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THE EFFECTS OF *lexA101, recB21, recF143* and *uvrD3* MUTATIONS ON LIQUID-HOLDING RECOVERY IN ULTRAVIOLET-IRRADIATED *Escherichia coli* K12 *recA56*

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Summary

Using an *Escherichia coli* K12 *recA* strain, we have tested the effects of incorporating additional mutations affecting deoxyribonucleic acid (DNA) repair on ultraviolet-radiation sensitivity and on the expression of liquid-holding recovery (LHR). (This laboratory had previously shown that a mutation at *uvrA*, *uvrB* or *uvrC* blocked LHR in a *recA* strain.) In the *recA56* background, an additional *lexA101* mutation had no effect on UV-radiation sensitivity or LHR. The addition of a *recB21* mutation to *recA56* did not alter UV-radiation sensitivity, but greatly increased the rate of LHR. The *recB* gene product (exonuclease V) appears to act as a competitive inhibitor both of excision repair and of photoreactivation under liquid-holding (LH) conditions. The *uvrD3* mutation increased the radiation sensitivity of a *recA* strain, and almost completely blocked LHR. The *recA uvrD* strain showed more DNA degradation and DNA double-strand breaks during LH than did the *recA* strain. The *recF143* mutation increased both UV-radiation sensitivity and LHR in a *recA* strain, suggesting that the *recF* gene product may also function in *recA*-independent pathways of DNA repair.

When certain strains of *Escherichia coli* are held in non-nutrient medium after ultraviolet irradiation prior to spreading on nutrient plates, they exhibit a higher survival than if plated immediately after UV irradiation. This phenomenon of enhanced survival has been termed liquid-holding recover (LHR). Based upon both genetic and biochemical evidence [5,6,8,9,19], LHR depends upon an efficient excision-repair system, but the *detection* of LHR in strains of *E. coli* K12 was reported to depend upon the presence of a *recA* mutation [5]. This *recA* deficiency is not required during liquid holding (LH), but is required

TABLE 1

E. coli STRAINS USED

Stanford radiology number	Repair associated markers	Other markers [a]	Source or derivation [b]
SR192	*lexA101*	*metE thyA36 deo(C2?) lacZ53 rpsL151*	DY99; [24]
SR248	+	*leuB19 metE70 thyA36 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151* Su^-	KH21, R.B. Helling
SR291	*uvrB*	*his Δ(uvrB—chlA)*	SA420, A.M. Campbell
SR299	*recF143*	*argE3 his-4 leuB6 proA2 thr-1 galK2 lacY1 mtl-1 xyl-5 tsx-33 rpsL31 supE44*	JC9239, A.J. Clark
SR300	*recF143*	*leuB19 thyA36 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151*	SR395 X P1 · SR299 (select Ilv^+)
SR304	*recF143*	*leuB19 thyA36 deo(C2?) lacZ53 malB45 rha-5 rpsL151*	SR300 X P1 · SR291 (select Bio^+)
SR321	*recA56 recF143*	*leuB19 deo(C2?) lacZ53 malB45 rha-5 rpsL151*	SR304 X SR400 (select Thy^+)
SR323	*recA56 recB21*	*leuB19 metE70 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151*	SR248 X SR627 (select Thy^+)
SR392	+	*leuB19 thyA36 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151*	DY174; [18]
SR393	*uvrD3*	*leuB19 thyA36 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151*	DY175; [18]
SR395	+	*ilv leuB19 thyA36 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151*	SR248 X P1 · SR462 (select Met^+)
SR440	*recA56*	HfrKL16 *ilv thr rpsE*	JC5088, J.D. Gross
SR462	+	HfrKL16 *ilv thr rpsE*	JG89, J.D. Gross
SR627	*recA56 recB21*	HfrKL16 *ilv thr thi rpsE rpsL*	JC7505, A.J. Clark
SR661	*recA56 uvrD3*	*leuB19 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151*	SR393 X SR440 (select Thy^+)
SR669	*recA56*	HfrPO45 *ilv-318 thr-300 srlA300::Tn10*(Tet^R) *rpsE300*	JC10240, A.J. Clark
SR688	*lexA101*	*leuB19 metE70 thyA36 deo(C2?) lacZ53 rha-5 bioA2 rpsL151*	SR248 X Plkc · SR192 (select Mal^+)
SR754	*recA56 uvrD3*	*leuB19 thyA deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151*	SR661 (select Tmp^R)
SR755	*recA56*	*leuB19 thyA36 deo(C2?) lacZ53 malB45 rha-5 srlA300::Tn10 bioA2 rpsL151*	SR392 X Pl::Tn*9c* · SR669 (select Tet^R)
SR756	*recA56 recB21*	*leuB19 metE70 thyA deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151*	SR323 (select Tmp^R)
SR757	*recA56 lexA101*	*leuB19 metE70 thyA36 deo(C?) lacZ53 rha-5 srlA300::Tn10 bioA2 rpsL151*	SR688 X Pl::Tn*9c* · SR669 (select Tet^R)

