JOURNAL OF BACTERIOLOGY, July 1980, p. 212-220
0021-9193/80/07-0212/09$02.00/0

Vol. 143, No. 1

Involvement of Genes *uvrD* and *recB* in Separate Mutagenic Deoxyribonucleic Acid Repair Pathways in *Escherichia coli* K-12 *uvrB5* and B/r *uvrA155*

NEIL J. SARGENTINI* AND KENDRIC C. SMITH

Department of Radiology, Stanford University School of Medicine, Stanford, California 94305

We compared the ultraviolet radiation-induced reversion of nonsense (*lacZ53*) and missense (*leuB19*) mutations in *uvrB5*, *uvrB5 uvrD3*, *uvrB5 recB21*, and *uvrB5 uvrD3 recB21* strains of *Escherichia coli* K-12. Nonsense (*trpE65*) reversion was also compared in similar derivatives of *E. coli* B/r *uvrA155*. The *uvrD* mutation reduced mutagenesis in every case, but had its main effect in cells ultraviolet irradiated with low fluences (<0.6 J m^{-2}). The effect of the *recB* mutation varied; it decreased Leu and Trp reversion, but had little effect on Lac reversion. The effect of the *uvrD recB* combination was a gross reduction in mutagenesis. Only in the case of Lac reversion was appreciable mutagenesis detected (at fluences >0.3 J m^{-2}). These results indicate that separate *uvrD*- and *recB*-controlled pathways exist for ultraviolet radiation mutagenesis.

Escherichia coli deals with UV radiation-induced DNA damage by employing three repair modes: photoreactivation (reviewed in reference 26), excision repair, and postreplication repair (reviewed in reference 11). The mechanism for photoreactivation appears to be error-free. Mutation fixation that is dependent on the *uvrA* gene (i.e., error-prone excision repair) has been demonstrated (7, 22, 23). However, excision-deficient strains, e.g., *uvrA* or *uvrB* strains (4, 15, 27), show enhanced UV radiation mutagenesis (1, 12, 17, 38). Presumably, DNA repair of UV radiation damage in *uvrA* and *uvrB* strains is more error-prone than in *uvr*$^+$ strains (6, 37).

Postreplication repair is accomplished by at least five separate genetically controlled pathways (43). One pathway is inhibitable by chloramphenicol (CAP) (25, 43) and is dependent on *recA*$^+$ (29), *uvrD*$^+$ (43), *recB*$^+$ (43), and *lexA*$^+$ (25, 43) functions. Since CAP also blocks the fixation of UV radiation-induced mutations in a *uvrA* strain (25), the CAP-sensitive pathway of postreplication repair is assumed to be responsible for UV radiation mutagenesis in excision-deficient strains (25, 43). The CAP-sensitive pathway employs the *recA*$^+$ and *lexA*$^+$ functions (25, 29, 43), which is consistent with the absolute requirement of these functions for UV radiation mutagenesis (16, 19, 36, 39, 40). However, the involvement of the *uvrD*$^+$ and *recB*$^+$ components of the CAP-sensitive pathway in mutagenesis has not been resolved (13, 19, 20, 21, 28, 40, 41).

We theorized that the putative mutagenic pathway of postreplication repair was composed of alternate pathways dependent on either the *uvrD*$^+$ or *recB*$^+$ function. If premutational lesions could be readily processed via either of these two branches, then neither deficiency alone would be capable of totally blocking error-prone repair in an excision-deficient strain. This model predicted that a *uvrB uvrD recB* triple mutant (deficient in both mutagenic pathways) would be refractory to UV radiation mutagenesis. Our data are consistent with the hypothesis that the *uvrD* and *recB* genes control alternate pathways of mutagenic DNA repair in excision-deficient strains.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* strains used in this study are listed in Table 1. The transduction techniques used bacteriophage P1 and P1::Tn*9*(Cm^r)cts and have been described (24, 42, 43). A trimethoprim resistance selection procedure (31) was used to isolate *thyA* mutants.

Media. *E. coli* K-12 cells were cultured in a 0.4% glucose-salts medium (10) supplemented with thiamine-hydrochloride 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 supplemented glucose-salts plates (27 ml) solidified with Noble agar (Difco Laboratories) (34) at 1.6%, containing lactose at 0.4% and glucose at 1,200 μg/ml (glu-1200). Plates lacking any glucose (glu-0) were used to quantitate spontaneous Lac$^+$ mutants present when the culture was harvested. The glucose in the glu-1200 plates was necessary to allow the expression of induced Lac$^+$ mutants, and also allowed relatively small numbers of Lac$^-$ cells to grow into countable colonies for the estimation of viability. Survival and reversion to Leu$^+$ were assayed as above on analogous plating media (i.e., leu-15 and leu-0). *E. coli* B/r cells were cultured in 0.4% glucose-salts medium

TABLE 1. *Strains of E. coli used*

Strain designation	Relevant genotype	Other genotype[a]	Source or derivation
SR250	*uvrB5*	*leuB19 metE70 thyA36 deo(C2?) rha-5 lacZ53 rpsL151*	DY145, (42)
SR255	*recB21*	*leuB6 thr-1 proA2 argE3 his-4 deoB16 lacY1 ara-14 galK2 mtl-1 xyl-5 thi-1 rpsL31 tsx-33 supE44*	(43)
SR257	*uvrB5 recB21*	*leuB19 metE70 deo(C2?) rha-5 lacZ53 rpsL151*	DY157, (43)
SR282	*uvrB5 uvrD3*	*leuB19 thyA36 deo(C2?) rha-5 lacZ53 rpsL151*	DY179, (43)
SR287	*uvrB5 uvrD3*	*leuB19 deo(C2?) rha-5 lacZ53 rpsL151*	P1·SR255 × SR282 (select Thy$^+$)[b]
SR288	*uvrB5 uvrD3 recB21*	*leuB19 deo(C2?) rha-5 lacZ53 rpsL151*	DY197, (43)
SR576	*uvrA155 uvrD3*	*trpE65 sulA1*	DY214, (28)
SR577	*uvrA155*	*trpE65 sulA1*	DY215, (28)
SR662	*uvrA155 uvrD3*	*trpE65 thyA sulA1*	SR576 (select for trimethoprim resistance)
SR663	*uvrA155 uvrD3*	*trpE65 sulA1*	P1::Tn*9c*·SR255 × SR662 (select Thy$^+$)
SR664	*uvrA155 uvrD3 recB21*	*trpE65 sulA1*	Same as SR663
SR665	*uvrA155*	*trpE65 thyA sulA1*	SR577 (select for trimethoprim resistance)
SR666	*uvrA155*	*trpE65 sulA1*	P1::Tn*9c*·SR255 × SR665 (select Thy$^+$)
SR667	*uvrA155 recB21*	*trpE65 sulA1*	Same as SR666

[a] Genotype symbols are those used by Bachmann et al. (2). All strains are F$^-$ and λ$^-$.
[b] Strain constructed by D. A. Youngs.

