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Effect of *recB21, uvrD3, lexA101* and *recF143* Mutations on Ultraviolet Radiation Sensitivity and Genetic Recombination in *ΔuvrB* Strains of *Escherichia coli* K-12

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Summary. The interaction of the *recB21, uvrD3, lexA101,* and *recF143* mutations on UV radiation sensitization and genetic recombination was studied in isogenic strains containing all possible combinations of these mutations in a *ΔuvrB* genetic background. The relative UV radiation sensitivities of the multiply mutant strains in the *ΔuvrB* background were: *recF recB lexA* > *recF recB uvrD lexA, recF recB uvrD* > *recA* > *recF uvrD lexA* > *recF recB, recF uvrD* > *recF lexA* > *recB uvrD lexA* > *recB uvrD* > *recB lexA, lexA uvrD* > *recB* > *lexA, uvrD* > *recF;* three of these strains were more UV radiation sensitive than the *uvrB recA* strain. There was no correlation between the degree of radiation sensitivity and the degree of deficiency in genetic recombination. An analysis of the survival curves revealed that the *recF* mutation interacts synergistically with the *recB, uvrD,* and *lexA* mutations in UV radiation sensitization, while the *recB, uvrD,* and *lexA* mutations appear to interact additively with each other. We interpret these data to suggest that there are two major independent pathways for postreplication repair; one is dependent on the *recF* gene, and the other is dependent on the *recB, uvrD,* and *lexA* genes.

Introduction

The two major systems for the dark repair of ultraviolet (UV) radiation damaged deoxyribonucleic acid (DNA) of *Escherichia coli* are excision repair and postreplication repair (Howard-Flanders 1968). The *uvrA* and *uvrB* strains of *E. coli* do not excise pyrimidine dimers from their DNA after UV irradiation (Howard-Flanders et al. 1966), since they are defective in the incision step of the excision repair process (Braun and Grossman 1974). Therefore, the major dark-repair system operating in the *uvrA* and *uvrB* strains is postreplication repair.

While little is known about the actual biochemical mechanisms of postreplication repair, any mutation that sensitizes a *uvrA* or *uvrB* strain to UV radiation (in the absence of photoreactivation) is assumed to act by blocking some step of postreplication repair. Such mutations occur in the *recA* (Howard-Flanders and Boyce 1966), *recB, recC* (Ganesan and Smith 1970), *recF* (Horii and Clark 1973), *recL* (Rothman and Clark 1977a), *lexA* (Mattern et al. 1966), *lexC* (Johnson 1977), *uvrD* (Ogawa et al. 1968), *umuC* (Kato and Shinoura 1977), *polA* (Barfknecht and Smith 1978), and *dam* (Marinus and Morris 1975) genes. A *recA* mutation can completely block genetic recombination (Clark 1967), "SOS" functions (Radman 1974; Sedgwick 1976; Witkin 1976), and the closure of gaps that arise in newly synthesized DNA after UV irradiation (Smith and Meun 1970). Mutations at *recB, lexA,* and *uvrD* result in a partial deficiency in the closure of DNA daughter-strand gaps (Youngs and Smith 1976), and when these mutations are combined the deficiency is increased. A similar response was observed for survival after UV irradiation (Youngs and Smith 1976). A mutation at *recF* results in a deficiency in the repair of DNA daughter-strand gaps (Ganesan and Seawell 1975; Rothman and Clark 1977b; Kato 1977), and it acts independently of a *recB* mutation in genetic recombination, in UV radiation sensitization (Horii and Clark 1973), and in postreplication repair (Rothman et al. 1975). Since a *recB recF* strain has a UV radiation sensitivity approaching that of a *recA* strain (Horii and Clark 1973; Kato et al. 1977), it has been proposed that there are two major independent pathways for the repair of UV radiation damaged DNA, one of them dependent on the *recF* gene, and the other dependent on the *recB(C)* genes (Horii and Clark 1973). It is not known, however, whether mutations at *lexA* and *uvrD* act independently of the *recF* mutation in sensitizing cells to UV radiation. The present report concerns the nature of the interaction (i.e., none, additive or synergistic) of *recB, lexA, uvrD,* and *recF* mutations on UV radiation sensitivity and on genetic recombination.

Materials and Methods

Bacterial Strains. The bacterial strains used in the experiments are derivatives of *E. coli* K-12 W3110, and are listed in Table 1. The derivatives used in strain construction are listed in Table 2. For studies on genetic recombination, strain SR96 (HfrH *thyA deo thi*) and strain SR865 (F'*lac*$^+$/*lacY thi*) were used as male donors of genetic markers. Strain SR865 (JC2625) was obtained from Dr. A.J. Clark.

