity found in extracts of *rec*$^+$ strains (2, 5, 23). The correlation between absence of the exonuclease and absence of high rate of UV-induced DNA breakdown in *recA*$^-$ *recB*$^-$ and *recA*$^-$ *recC*$^-$ double mutants suggests that the exonuclease is the enzyme responsible for the high rate of breakdown. We have observed (Moody, Mount, and Low, *in preparation*) that a considerably slower rate of degradation is observed in irradiated cultures of *lex*$^-$ *recB*$^-$ and *lex*$^-$ *recC*$^-$ double mutants compared to the *lex*$^-$ single mutant. This observation suggests that the ATP-dependent nuclease is probably present in Lex$^-$ mutant strains and also degrades their DNA when they are irradiated.

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