(10) supplemented with 1 mM L-tryptophan. Survival and reversion to Trp$^+$ were assayed on 0.4% glucose-salts medium plates supplemented with nutrient broth (Difco) at 200 μg/ml in place of tryptophan [i.e., trp(NB-200)]. Plates lacking nutrient broth (trp-0) were used in the same fashion as the glu-0 and leu-0 plates.

UV radiation mutagenesis procedure. Cells in logarithmic growth phase were centrifuged (6,000 × *g* for 6 min), washed once, and suspended in 67 mM phosphate buffer, pH 7.0 (9), to an optical density at 650 nm of 0.2 in a Zeiss PMQII spectrophotometer. This optical density corresponded to 1×10^8 to 3×10^8 colony-forming units per ml, depending on the strain being assayed.

Cells were UV irradiated as described previously (24). Briefly, the cells were UV irradiated (254 nm) as required with an average-incident fluence rate (corrected for cell density [24]) of ~0.2 or ~0.04 J m^{-2} s^{-1}. These cells were concentrated 1-, 10-, or 100-fold by centrifugation, depending on the mutant assay system, and plated for mutants and viability. Mutant colonies were counted when their number no longer increased (after 3 to 5 days at 37°C, depending on the strain). Data were compiled generally from four or more experiments per UV radiation fluence, with four or more mutant selection plates and three viability plates per fluence.

Quantitation of mutagenesis. The UV radiation-induced mutant frequency (MF) was calculated per average mutant selection plate according to a rearranged version of the formula of Bridges (5):

$$MF = (M_x)(1 \times 10^8)/(S_c)(\text{volume plated})$$

where $M_x = M_t - M_{p0} + M_0(1 - SF_c)$, and M_t is the number of mutant colonies arising from irradiated cells on mutant selection plates [i.e., glu-1200, leu-15, or trp(NB-200)], M_{p0} is the number of mutant colonies arising from nonirradiated cells on mutant selection plates, M_0 is the number of mutant colonies arising from nonirradiated cells on plates lacking the growth-limiting nutrient (i.e., glucose, leucine, or nutrient broth), SF_c is the surviving fraction of irradiated cells as influenced by the concentration technique used, M_x is the number of radiation-induced mutants per mutant selection plate, 1×10^8 is the factor included to normalize mutant frequencies to the number of mutants per 1×10^8 survivors, volume plated is the volume of cell suspension plated (e.g., 0.2 ml) in the mutant assays, and S_c is the colony-forming units per milliliter in this suspension.

RESULTS

Effect of the *uvrD3* and *recB21* mutations on UV radiation survival. As observed previously (43), an *E. coli* K-12 *uvrB5 uvrD3 recB21* strain is more sensitive to UV radiation than related *uvrB5 uvrD3* or *uvrB5 recB21* strains (Fig. 1a), suggesting that the *recB* and *uvrD* genes affect separate pathways of DNA repair. This observation was extended here for the corresponding *E. coli* B/r *uvrA155* strains (Fig. 1b).

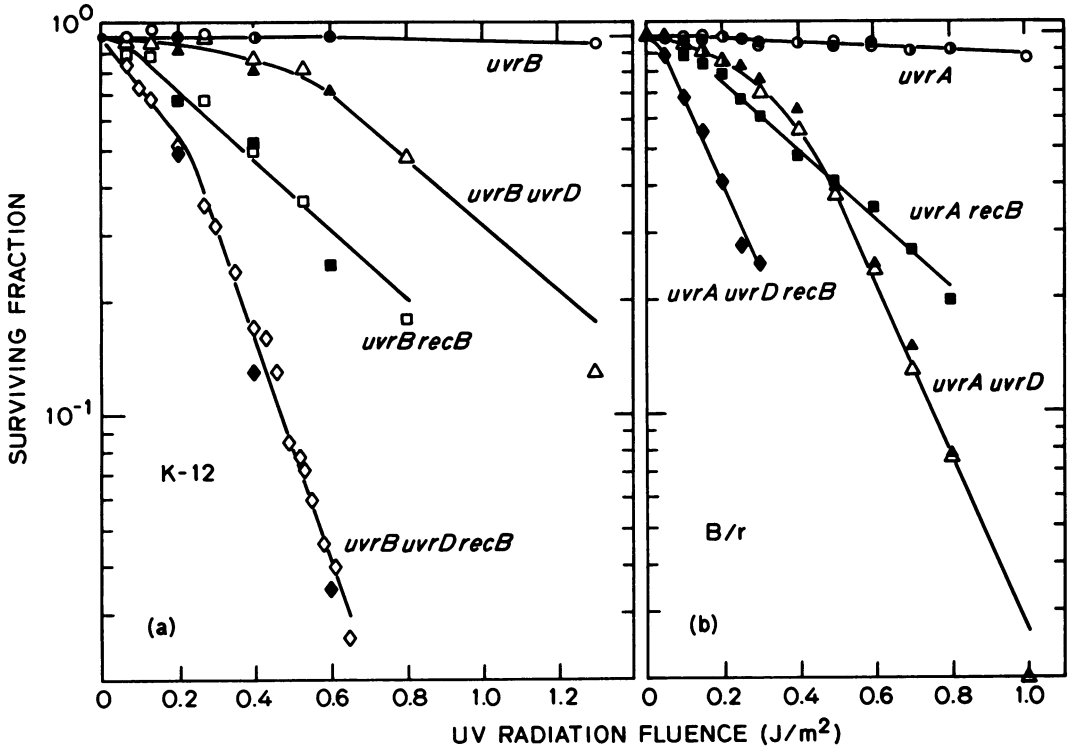

FIG. 1. *UV radiation survival of E. coli strains. Cells were UV irradiated, diluted, and spread on three plates per fluence tested, with three or more experiments per data point. In (a), the cells were spread on either glu-1200 (open symbols) or leu-15 (closed symbols) plates. In (b), the cells were spread on trp(NB-200) plates. E. coli K-12 strains are: SR250 (uvrB5), SR257 (uvrB5 recB21), SR287 (uvrB5 uvrD3), and SR288 (uvrB5 uvrD3 recB21). E. coli B/r strains are: SR576 (uvrA155 uvrD3), △; SR577 (uvrA155), ○; SR663 (uvrA155 uvrD3), ▲; SR664 (uvrA155 uvrD3 recB21), ◆; SR666, (uvrA155), ●; and SR667 (uvrA155 recB21), ■.*

The UV radiation survival for *E. coli* K-12 was similar regardless of the Lac^+ or Leu^+ mutant selection plating medium used here (Fig. 1a).