Media. The media used for the growth of cells were either LB medium (Difco tryptone, 10 g; Difco yeast extract, 5 g; NaCl, 10 g; H_2O, 1 liter) or minimal medium (MM) (Ganesan and Smith 1968) supplemented with 0.5 μg/ml of thiamine·HCl and, when necessary, thymine at 10 μg/ml and L-amino acids at 1 mM. YENB agar (7.5 g Difco yeast extract, 23 g Difco nutrient agar per liter of H_2O) and MM agar [MM medium was solidified with 1.6% Difco Noble agar, since less pure grades of agar have been shown to inhibit the *recA* gene-dependent pathway of excision repair (Van der Schueren et al. 1974)] were used to determine colony-forming units. The lactose-MM, maltose-MM, and rhamnose-MM agar used in genetic experiments contained 0.4% of these sugars instead of glucose. MM agar with necessary supplements and streptomycin (200 μg/ml) was used to select recombinants from a cross of an Hfr with an F$^-$ recipient. Lactose-MM agar with necessary supplements and streptomycin (200 μg/ml) was used

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Table 1. A list of *Escherichia coli* K-12 *Δ(uvrB-chlA)* derivatives used[a]

Stanford radiology No.	Relevant genotype	Source or derivation
SR617[b]	+	D.A. Youngs (DY274)
SR902[c]	+	$P1_{kc}$·SR257 × SR898 (select Thy$^+$)
SR903[c]	*recB21*	$P1_{kc}$·SR257 × SR898 (select Thy$^+$)
SR904[c]	*lexA101*	$P1_{kc}$·SR257 × SR899 (select Thy$^+$)
SR906[c]	*uvrD3*	$P1_{kc}$·SR257 × SR900 (select Thy$^+$)
SR613[b]	*recF143*	D.A. Youngs (DY268)
SR614[b]	*recF143 recB21*	D.A. Youngs (DY269)
SR404[d]	*recF143 uvrD3*	$P1_{kc}$·SR474 × SR401 (select Met$^+$)
SR409[c]	*recF143 lexA101*	$P1_{kc}$·SR441 × SR625 (select Thy$^+$)
SR908[c]	*uvrD3 lexA101*	$P1_{kc}$·SR257 × SR901 (select Thy$^+$)
SR907[c]	*recB21 uvrD3*	$P1_{kc}$·SR257 × SR900 (select Thy$^+$)
SR905[c]	*recB21 lexA101*	$P1_{kc}$·SR257 × SR899 (select Thy$^+$)
SR909[c]	*uvrD3 lexA101 recB21*	$P1_{kc}$·SR257 × SR901 (select Thy$^+$)
SR411[c]	*recF143 recB21 lexA101*	$P1_{kc}$·SR441 × SR625 (select Thy$^+$)
SR842[c]	*recF143 recB21 uvrD3*	$P1_{kc}$·SR441 × SR408 (select Thy$^+$)
SR415	*recF143 lexA101 uvrD3*	$P1_{kc}$·SR441 × SR406 (select Thy$^+$)
SR844	*recF143 lexA101 uvrD3 recB21*	$P1_{kc}$·SR441 × SR406 (select Thy$^+$)
SR826[e]	*recA56*	P1::Tn*9c*·SR669 × SR617 (select Tc^r)

[a] All strains are F$^-$ and λ^-, and carry *leuB lacZ rpsL deo(C2?)*. Genotype symbols are those used by Bachmann and Low (1980)
[b] Also carries *rha malB*
[c] Also carries *rha*
[d] Also carries *malB*
[e] Also carries *rha malB srlA*::Tn*10*

to select Lac$^+$ recombinants from a cross of an F′*lac*$^+$ with a F recipient. For selecting the tetracycline resistant (Tc^r) colonies used in the construction of strain SR826, YENB agar containing tetracycline at 25 μg/ml was used. DTM buffer, which is MM medium without glucose or supplements, was used for washing and resuspending cells.

Construction of Strains. The transduction technique used was similar to that described by Miller (1972). Cells were grown in LB medium at 37° C overnight, centrifuged, and resupended in 1/5 of the original volume in MC buffer (0.1 M $MgSO_4$, 5 mM $CaCl_2$). After aerating at 37° C for 15 min, the phage lysate was added at a multiplicity of infection of 0.1 to 1, and incubated at 37° C for 20 min. An equal volume of 1 M sodium citrate was added at the end of the incubation, and a sample was diluted, and plated on selective media. In general, the co-transfer of a repair-deficient marker with a selected nutritional marker was tested by comparing the UV radiation sensitivities of recombinants with their parental strains.

Spontaneous *thyA* mutations were obtained by trimethoprim selection (Stacey and Simson 1965).

Irradiation. The source for UV radiation was a General Electric germicidal lamp (8 W) emitting primarily at 254 nm. The fluence rate for UV irradiation was determined with an International Light germicidal photometer (No. IL-254). For survival studies, cultures were grown exponentially at 37° C in supplemented MM until reaching a density of about 2×10^8 cells/ml. The cultures were centrifuged (10 min at 6,000 × g), washed three times with DTM buffer, and resuspended in DTM buffer at $OD_{650} = 0.1$ (Zeiss PMQII spectrophotometer) (about 1×10^8 cells/ml). Two ml of cell suspension was UV irradiated with mixing at room temperature (~23° C) in uncovered 6 cm Pyrex Petri dishes. Cells were diluted in 0.067 M sodium-potassium phosphate buffer (Na_2HPO_4 at 5.83 g/liter and KH_2PO_4 at 3.53 g/liter) (pH 7), and spread onto both supplemented MM agar and YENB agar to determine colony-forming units. All experiments involving UV irradiation were done under yellow light to avoid photoreactivation.

Determination of Genetic Recombination Ability. Cells were grown exponentially at 37° C in LB medium to about 10^8 cells/ml ($OD_{650} = 0.25$, Zeiss PMQII spectrophotometer). They were mixed at a donor to recipient ratio of 1:20, and incubated at 37° C for 40 min. Mating pairs were disrupted by vortexing at maximal speed for 30 s, and samples were spread onto selective media. Recombinants were scored after incubation at 37° C for 2–3 days.

Results

As a first step towards understanding the genetic control of postreplication repair, isogenic strains containing different combinations of the *recB21*, *recF143*, *lexA101*, and *uvrD3* mutations were constructed in the *ΔuvrB* background and tested for their sensitivities to UV radiation. The survival curves are shown in Fig. 1 and 2, and the F_{10} values (UV radiation fluences required to yield a surviving fraction of 0.1) are summarized in Table 3.

For convenience in presenting the data, the multiply mutant strains are grouped into four classes. The class I strains (Fig. 1), which contain one sensitizing mutation (i.e., either *recB*, *recF*, *lexA*, or *uvrD*) in addition to *uvrB*, were sensitized about 4 6 fold to killing by UV radiation as compared to their parental *uvrB* strain. The degree of sensitization by the *recB*, *uvrD*, and *lexA* mutations in our *ΔuvrB* genetic background was similar to that reported for these mutations in a *uvrB5* genetic background (Youngs and Smith 1976). The degree of sensitization by a *recF* mutation in our strain was similar to that reported in the literature (Ganesan and Seawell 1975). The class II strains (Fig. 1), which contain two sensitizing mutations in addition to *uvrB*, were further sensitized to killing by UV radiation as compared to their parental class I strains. Although the shapes of the survival curves differed for some of these strains, the degree of UV radiation sensitivity of these strains fell into two categories; those containing any two sensitizing mutations of *recB*, *uvrD*, and *lexA* (i.e., *uvrB recB lexA*, *uvrB recB uvrD*, *uvrB lexA uvrD*) were quite resistant to killing by UV radiation, while those strains containing the *recF* mutation and any one of the *recB*, *uvrD*, or *lexA* mutations were much more sensitive to killing by UV radiation. All of the class II strains were more resistant to UV radiation killing than was the *uvrB recA* strain (Fig. 1). The class III strains (Fig. 2), which contain three sensitizing mutations in addition to *uvrB*, were further sensitized to

Table 2. *Escherichia coli* K-12 derivatives used for strain construction[a]

Stanford radiology No.	Genotype	Source or derivation
SR248	*leuB metE rha lacZ bio thyA deo(C2?) malB rpsL*	R.B. Helling (KH21)
SR260	*Δ(uvrB-chlA) leuB metE rha lacZ thyA deo(C2?) malB rpsL*	D.A. Youngs (DY168)
SR669	Hfr PO45 *recA56 thr ilv rpsE srlA*::Tn*10* (Tc^r)	A.J. Clark (JC10240)
SR441	Hfr KL16 *recB21thr ilv rpsE*	J.D. Gross (JC5412)
SR474	*uvrD3 trp gal rpsL*	H. Ogawa (N14-4)
SR752	*metE thyA deo(C2?) lacZ rpsL*	D.A. Youngs (DY165)
SR625	*Δ(uvrB-chlA) recF143 lexA101 leuB rha lacZ rpsL thyA deo(C2?)*	D.A. Youngs (DY284)
SR257	*uvrB5 recB21 leuB metE rha lacZ rpsL deo(C2?)*	D.A. Youngs (DY157)
SR401	*Δ(uvrB-chlA) recF143 leuB metE lacZ rpsL deo(C2?) malB*	P1·SR752 × SR613 (select Rha^+)
SR402	*Δ(uvrB-chlA) recF143 lexA101 leuB metE lacZ rpsL thyA deo(C2?)*	P1·SR752 × SR625 (select Rha^+)
SR406	*Δ(uvrB-chlA) recF143 lexA101 uvrD3 leuB lacZ thyA deo(2?) rpsL*	$P1_{kc}$·SR474 × SR402 (select Met^+)
SR408	*Δ(uvrB-chlA) recF143 uvrD3 leuB lacZ thyA deo(C2?) malB rpsL*	spontaneous *thyA* from SR404
SR896	*Δ(uvrB-chlA) leuB rha lacZ thyA deo(C2?) malB rpsL*	$P1_{kc}$·SR404 × SR260 (select Met^+)
SR897	*Δ(uvrB-chlA) uvrD3 leuB rha lacZ thyA deo(C2?) malB rpsL*	$P1_{kc}$·SR404 × SR260 (select Met^+)
SR898	*Δ(uvrB-chlA) leuB rha lacZ thyA deo(C2?) rpsL*	$P1_{kc}$·SR409 × SR896 (select Mal^+)
SR899	*Δ(uvrB-chlA) lexA101 leuB rha lacZ thyA deo(C2?) rpsL*	$P1_{kc}$·SR409 × SR896 (select Mal^+)
SR900	*Δ(uvrB-chlA) uvrD3 leuB rha lacZ thyA deo(C2?) rpsL*	$P1_{kc}$·SR409 × SR897 (select Mal^+)
SR901	*Δ(uvrB-chlA) uvrD3 lexA101 leuB rha lacZ thyA deo(C2?) rpsL*	$P1_{kc}$·SR409 × SR897 (select Mal^+)