Optimization of conditions for assaying UV radiation mutagenesis. In our initial attempt to ascertain whether the *uvrB uvrD recB* strain was nonmutable, we chose to concentrate by 100-fold the cells to be plated in the mutagenesis assays. The goal was to screen large numbers of cells for mutagenesis. However, we used a mutant selection medium (glu-300) that had been formulated for the optimal detection of UV radiation mutagenesis in a related control strain (i.e., nonconcentrated *uvrB5* cells). This UV radiation mutagenesis assay indicated that the *uvrB uvrD recB* strain was nonmutable by UV radiation (30). A problem associated with plating highly concentrated UV-irradiated cells on mutant selection medium is indicated in Table 2, which shows that the *uvrB uvrD recB* strain appeared to change from nonmutable to mutable when the concentration of growth-limiting nutrient (i.e., glucose) was increased. Therefore, we designed a more proper protocol to be used in this paper to test whether a strain was deficient in mutability relative to a control strain. When comparing a given set of strains, standard irradiation fluences, cell concentration factors, and plating conditions were used. These mutagenesis assay conditions were optimized for the *uvrD* and *recB* control strains in each set shown here. For example, a glucose concentration of 1,200 μg/ml appeared to be necessary to obtain maximal expression of UV radiation mutagenesis (*lacZ53* → Lac^+) when 0.2 ml of 10-fold-concentrated cells was spread per plate (Fig. 2). Spontaneous mutagenesis (plate mutants corrected for preexisting mutants) increased linearly with glucose concentration (data not shown) in experiments such as those described in Fig. 2.

UV radiation mutagenesis in *E. coli* K-12 *uvrB5* strains. Experiments comparing the effects of the *uvrD3* and *recB21* mutations on UV radiation mutagenesis in *E. coli* K-12 *uvrB5* cells had the following results: (i) the *recB* mutation

reduced mutagenesis by ~60% for Leu reversion (*leuB19* → Leu$^+$; Table 3), whereas Lac reversion (*lacZ53* → Lac$^+$; Table 4) was only slightly reduced; (ii) the *uvrD* mutation reduced mutagenesis by ~90% for Leu reversion (Table 3) and by ~80% for Lac reversion (Table 4); (iii) UV radiation-induced Leu reversion was generally not detected in the *uvrB uvrD recB* strain (Table 3), whereas considerable UV radiation-induced Lac reversion (Table 4) (especially at fluences greater than ~0.3 J m^{-2}) was detected in this strain. This residual mutagenesis was not detected in a related *uvrB5 lexA101* strain (this strain is slightly less radiosensitive than the *uvrB uvrD recB* strain) (data not shown), and therefore is probably not an artifact of the Lac reversion system. The UV radiation mutagenesis deficiencies noted for Lac reversion in the *uvrB uvrD* and *uvrB uvrD recB* strains are more easily compared in Fig. 3, which shows that the deficiency in these two strains is evident only at fluences less than ~0.5 J m^{-2}.

UV radiation mutagenesis in *E. coli* B/r strains. UV radiation mutagenesis (*trpE65* → Trp$^+$) was reduced by ~80% in both the *uvrA155 uvrD3* and *uvrA155 recB21* strains relative to the *uvrA155* strain, and the *uvrA155 uvrD3 recB21* strain was grossly deficient in Trp reversion (Table 5).

DISCUSSION

To test our hypothesis that the *recB* and *uvrD* genes control alternate pathways of error-prone DNA repair, we examined (i) the effect of the single *recB* and *uvrD* mutations on UV radiation mutagenesis in *uvrA* and *uvrB* strains and (ii) the ability of the *uvrB uvrD recB* strain to undergo UV radiation mutagenesis.

TABLE 2. *Effect of glucose concentration in mutant selection plates on the yield of UV radiation-induced Lac$^+$ mutants of E. coli K-12 uvrB5 uvrD3 recB21 (SR288)*[a]

Glucose concn (mg/ml)	M_t	M_{p0}	M_x	MF (Lac$^+$/10^8 cells)
0.0	1.5	7.4	−0.3	−0.08
0.3	7.2	22.8	−10.0	−2.8
1.2	50.4	57.9	−1.9	−5.3
2.0	99.5	90.6	14.5	4.0
3.0	148.5	107.6	46.5	12.9

[a] Data compiled from two experiments per mutant selection plating medium in which the glucose concentration (and hence the growth yield) was varied. All terms are defined in the text. MF was calculated by using M_0 = 7.4, SF_c = 0.24, and S_c = 1.8 × 10^9 colony-forming units/ml, and represents the mutant frequency induced by 0.33 J m^{-2} when cells concentrated 100-fold were plated on mutant selection medium.