[a] Genotype symbols are those used by Bachmann and Low (1980). All strains are F⁻ and λ⁻, unless otherwise noted

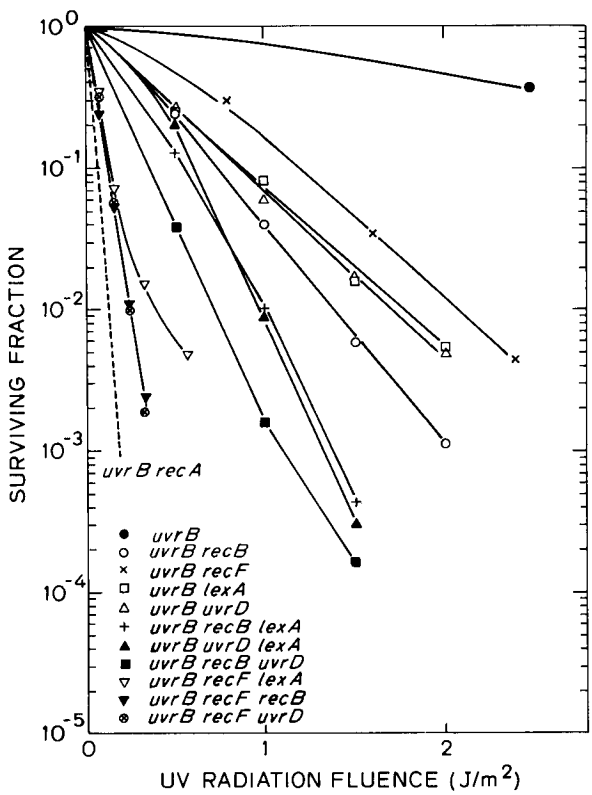

Fig. 1. The survival of *uvrB* strains of *E. coli* K-12 after UV irradiation. All strains were grown and treated as described in Materials and Methods. Symbols: *uvrB* (●), *uvrB recF* (×), *uvrB lexA* (□), *uvrB uvrD* (△), *uvrB recB* (○), *uvrB recB lexA* (+), *uvrB uvrD lexA* (▲), *uvrB recB uvrD* (■), *uvrB recF lexA* (▽), *uvrB recF recB* (▼), *uvrB recF uvrD* (×). The dashed curve is the survival of strain *uvrB recA* taken from Fig. 2 for comparison. All points are the average of at least two experiments

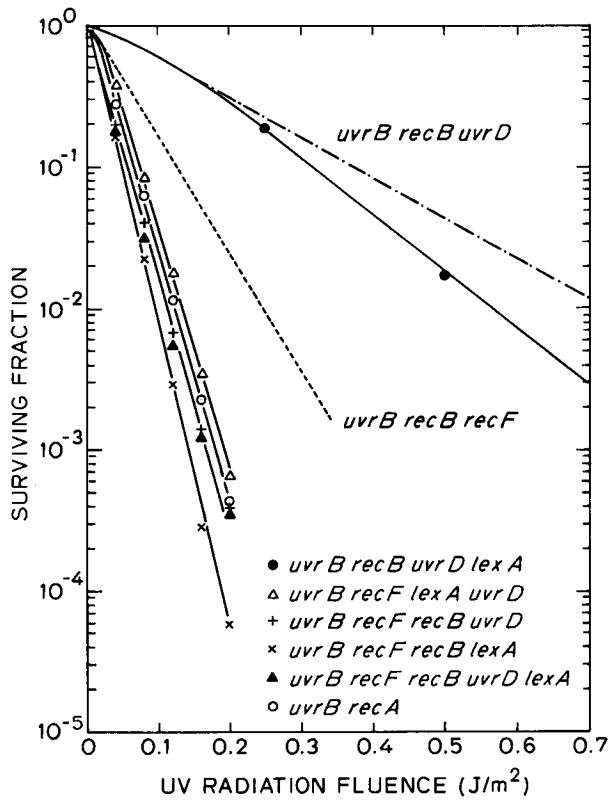

Fig. 2. The survival of *uvrB* strains of *E. coli* K-12 after UV irradiation. Symbols: *uvrB recB uvrD lexA* (●), *uvrB recF lexA uvrD* (△), *uvrB recF recB uvrD* (+), *uvrB recF recB lexA* (×), *uvrB recF recB lexA uvrD* (▲), and *uvrB recA* (○). For comparison, the dashed curves are survival curves for *uvrB recB recF* (– – –) and *uvrB recB uvrD* (· · · ·) taken from Fig. 1. All points are the average of at least two experiments

Table 3. Effect of *recB21*, *uvrD3*, *lexA101* and *recF14* mutations on UV radiation sensitivity and genetic recombination in *ΔuvrB* strains of *E. coli* K-12

uvrB strains	Relevant genotype	F_{10}[a] (J/m^2)	Relative UV radiation sensitivity $\left[\frac{(F_{10})wt}{(F_{10})mut}\right]$	Recombination deficiency indices[b]		
				Leu$^+$ Sm^r (×SR96, HfrH)	Lac$^+$ Sm^r (×SR865, F′*lac*$^+$)	$\frac{\text{Leu}^+\ \text{Sm}^r}{\text{Lac}^+\ \text{Sm}^r}$
SR617	+	4.75	1	1	1	1
SR613	*recF*	1.20	4.0	0.74	0.81	0.91
SR903	*recB*	0.75	6.3	1.4×10^3	3.9	3.6×10^2
SR906	*uvrD*	0.81	5.7	10	2.5	4.0
SR904	*lexA*	0.83	5.8	3.8	0.96	3.9
SR404	*recF uvrD*	0.13	37	3.2	1.6	2.0
SR409	*recF lexA*	0.14	34	4.3	1.6	2.7
SR908	*uvrD lexA*	0.61	7.8	20	1.3	15
SR614	*recB recF*	0.12	40	1.5×10^3	6.3	2.4×10^2
SR907	*recB uvrD*	0.35	14	$>2\times10^3$	9.1	$>2.2\times10^2$
SR905	*recB lexA*	0.56	8.5	$>2\times10^3$	1.7	$>1.2\times10^3$
SR826	*recA*	0.066	72	$>3\times10^3$	4.3	$>6.9\times10^2$
SR909	*recB lexA uvrD*	0.32	15	–		–
SR415	*recF uvrD lexA*	0.072	66			–
SR842	*recF recB uvrD*	0.049	97		–	
SR411	*recF recB lexA*	0.048	99	–		–
SR844	*recF recB lexA uvrD*	0.049	97		–	

[a] The UV radiation fluence that is required to inactivate 90% of the cell population

[b] A deficiency index was calculated by dividing the frequency at which progeny were produced in a cross of male donor (SR96 or SR865) with a Rec$^+$ recipient (SR617) by the frequency obtained with a tester strain recipient. The first column lists the Recombination Deficiency Index when scoring for Leu$^+$ Sm^r recombinants from a cross of an Hfr with an F$^-$ recipient; it monitors the deficiency in the recombination between incoming donor DNA and recipient chromosomal DNA. The second column lists the Recombination Deficiency Index when scoring for Lac$^+$ Sm^r recombinants from a cross of an F′*lac*$^+$ with an F$^-$ recipient; it monitors the deficiency in the uptake of donor F′ DNA into the recipient. In the third column a correction is made for the possible deficiency in the entry of donor DNA during conjugation; these values are taken as the true genetic recombination deficiency indices for the tester strains

UV radiation killing. The *uvrB recB uvrD lexA* strain, although more UV radiation-sensitive than any of the class II parental strains lacking the *recF* mutation (i.e., *uvrB recB uvrD*, *uvrB recB lexA*, or *uvrB lexA uvrD*), was far more resistant to UV irradiation than the class II strains that carried the *recF* mutation (i.e., *uvrB recF recB*, *uvrB recF uvrD*, and *uvrB recF lexA*). On the other hand, the class III strains that carried the *recF* mutation (i.e., *uvrB recF lexA uvrD*, *uvrB recF recB lexA*, and *uvrB recF recB uvrD*) were all more UV radiation sensitive than are any of the class II strains. In fact, the *uvrB recF recB lexA* and *uvrB recF recB uvrD* strains were even more UV radiation-sensitive than the *uvrB recA* strain, while the *uvrB recF uvrD lexA* strain was only slightly more resistant to UV radiation than was the *uvrB recA* strain. The class IV strain (Fig. 2), which contains all of the four sensitizing mutations in addition to *uvrB*, had approximately the same UV radiation sensitivity as the *uvrB recB recF uvrD* and *uvrB recB recF lexA* strains when survival was assayed on supplemented MM agar. This strain, however, was slightly more UV radiation sensitive than any of the class III strains or the *uvrB recA* strain, when survival was assayed on YENB agar (data not shown).

To test whether the recombinational process may play a major role in postreplication repair, the effect of these radiation-sensitizing mutations on genetic recombination was examined by conjugation, and the results are shown in Table 3. The class I strains, which have approximately the same sensitivity to UV radiation, showed a big variation in their ability to carry out gentic recombination. A mutation at *recF* did not cause a deficiency in genetic recombination, mutations at *uvrD* or *lexA* produced a moderate deficiency, and a *recB* mutation caused a large deficiency in genetic recombination.

In the class II strains, an additional *recF* mutation did not cause any further deficiency in genetic recombination in the *recB*, *uvrD* or *lexA* strains. In fact, these strains appear to be slightly more proficient in genetic recombination than their parental class I strains, yet they are highly sensitive to UV radiation. On the other hand, an additional *lexA* mutation further increased the deficiency of both the *uvrD* and *recB* strains to perform genetic recombination, yet had only a small effect on their sensitivity to UV radiation.

Discussion

Studies on *recF* strains by Horii and Clark (1973) led them to suggest that there are independent *recF* and *recB(C)* gene-controlled pathways for the repair of UV radiation damage. Subsequently, Youngs and Smith (1976) observed that the *uvrD*, *lexA*, and *recB* mutations interact with each other to further sensitize cells to UV radiation, and from their analysis of survival curves and by measuring the repair of DNA daughter-strand gaps, they reached the conclusion that the *recB*, *lexA*, and *uvrD* genes act independently of each other in postreplication repair. In order to determine the nature of the interaction between the *recF*, *recB*, *uvrD*, and *lexA* mutations, we have constructed isogenic strains containing all possible combinations of the *recB21*, *uvrD3*, *lexA101*, and *recF143* mutations in a *ΔuvrB* genetic background, and have studied the interaction of these mutations on postreplication repair by survival analysis.