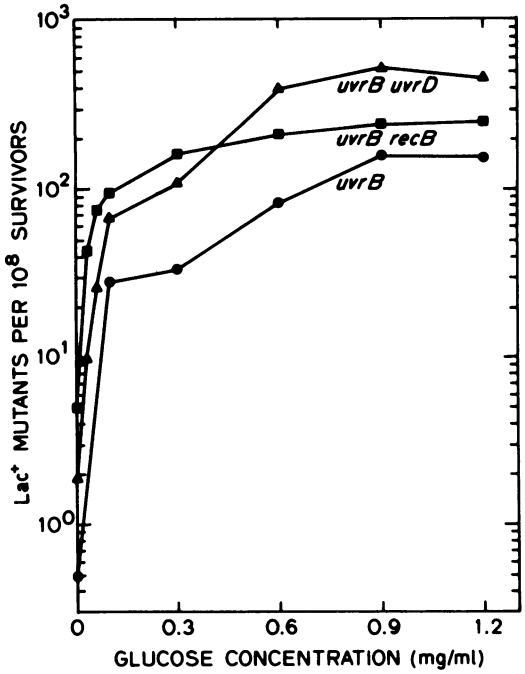

FIG. 2. *Effect of glucose concentration (in the mutant selection plates) on UV radiation mutagenesis (lacZ53 → Lac$^+$) in E. coli K-12 uvrB, uvrB recB, and uvrB uvrD strains. Strains: SR250 (uvrB5), 0.4 J m^{-2}; SR257 (uvrB5 recB21), 0.9 J m^{-2}; and SR287 (uvrB5 uvrD3), 1.2 J m^{-2}. Cells concentrated 10-fold were spread (0.2 ml) upon each of four plates per glucose concentration per experiment. Each point is the average of data from two experiments, except for those for strain SR257, which represent a single experiment.*

Effect of exonuclease V (*recB* or *recC*) deficiencies on UV radiation mutagenesis. The effect of *recB* or *recC* deficiencies on UV radiation mutagenesis has not been resolved. Witkin (40, 41) reported that the *recB21* allele reduced UV radiation mutagenesis by ~90%. Hill and Nestmann (13) ascribed this decrease in mutagenesis to the lethal-sectoring phenomenon (i.e., in *recB* mutants not all daughter cells are viable; reviewed in reference 8), since they obtained a *rec*$^+$ level of mutagenesis with their *recC22* strain under nonselective mutation assay conditions. However, Morse and Pauling (21) noted a reduction in mutations in bacteriophage λ when plated on *lig-7* (polynucleotide ligase-deficient) cells if an additional *recB21* mutation was added to the host. They concluded that the *recB21* deficiency resulted in reduced mutagenesis under circumstances not affected by the

TABLE 3. *UV radiation mutagenesis (leuB19 → Leu⁺) of E. coli K-12 uvrB5 strains*[a]

Strain	UV radiation fluence (J m^{-2})	Surviving fraction	M_t	M_{p0}	$M_0(1 - SF_c)$	$(M_x)^b$	S_c	Leu⁺ mutants/10⁸ survivors[c]
SR250	0.2	1.0	134	70	61 (1 − 0.89)	71	9.9×10^9	3.8 ± 0.8 (3)
(*uvrB5*)	0.4	1.0	263	70	61 (1 − 0.88)	200	1.01×10^{10}	9.8 ± 0.9 (3)
	0.6	1.0	438	70	61 (1 − 0.94)	372	1.06×10^{10}	17.8 ± 4.6 (3)
SR257	0.2	0.68	19.2	2.4	0.2 (1 − 0.80)	16.8	5.9×10^9	1.5 ± 1.0 (3)
(*uvrB5*	0.4	0.52	32.1	2.4	0.2 (1 − 0.58)	29.8	4.2×10^9	3.7 ± 1.9 (3)
recB21)	0.6	0.25	36.5	2.4	0.2 (1 − 0.30)	34.2	2.2×10^9	7.9 ± 1.8 (3)
SR287	0.2	0.92	8.1	2.4	0.6 (1 − 0.91)	5.8	1.08×10^{10}	0.3 ± 0.1 (5)
(*uvrB5*	0.4	0.81	24.6	2.4	0.6 (1 − 0.80)	22.3	9.5×10^9	1.2 ± 0.3 (5)
uvrD3)	0.6	0.72	34.0	2.4	0.6 (1 − 0.64)	31.8	7.7×10^9	2.1 ± 0.8 (5)
SR288	0.2	0.51	0.8	1.4	0.2 (1 − 0.51)	−0.5	3.7×10^9	−0.08 ± 0.09 (4)
(*uvrB5*	0.4	0.31	1.4	1.4	0.2 (1 − 0.17)	0.17	1.24×10^9	0.03 ± 0.34 (4)
uvrD3	0.6	0.035	0.9	1.4	0.2 (1 − 0.035)	−3.1	2.5×10^8	−0.9 ± 1.3 (4)
recB21)								

[a] All terms are defined in the text. All data (except last column) are averages compiled from three to five experiments (per strain), each one using four mutant selection plates and three viability plates per UV radiation fluence.

[b] Average UV radiation-induced Leu⁺ mutants per 0.2 ml, from irradiated cell suspensions that had been concentrated 100-fold before being spread upon leu-15 plates.

[c] Average mutant frequency ± standard deviation. The number in parentheses indicates the total experiments included in this compilation. These data are averages of the calculated mutant frequencies from the individual experiments rather than a direct calculation of the averaged data in this table.

lethal-sectoring phenomenon. Miura and Tomizawa (20) reported normal UV radiation mutagenesis of bacteriophage λ when plated on *recB21* or *recC22* hosts.

Under conditions designed to minimize the effects of lethal sectoring, i.e., extended incubation of mutant selection plates (13), we found that the effect of the *recB21* mutation on UV radiation mutagenesis was variable. For example, mutagenesis was only slightly reduced when scoring Lac reversion (Table 4), but was reduced by ~60% when scoring Leu reversion (Table 3) in the same *E. coli* K-12 *uvrB recB* strain over the same UV radiation fluence range. The *recB* mutation also reduced mutagenesis in the *E. coli* B/r *uvrA* strain, when scoring Trp reversion (Table 5), to a degree similar to that reported by Witkin (41). We suggest that the apparently contradictory mutagenesis data for *recB* strains, reported here and in the literature, are consistent with exonuclease V playing a role in error-prone repair only in certain situations (e.g., those involving certain types of premutational lesions or chromosomal regions or both).

Effect of the *uvrD3* deficiency on UV radiation mutagenesis. Miura and Tomizawa (19) assayed clear-plaque mutations in bacteriophage λ at a UV radiation fluence of 40 J m^{-2} incident upon the *uvrD3* host cells, and concluded that the *uvrD* gene played no obvious role in UV radiation mutagenesis. Smith (28) reached a similar conclusion from UV radiation mutagenesis data (compared relative to UV radiation fluence) on *E. coli* K-12 *uvrB5 uvrD3* cells when scoring for Leu reversion (*leuB19* → Leu⁺), and in *E. coli* B/r *uvrA155 uvrD3* cells when scoring for Trp reversion (*trpE65* → Trp⁺).