The mathematical formulas used for our survival analysis are derived in the Appendix, and the results from such an analysis are shown in Fig. 3. It is clear that the *recF* mutation interacts synergistically with the *recB*, *uvrD*, and *lexA* mutations (Fig. 3a,

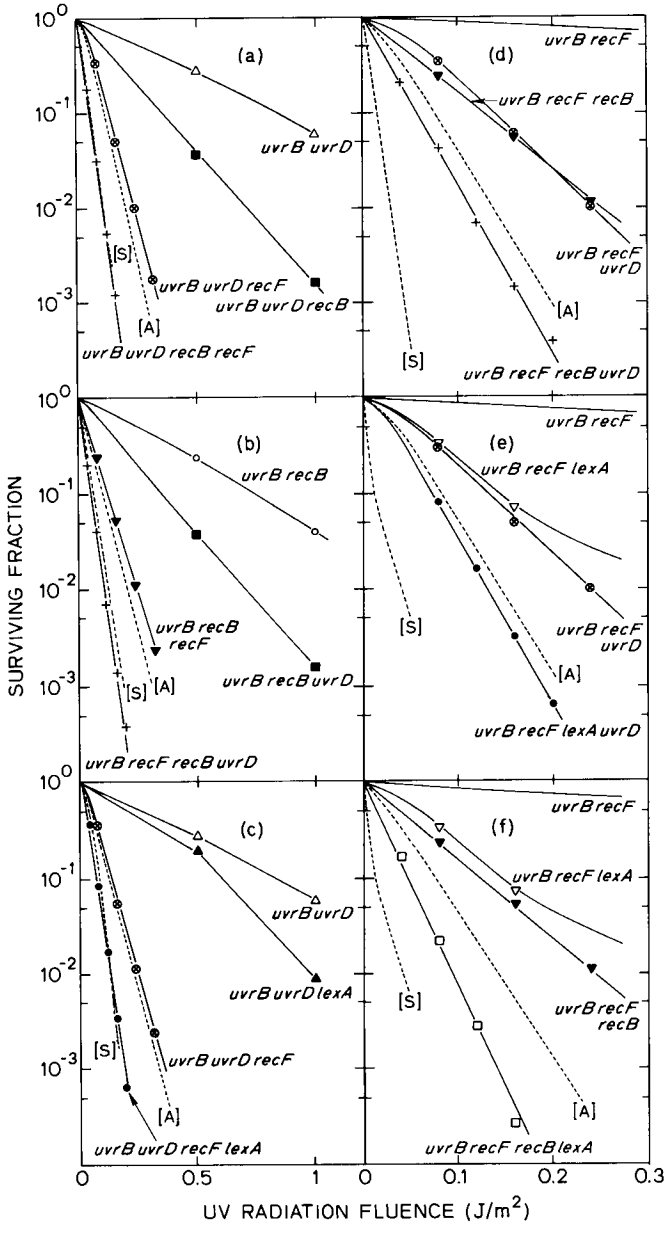

Fig. 3a–f. Analysis of the interaction of two UV radiation sensitizing mutations on cell survival. The *uvrB recF recB uvrD* (+), *uvrB recF uvrD lexA* (●), and *uvrB recF recB lexA* (□) strains assumed to be repair-less. From Eq. 7 and Eq. 18 in the Appendix, the predicted survival curves were calculated from the actual survival curves for synergistic interaction (dashed curve labeled with S) and for additive interaction (dashed curve labeled with A). Interaction between mutations: (**a**) *recF* and *recB*, (**b**) *recF* and *uvrD*, (**c**) *recF* and *lexA*, (**d**) *recB* and *uvrD*, (**e**) *uvrD* and *lexA*, and (**f**) *recB* and *lexA*

b, c). On the other hand, our analysis of the combined effects of the *recB, uvrD*, and *lexA* mutations on UV radiation sensitization indicates interactions that are neither totally synergistic nor totally additive. As shown in Fig. 3d, e, f, the survival curves for the doubly mutant strains are slightly more sensitive than that predicted for an additive interaction, but are considerably more UV radiation-resistant than that predicted for a synergistic interaction. According to Brendel and Haynes (1973), these results should indicate that the *recB, uvrD*, and *lexA* mutations interact synergistically with each other on radiation sensitization, a conclusion reached by Youngs and Smith (1976). However, we feel that it is more appropriate to conclude from such an analysis that the *recB, uvrD*, and *lexA* mutations interact mostly additively with each other, because the survival curves of the doubly-mutant strains are far more resistant than that expected for a synergistic interaction.

As discussed in the Appendix, we interpret a synergistic interaction between two sensitizing mutations to mean that the two repair functions act independently of each other, and compete for the same substrate. Therefore, our data suggest that there are two major independent pathways for postreplication repair, one of them dependent on the *recF* gene, and the other dependent on the *recB, uvrD*, and *lexA* genes. It is of interest to note that the *uvrB recF recB uvrD*, *uvrB recF recB lexA* and *uvrB recF recB uvrD lexA* strains are more sensitive to UV radiation than is the *uvrB recA* strain (Fig. 2). If the *recA56* mutation is not leaky, this would suggest the existence of a *recA*-independent mode of postreplication repair.

A major question that arises from the present work is how can the *recB, uvrD*, and *lexA* mutations interact additively, and yet affect the same pathway of postreplication repair. A plausible explanation for an additive interaction may be that these gene products are involved directly or indirectly (i.e., a regulatory function) in a DNA repair complex, such that a mutation in one of the genes results in a partial deficiency in the function of the repair complex. An additional mutation would result in an even greater deficiency in repair, until at some point the complex would not function at all.

Although a major part of postreplication repair appears to involve some kind of recombinational process (Rupp et al. 1971; Ganesan 1974), there is no good correlation between the degree of UV radiation sensitivity of our strains and the degree of deficiency in carrying out genetic recombination (Table 3). For example, the class I strains have approximately the same UV radiation sensitivity, but they show a big variation in ability to carry out genetic recombination. Similarly, the *uvrB recF uvrD, uvrB recF lexA*, and *uvrB recF recB* strains all have approximately the same UV radiation sensitivity, but the *uvrB recF recB* strain showed a barely detectable genetic recombination, while the *uvrB recF uvrD* and *uvrB recF lexA* strains were only slightly deficient in genetic recombination. Recently, Clark (1980) proposed that the *recB(C)* pathway of genetic recombination results in double-strand DNA substitution and/or integration, while the *recF* pathway results in single-strand DNA substitution. It has been suggested that the major contribution of the *recB recC* enzyme, exonuclease V, to conjugational recombination is its strand unwinding function (Rosamond et al. 1979), which creates single-stranded DNA in the exogenote that is used in a *recA* protein catalyzed synapse with the chromosome. By contrast, the *recF* pathway may use single-stranded regions of chromosomal DNA to interact with double-stranded regions of the exogenote during conjugational recombination. Since the recombination process that is utilized in the postreplication repair of DNA daughter-strand gaps may not need some of the functions that are required in conjugational recombination, this may explain why there is no good correlation between UV radiation sensitivity and genetic recombination as tested by conjugation.

Our data on genetic recombination (Table 3) do not reveal an effect of the *recF* mutation on the efficiency of *recB* cells to carry out genetic recombination, as has been reported by Horii and Clark (1973) and Kato et al. (1977). This discrepancy may be due to the fact that our *recB* strain had a greater deficiency in genetic recombination then the *recB* strains used by

these workers, thus possibly obscuring the effect of the *recF* mutation on genetic recombination in our study. Alternatively, we calculated the deficiency indices for recombination by making a correction for a possible deficiency in the uptake of donor DNA during conjugation (see Table 3, footnote); this correction was not made by the above authors.

At present, little can be said about the molecular processes involved in the two pathways of postreplication repair, because the functions of the *recF, uvrD* and *lexA* gene products are not known. Several other mutations (e.g., *ssb, umuC, dam3*) sensitize *uvrA(B)* strains to UV radiation, and the products of these genes must play a role in postreplication repair. Studies on these and other mutations that affect postreplication repair are underway, and should provide a better understanding of the genetic control of postreplication repair, with the ultimate goal of understanding the molecular basis of this complex repair process.

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Appendix

In this analysis we assume, as Brendel and Haynes (1973) did, that lethality is an all-or-nothing response governed by Poisson

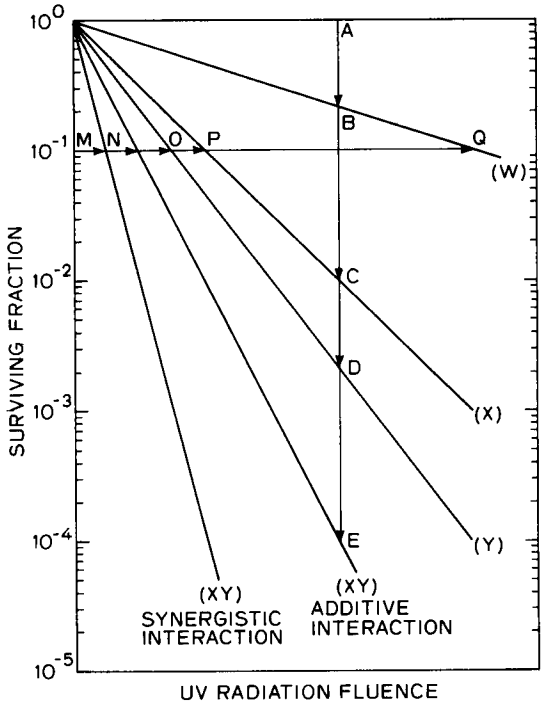

Fig. 4. Theoretical prediction for the effect of the interaction of two radiation sensitizing mutations on survival curves. The doubly mutant strain XY is assumed to be repair-less and the single mutants X and Y possess a repair function that involves gene products Y and X, respectively, and the wild-type, W, possesses both repair functions. The points B, C, and D represent the survival at a given UV radiation fluence, f, for the wild-type and two single mutants that have different radiation sensitivities. If no interaction occurs between the two mutations, the survival curve for the doubly mutant strain, XY, will be no more sensitive than its more sensitive parental strain, Y; if the two mutations interact additively, the survival point for the doubly mutant strain, XY, at the same UV radiation fluence will follow Eq. 7 in the Appendix such that AE=AC+BD. If the two mutations interact synergistically, the UV radiation fluence, MN, that is required to inactivate the doubly mutant strain to the survival level S_f should follow Eq. 18 in the Appendix such that MN=(MO)(MP)/(MQ), where MQ, MP and MO represent the UV radiation fluences that are required to inactivate the wild-type and the two singly mutant strains, respectively, to the same survival level, S_f

statistics so that survival curves can be expressed in the general form,

$-\ln S=$(number of unrepaired lethal hits per cell)

where S is the surviving fraction of cells in the irradiated population, as determined by a plating assay.