Data presented in this study show that the *uvrD3* mutation does reduce UV radiation mutagenesis when measured by Leu reversion (*leuB19* → Leu⁺) in *E. coli* K-12 *uvrB5 uvrD3* (Table 3) or by Trp reversion (*trpE65* → Trp⁺) in *E. coli* B/r *uvrA155 uvrD3* (Table 5). Mutagenesis in the *E. coli* K-12 *uvrB uvrD* strain was also reduced when measured by Lac reversion (Table 4). A reexamination of the earlier study (28) showed that those data and our data are consistent with regard to the effect of the *uvrD3* mutation on mutagenesis, when compared over the same UV radiation fluence range used here. Therefore, the *uvrD* gene product (function unknown) must play a role in UV radiation mutagenesis at least in *uvrA* and *uvrB* strains. The effect of *uvrD* mutations in *uvrA*⁺ and *uvrB*⁺ strains is less clear. Venturini and Monti-Bragadin (35) indicated a *uvrD3*-related reduction (by ~50%) in UV radiation mutagenesis, whereas enhanced UV radiation mutagenesis has been observed for *uvrD101* mutants (3, 32). Other evidence supporting a role for the *uvrD* locus in error-prone repair comes from a recent report (18) showing that *uvrE* (33) and *mutU* (14)

TABLE 4. *UV radiation mutagenesis (lacZ53 → Lac⁺) of E. coli K-12 uvrB5 strains*[a]

Strain	UV radiation fluence (J m^{-2})	Surviving fraction	M_t	M_{p0}	$M_0(1 - SF_c)$	$(M_x)^b$	S_c	Lac⁺ mutants/10^8 survivors[c]	
SR250	0.068	1.03	862	780	122 (1 − 0.93)	91	1.35×10^9	35 ± 26	(10)
(*uvrB5*)	0.13	1.07	953	780	116 (1 − 0.98)	175	1.42×10^9	62 ± 28	(12)
	0.27	1.06	1,094	780	122 (1 − 0.95)	320	1.38×10^9	118 ± 36	(10)
	0.40	1.04	1,265	780	122 (1 − 0.98)	487	1.42×10^9	178 ± 58	(10)
SR257	0.068	0.91	674	632	30 (1 − 0.89)	45	1.02×10^9	18 ± 53	(8)
(*uvrB5*	0.13	0.86	717	604	28 (1 − 0.91)	116	1.00×10^9	53 ± 50	(9)
recB21)	0.27	0.70	789	632	30 (1 − 0.71)	166	8.0×10^8	100 ± 87	(8)
	0.40	0.49	788	632	30 (1 − 0.50)	171	5.9×10^8	134 ± 110	(8)
	0.53	0.38	830	632	30 (1 − 0.38)	217	4.3×10^8	251 ± 125	(8)
	0.65	0.26	829	634	23 (1 − 0.28)	212	2.7×10^8	488 ± 555	(4)
	0.80	0.18	801	632	30 (1 − 0.17)	194	1.99×10^8	512 ± 288	(8)
SR287	0.068	0.97	308	284	26 (1 − 0.94)	26	1.77×10^9	7 ± 5	(6)
(*uvrB5*	0.13	0.97	355	297	25 (1 − 0.93)	60	1.78×10^9	17 ± 10	(7)
uvrD3)	0.27	0.90	399	284	26 (1 − 0.82)	120	1.50×10^9	41 ± 8	(6)
	0.40	0.74	494	297	26 (1 − 0.71)	205	1.29×10^9	88 ± 22	(6)
	0.53	0.58	548	284	26 (1 − 0.55)	276	9.6×10^8	147 ± 33	(6)
	0.80	0.31	624	284	26 (1 − 0.29)	358	4.9×10^8	391 ± 82	(6)
	1.3	0.099	506	284	26 (1 − 0.069)	246	1.19×10^8	1,045 ± 422	(6)
SR288	0.068	0.85	79.5	80.4	11.9 (1 − 0.80)	1.5	7.7×10^8	1.7 ± 8.3	(10)
(*uvrB5 uvrD3*	0.10	0.81	45.2	48.4	7.4 (1 − 0.76)	−1.4	8.1×10^8	−1.3 ± 7.3	(5)
recB21)	0.13	0.69	79.8	80.7	11.3 (1 − 0.66)	2.9	6.3×10^8	2.8 ± 8.8	(11)
	0.20	0.53	49.4	48.4	7.4 (1 − 0.44)	5.1	4.6×10^8	3.8 ± 9.8	(5)
	0.27	0.36	79.8	80.4	11.9 (1 − 0.37)	6.9	3.6×10^8	10.7 ± 18.7	(10)
	0.30	0.33	47.2	48.4	7.4 (1 − 0.31)	3.9	3.3×10^8	6.0 ± 13.2	(5)
	0.35	0.24	44.2	48.4	7.4 (1 − 0.22)	1.6	2.3×10^8	3.2 ± 33	(5)
	0.40	0.17	68.3	69.8	10.4 (1 − 0.17)	7.1	1.68×10^8	24.3 ± 35.2	(15)
	0.43	0.15	46.0	48.4	7.4 (1 − 0.14)	4.0	1.52×10^8	9.5 ± 59.4	(5)
	0.46	0.12	56.3	51.1	9.1 (1 − 0.12)	13.2	1.30×10^8	56.3 ± 37.7	(4)
	0.49	0.094	54.7	48.4	7.4 (1 − 0.090)	13.0	9.4×10^7	68.4 ± 70.0	(5)
	0.52	0.076	62.8	50.2	7.9 (1 − 0.086)	19.8	9.2×10^7	111 ± 14	(4)
	0.53	0.072	84.5	80.4	11.9 (1 − 0.064)	15.2	6.3×10^7	156 ± 201	(10)
	0.55	0.060	67.9	54.4	10.3 (1 − 0.069)	23.1	7.6×10^7	150 ± 60	(3)
	0.58	0.048	61.6	48.4	7.4 (1 − 0.046)	20.3	4.9×10^7	200 ± 134	(5)
	0.61	0.038	57.2	48.4	7.4 (1 − 0.041)	15.9	4.3×10^7	174 ± 161	(5)
	0.65	0.026	54.0	48.4	7.4 (1 − 0.029)	12.8	3.0×10^7	198 ± 282	(5)

[a] All terms are defined in the text. All data (except last column) are averages compiled from 4 to 15 experiments (per strain), each one using four mutant selection plates and three viability plates per UV radiation fluence.

[b] Average radiation-induced Lac⁺ mutants per 0.2 ml, from irradiated cell suspensions that had been concentrated 10-fold before being spread upon glu-1200 plates.

[c] See Table 3.

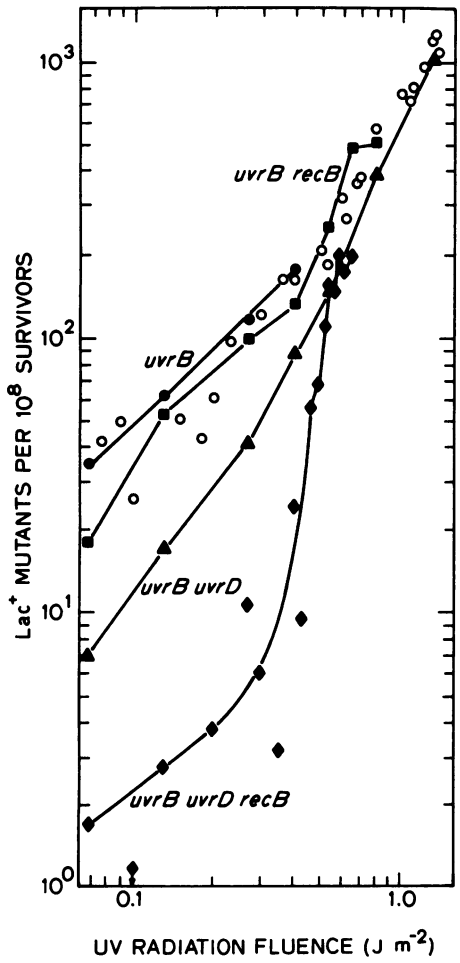

FIG. 3. *UV radiation mutagenesis to Lac$^+$ of E. coli K-12 uvrB5 strains. Ten-fold-concentrated cells (closed symbols) or nonconcentrated cells (open symbols) were spread (0.2 ml, four plates per fluence per experiment) on glu-1200 or glu-300 plates, respectively. Symbols: SR250 (uvrB5), ●, ○; SR257 (uvrB5 recB21), ■; SR287 (uvrB5 uvrD3), ▲; SR288 (uvrB5 uvrD3 recB21), ◆. The data for strain SR250 are from reference 24.*

mutations are mutator alleles of the *uvrD* gene.

Effect of a combination of the *uvrD3* and *recB21* deficiencies on UV radiation mutagenesis. The data discussed to this point generally show the involvement of the *recB* and *uvrD* genes in UV radiation mutagenesis. This is consistent with evidence showing the involvement of these genes in the CAP-sensitive pathway of postreplication repair (43), a pathway that has been proposed to be involved in UV radiation mutagenesis (25, 43). Since mutations at either locus, i.e., *uvrD3* and *recB21*, did not totally prevent UV radiation mutagenesis in the fashion of some *recA* and *lexA* mutations (16, 19, 39, 40), we theorized that alternate pathways, i.e., a *recB* pathway and a *uvrD* pathway, may exist in the context of error-prone repair. This hypothesis for alternate error-prone repair pathways suggested that a *uvrB uvrD recB* strain should not be mutable by UV radiation.

UV radiation mutagenesis was generally not detected in the *uvrB5 uvrD3 recB21* strain of *E. coli* K-12 when assayed for Leu reversion (Table 3), and reversion to Lac$^+$ was greatly diminished (Table 4, Fig. 3), as predicted by the alternate error-prone pathway hypothesis. However, this was only true at UV radiation fluences less than ~0.3 J m^{-2}. At fluences greater than ~0.5 J m^{-2}, the Lac$^+$ mutant frequency was similar to that for the other control strains (Fig. 3).

The *uvrD3 recB21* combination also caused the *E. coli* B/r *uvrA155* strain to be generally nonmutable by UV radiation (Table 5). Thus, the general conclusion from studying *uvrA* (or *uvrB*) *uvrD recB* mutants is that the *uvrD* and *recB* genes affect alternate pathways involved in error-prone repair of UV radiation damage. The residual mutagenesis seen in the *uvrB uvrD recB* strain (Fig. 3) (but not in a *uvrB5 lexA101* strain) indicates that other error-prone mechanisms not strictly blocked by *uvrD3* and *recB21* mutations can apparently operate (at least for Lac reversion) at fluences >0.3 J m^{-2}. Besides this residual mutagenesis, other *uvrD recB*-independent mechanism(s) appear to operate in *uvrA$^+$ uvrB$^+$* cells (unpublished observation; 3) and will be the topic of a future publication.

Conclusion. We have examined Lac, Leu, and Trp reversion in *uvrA* and *uvrB* strains of *E. coli* and found, in every case, evidence consistent with the notions that not only are the *recB* and *uvrD* genes involved in UV radiation mutagenesis, but also their involvement is on alternate error-prone repair pathways. This model provides an explanation for the apparently contradictory UV radiation mutagenesis data in the literature (13, 20, 21, 40, 41) and in this study concerning the involvement of exonuclease V in UV radiation mutagenesis. In addition, our data suggest the existence of at least one error-prone DNA repair pathway that is *recB uvrD*-independent (exemplified by the residual UV radiation-induced Lac reversion in the *uvrB uvrD recB* strain).

TABLE 5. *UV radiation mutagenesis (trpE65 → Trp⁺) of E. coli B/r uvrA155 strains*[a]