Let the number of potentially lethal hits formed initially by radiation fluence, x, be $F(x)$. Then for a repair-less cell, the survival after a radiation fluence, x, becomes:

$$-\ln S=F(x). \tag{1}$$

Now, assume the exsistence of a single repair process capable of eliminating a fraction, $r(x)$, of the initial hits prior to colony formation on growth medium. The probability that a hit escapes repair is $1-r(x)$, therefore, the survival of a cell possessing such a repair process becomes:

$$-\ln S=[1-r(x)]F(x)=F(x)-r(x)F(x) \tag{2}$$

or

$$-\ln S=F(x)-R(x) \tag{3}$$

where $R(x)=r(x)F(x)$ is the number of potentially lethal hits removed by repair.

Next, assume the existence of two repair processes, R_1 and R_2. According to Brendel and Haynes (1973), if R_1 and R_2 compete for the same substrate, then the survival of a cell possessing both repair processes becomes:

$$-\ln S=F(x)-R_1(x)-R_2(x)+R_1(x)R_2(x)/F(x). \tag{4}$$

On the other hand, if the two repair processes do not compete for the same substrate and they act independently on different components, F_1 and F_2, of the initial damage, where $F(x)=F_1(x)+F_2(x)$, then the survival of a cell becomes:

$$-\ln S=[1-r_1(x)]F_1(x)+[1-r_2(x)]F_2(x) \tag{5}$$

or

$$-\ln S=F(x)-R_1(x)-R_2(x). \tag{6}$$

According to Eq. (1), (3), (4) and (6), the relative shifts in survival curves of strains either singly or doubly mutant in processes R_1 and R_2 can be predicted, as shown in Fig. 4. For two repair processes, R_1 and R_2, that are independent and noncompeting [Eq. (6)], let the points C, D, and E (Fig. 4) represent the survival at a given UV radiation fluence for strains singly mutant in R_1 and R_2, and doubly mutant in both R_1 and R_2, respectively, then the survival point B for a wild-type cell at the same UV radiation fluence can be predicted to follow

$$AE=AC+BD=AD+BC \tag{7}$$

as discussed by Brendel and Haynes (1973).

For two repair processes, R_1 and R_2, that compete for the same substrate, let the distances MP, MO, and MN (Fig. 4) represent the UV radiation fluences x_2, x_1 and x_0, respectively, that are required to inactivate cells to the same survival level, S_f, for strains singly mutant in R_1 and R_2, and doubly mutant in both R_1 and R_2, respectively. Therefore,

single mutant X (R_1):

$$-\ln S_f=F(x_2)-R_2(x_2) \tag{8}$$

single mutant Y (R_2):

$$-\ln S_f=F(x_1)-R_1(x_1) \tag{9}$$

double mutant XY:

$$-\ln S_f=F(x_0). \tag{10}$$

If the survival curves are exponential, the fraction (r_1, r_2) of initial hits removed by repair processes R_1 and R_2 can be determined, since

$$F(x_0)=F(x_1)-r_1F(x_1)=F(x_2)-r_2F(x_2) \tag{11}$$

or

$$r_1=1-[F(x_0)/F(x_1)]=1-(MN/MO) \tag{12}$$

$$r_2=1-[F(x_0)/F(x_2)]=1-(MN/MP). \tag{13}$$

From Eq. 4, the UV radiation fluence x_3 (MQ) that is required to inactivate a wild-type cell to survival level S_f becomes:

$$-\ln S_f = F(x_3) - R_1(x_3) - R_2(x_3) + R_1(x_3)R_2(x_3)/F(x_3) \quad (14)$$

or

$$-\ln S_f = F(x_3) - r_1F(x_3) - r_2F(x_3) + r_1r_2F(x_3) = F(x_0) \quad (15)$$

or

$$MQ = MN + (r_1 + r_2)\,MQ - r_1r_2\ MQ. \quad (16)$$

Substituting Eq. (12) and (13) into Eq. (16):

$$MQ = MN + MQ[(1 - MN/MP) + (1 - MN/MO)] - MQ[(1 - MN/MP)(1 - MN/MO)] \quad (17)$$

solving Eq. (17):

$$MQ = (MO)(MP)/MN. \quad (18)$$

However, if the survival curves are not exponential, it becomes difficult to predict the expected survival curves from Eq. 4, since $r_1(x)$ and $r_2(x)$ change as a function of UV radiation fluence (x). Although, in principle, one should be able to read from the actual survival curves and test whether R_1 and R_2 competes for the same substrate according to Eq. (15), in reality, this is only possible at low UV radiation fluences were the survival for doubly mutant strains is still measureable. In any case, as discussed by Brendel and Haynes (1973), a doubly mutant strain devoid of two repair processes that compete for the same substrate is expected to be more sensitive than a doubly mutant strain devoid of two repair processes that act additively (i.e., noncompetatively).

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