Strain	UV radiation fluence (J m^{-2})	M_t	M_{p0}	$M_0(1 - SF_c)$[b]	(M_z)[c]	S_c	Trp⁺ mutants/10^8 survivors[d]	
SR666	0.05	43.0	31.8	2.4 (1 − 0.98)	11.2	3.17×10^8	18 ± 5	(4)
(*uvrA155*)	0.10	54.8	31.8	2.4 (1 − 0.98)	23.0	3.17×10^8	36 ± 7	(4)
	0.15	76.9	31.8	2.4 (1 − 0.98)	45.1	3.20×10^8	70 ± 9	(4)
	0.20	100.9	31.8	2.4 (1 − 0.99)	69.0	3.21×10^8	108 ± 10	(4)
	0.25	126.9	31.8	2.4 (1 − 0.97)	95.2	3.17×10^8	151 ± 12	(4)
	0.30	173.0	33.1	1.6 (1 − 0.96)	140.0	3.20×10^8	219 ± 25	(3)
	0.40	248.9	33.1	1.6 (1 − 0.96)	215.9	3.17×10^8	341 ± 18	(3)
	0.50	348.1	33.1	1.6 (1 − 0.95)	315.1	3.19×10^8	498 ± 72	(3)
	0.60	476.0	33.1	1.6 (1 − 0.94)	443.0	3.14×10^8	707 ± 28	(3)
	0.70	556.1	33.1	1.6 (1 − 0.91)	523.1	3.01×10^8	873 ± 144	(3)
	0.80	698.6	33.1	1.6 (1 − 0.92)	665.6	3.07×10^8	1,084 ± 159	(3)
SR667	0.05	11.0	9.7	0.3 (1 − 0.98)	1.3	1.27×10^8	5 ± 6	(5)
(*uvrA155*	0.10	11.6	9.7	0.3 (1 − 0.89)	1.9	1.20×10^8	9 ± 11	(5)
recB21)	0.15	11.7	9.7	0.3 (1 − 0.85)	2.0	1.12×10^8	9 ± 5	(5)
	0.20	13.0	9.7	0.3 (1 − 0.79)	3.4	1.01×10^8	16 ± 6	(5)
	0.25	15.3	9.7	0.3 (1 − 0.68)	5.7	9.7×10^7	31 ± 12	(5)
	0.30	17.8	9.7	0.3 (1 − 0.61)	8.2	7.3×10^7	56 ± 9	(3)
	0.40	18.2	9.7	0.3 (1 − 0.48)	8.7	5.8×10^7	75 ± 13	(3)
	0.50	21.2	9.7	0.3 (1 − 0.40)	11.7	4.8×10^7	123 ± 18	(3)
	0.60	21.1	9.7	0.3 (1 − 0.35)	11.6	4.1×10^7	140 ± 28	(3)
	0.70	22.2	9.7	0.3 (1 − 0.27)	12.7	3.25×10^7	189 ± 37	(3)
	0.80	21.3	9.7	0.3 (1 − 0.20)	11.8	2.46×10^7	240 ± 6	(3)
SR663	0.05	19.4	17.9	0.4 (1 − 1.01)	1.5	3.42×10^8	2 ± 4	(5)
(*uvrA155*	0.10	23.2	17.9	0.4 (1 − 0.97)	5.3	3.29×10^8	9 ± 7	(5)
uvrD3)	0.15	26.2	17.9	0.4 (1 − 0.91)	8.3	3.08×10^8	14 ± 4	(5)
	0.20	30.0	17.9	0.4 (1 − 0.86)	12.2	2.92×10^8	21 ± 7	(5)
	0.25	35.9	17.9	0.4 (1 − 0.84)	18.1	2.83×10^8	32 ± 7	(5)
	0.30	46.5	18.8	0.4 (1 − 0.77)	27.8	2.66×10^8	52 ± 12	(3)
	0.40	51.9	18.8	0.4 (1 − 0.64)	33.2	2.22×10^8	74 ± 24	(3)
	0.50	60.2	18.8	0.4 (1 − 0.40)	41.6	1.38×10^8	145 ± 53	(3)
	0.60	67.8	18.8	0.4 (1 − 0.25)	49.3	8.4×10^7	290 ± 44	(3)
	0.70	56.6	18.8	0.4 (1 − 0.15)	38.1	5.3×10^7	352 ± 110	(3)
	0.80	43.5	18.8	0.4 (1 − 0.78)	25.1	2.74×10^7	444 ± 181	(3)
SR664	0.05	7.3	7.0	0.7 (1 − 0.89)	0.38	1.18×10^8	0.9 ± 4.2	(5)
(*uvrA155 uvrD3*	0.10	7.1	7.0	0.7 (1 − 0.69)	0.32	9.1×10^7	1.4 ± 10.8	(5)
recB21)	0.15	6.7	7.0	0.7 (1 − 0.56)	0.008	7.4×10^7	0.3 ± 11.4	(5)
	0.20	6.5	7.0	0.7 (1 − 0.41)	−0.09	5.5×10^7	−2.2 ± 6.2	(5)
	0.25	6.6	7.0	0.7 (1 − 0.28)	0.10	3.9×10^7	2.2 ± 19.4	(5)
	0.30	8.0	8.3	0.7 (1 − 0.25)	0.30	3.6×10^7	1.7 ± 10.9	(3)

[a] See Table 3.

[b] Since, in these experiments, cells were not concentrated before plating, SF_c can also be used as an accurate estimate of the UV radiation-induced surviving fraction.

[c] Average UV radiation-induced Trp⁺ mutants per 0.2 ml, from irradiated cell suspensions that had been spread upon trp(NB-200) plates.

[d] See footnote *c*, Table 3.

ACKNOWLEDGMENTS

We thank D. A. Youngs for valuable advice and discussion during the early phases of this work.

This investigation was supported by Public Health Service research grant CA-02896 and research program grant CA-10372 from the National Cancer Institute.

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. **Bardwell, E., and P. Howard-Flanders.** 1980. Influence of *recC* and *uvrD* on ultraviolet-induced mutation to valine resistance in *E. coli* K12. Mutat. Res. **69**:13–17.
4. **Boyce, R. P., and P. Howard-Flanders.** 1964. Release of ultraviolet light-induced thymine dimers from DNA in *E. coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **51**:293–300.
5. **Bridges, B. A.** 1972. Simple bacterial systems for detecting mutagenic agents. Lab. Pract. **21**:413–419.
6. **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.

7. **Bridges, B. A., and R. Mottershead.** 1971. *RecA*$^+$-dependent mutagenesis occurring before DNA replication in UV- and γ-irradiated *Escherichia coli*. Mutat. Res. **13**:1-8.
8. **Capaldo, F. N., and S. D. Barbour.** 1975. The role of the *rec* genes in the viability of *Escherichia coli* K12, p. 405-418. *In* P. C. Hanawalt and R. B. Setlow (ed.), Molecular mechanisms for repair of DNA. Plenum Publishing Corp., New York.
9. **Castellani, A., J. Jagger, and R. B. Setlow.** 1964. Overlap of photoreactivation and liquid holding recovery in *Escherichia coli* B. Science **143**:1170-1171.
10. **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.
11. **Hanawalt, P. C., P. K. Cooper, A. K. Ganesan, and C. A. Smith.** 1979. DNA repair in bacteria and mammalian cells. Annu. Rev. Biochem. **48**:783-836.
12. **Hill, R. F.** 1965. Ultraviolet-induced lethality and reversion to prototrophy in *Escherichia coli* strains with normal and reduced dark repair ability. Photochem. Photobiol. **4**:563-568.
13. **Hill, R. F., and E. R. Nestmann.** 1973. Effect of the *recC* gene in *Escherichia coli* on frequencies of ultraviolet-induced mutants. Mutat. Res. **17**:27-36.
14. **Horiuchi, T., and T. Nagata.** 1973. Mutations affecting growth of the *Escherichia coli* cell under a condition of DNA polymerase I-deficiency. Mol. Gen. Genet. **123**: 89-110.
15. **Howard-Flanders, P., R. P. Boyce, and L. Theriot.** 1966. Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics **53**:1119-1136.
16. **Kondo, S.** 1968. Mutagenicity versus radiosensitivity in *Escherichia coli*. Proc. 12th Intern. Congr. Genet. **2**: 126-127.
17. **Kondo, S., and T. Kato.** 1966. Action spectra for photoreactivation of killing and mutation to prototrophy in U.V.-sensitive strains of *Escherichia coli* possessing and lacking photoreactivating enzyme. Photochem. Photobiol. **5**:827-837.
18. **Kushner, S. R., J. Shepherd, G. Edwards, and V. F. Maples.** 1978. *uvrD*, *uvrE* and *recL* represent a single gene, p. 251-254. *In* P. C. Hanawalt, E. C. Friedberg, and C. F. Fox (ed.), DNA repair mechanisms. Academic Press, Inc., New York.
19. **Miura, A., and J.-I. Tomizawa.** 1968. Studies on radiation-sensitive mutants of *E. coli*. III. Participation of the Rec system in induction of mutation by ultraviolet irradiation. Mol. Gen. Genet. **103**:1-10.
20. **Miura, A., and J.-I. Tomizawa.** 1970. Mutation and recombination of bacteriophage lambda: effect of ultraviolet radiation. Proc. Natl. Acad. Sci. U.S.A. **67**:1722-1726.
21. **Morse, L. S., and C. Pauling.** 1975. Induction of error-prone repair as a consequence of DNA ligase deficiency in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **72**: 4645-4649.
22. **Nishioka, H., and C. O. Doudney.** 1969. Different modes of loss of photoreversibility of mutation and lethal damage in ultraviolet-light resistant and sensitive bacteria. Mutat. Res. **8**:215-228.
23. **Nishioka, H., and C. O. Doudney.** 1970. Different modes of loss of photoreversibility of ultraviolet light-induced true and suppressor mutations to tryptophan independence in an auxotrophic strain of *Escherichia coli*. Mutat. Res. **9**:349-358.
24. **Sargentini, N. J., and K. C. Smith.** 1979. Multiple, independent components of ultraviolet radiation mutagenesis in *Escherichia coli* K-12 *uvrB5*. J. Bacteriol. **140**:436-444.
25. **Sedgwick, S. G.** 1975. Inducible error-prone repair in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **72**:2753-2757.
26. **Setlow, J. K.** 1966. The molecular basis of biological effects of ultraviolet radiation and photoreactivation, p. 195-248. *In* M. Ebert and A. Howard (ed.), Current topics in radiation research, vol. 2. North Holland, Amsterdam.
27. **Setlow, R. B., and W. L. Carrier.** 1964. The disappearance of thymine dimers from DNA: an error-correcting mechanism. Proc. Natl. Acad. Sci. U.S.A. **51**:226-231.
28. **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.
29. **Smith, K. C., and D. H. C. Meun.** 1970. Repair of radiation-induced damage in *Escherichia coli*. I. Effect of *rec* mutations on post-replication repair of damage due to ultraviolet radiation. J. Mol. Biol. **51**:459-472.
30. **Smith, K. C., D. A. Youngs, E. Van der Schueren, K. M. Carlson, and N. J. Sargentini.** 1978. Excision repair and mutagenesis are complex processes, p. 247-250. *In* P. C. Hanawalt, E. C. Friedberg, and C. F. Fox (ed.), DNA repair mechanisms. Academic Press, Inc., New York.
31. **Stacey, K. A., and E. Simson.** 1965. Improved method for the isolation of thymine-requiring mutants of *Escherichia coli*. J. Bacteriol. **90**:554-555.
32. **Todd, P. A., and B. W. Glickman.** 1979. UV protection and mutagenesis in *uvrD*, *uvrE* and *recL* strains of *Escherichia coli* carrying the pKM101 plasmid. Mutat. Res. **62**:451-457.
33. **Van de Putte, P., C. A. Van Sluis, J. van Dillewijn, and A. Rorsch.** 1965. The location of genes controlling radiation sensitivity in *Escherichia coli*. Mutat. Res. **2**: 97-110.
34. **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.
35. **Venturini, S., and C. Monti-Bragadin.** 1978. R plasmid-mediated enhancement of mutagenesis in strains of *Escherichia coli* deficient in known repair functions. Mutat. Res. **50**:1-8.
36. **Volkert, M. R., D. L. George, and E. M. Witkin.** 1976. Partial suppression of the LexA phenotype by mutations (*rnm*) which restore ultraviolet resistance but not ultraviolet mutability to *Escherichia coli* B/r *uvrA lexA*. Mutat. Res. **36**:17-28.
37. **Witkin, E. M.** 1966. Mutation and the repair of radiation damage in bacteria. Radiat. Res. Suppl. **6**:30-53.
38. **Witkin, E. M.** 1966. Radiation-induced mutations and their repair. Science **152**:1345-1353.
39. **Witkin, E. M.** 1967. Mutation-proof and mutation-prone modes of survival in derivatives of *Escherichia coli* B differing in sensitivity to ultraviolet light. Brookhaven Symp. Biol. **20**:17-55.
40. **Witkin, E. M.** 1969. The mutability toward ultraviolet light of recombination-deficient strains of *Escherichia coli*. Mutat. Res. **8**:9-14.
41. **Witkin, E. M.** 1972. Ultraviolet mutagenesis in repair-deficient derivatives of *Escherichia coli* B/r: *uvrA recB* and *uvrA recC* strains. Mutat. Res. **16**:235-242.
42. **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.
43. **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.