[a] Nomenclature is that used by Bachmann et al. [1]. All strains are F^-, λ^- unless otherwise noted.

[b] Strains SR300, SR304, SR321, SR323 and SR395 were constructed by D.A. Youngs. Strains SR661 and SR688 were constructed by N.J. Sargentini. Tet^R, resistant to tetracycline; Tmp^R, resistant to trimethorpim.

when the cells are returned to growth medium [20]. These results for *recA*, and those for additional strains carrying single mutations affecting DNA repair (i.e., *recB*, *recF* and *lexA*), demonstrate that the detection of LHR requires that the strain be at least partially defective in growth medium-dependent DNA repair [20].

Using *E. coli* K12 *recA*, a strain that is deficient in growth medium-dependent repair [20, and refs. therein], we have incorporated additional mutations that control DNA repair to see what effect they might have on UV-radiation sensitivity and LHR in the *recA* background. We found that the *recA*, *recA recB*, and *recA lexA* strains have similar UV-radiation sensitivities, but after 24 h of LH, the *recA recB* strain showed a much higher LHR than did the *recA* and *recA lexA* strains, which showed the same amount of LHR. In contrast, the *recA uvrD* strain was more sensitive to UV radiation than the *recA* strain, but showed little LHR. The *recA recF* strain showed both increased UV-radiation sensitivity and increased LHR. The effects at the molecular level of the *recB*, *recF* and *uvrD* mutations on LHR in *recA* strains are explored.

Materials and methods

Bacterial strains. The *E. coli* strains used are listed in Table 1.

Growth conditions. The growth medium used was a minimal salts medium (DTM) [11] (4×10^{-2} M K_2HPO_4, 1.5×10^{-2} M KH_2PO_4, 4.1×10^{-4} M $MgSO_4$, 7.6×10^{-3} M $(NH_4)_2SO_4$, 1.4×10^{-3} M sodium citrate, 3.4×10^{-5} M $CaCl_2$, 9.0×10^{-7} M $FeSO_4$) supplemented with glucose [at 0.4% (w/v)], L-methionine (at 10^{-3} M), L-leucine (at 10^{-3} M), thymine (at 4 μg/ml), and biotin (at 0.5 μg/ml), as required. Bacteria were grown exponentially in supplemented minimal medium at 37° C for at least 5 generations to a titer of $1-3 \times 10^8$ cells/ml. The cultures were collected on Millipore filters (HA, 0.45-μm pore size), and washed 3 times with and resuspended in the same volume of DTM. The cell suspensions were kept at 37° C in a shaking water bath for 2 h prior to experimental treatment.

UV irradiation and liquid holding. A cell suspension ($\sim 2 \times 10^8$ cells/ml in DTM) was placed in an open glass petri dish on a rotary shaker, and irradiated at room temperature with an 8-W General Electric germicidal lamp (254 nm). The fluence rate was reduced by placing a wire grid between the source and cell sample. The fluence rate was determined with a Germicidal Photometer (International Light Co., Model IL-254). The UV-radiation fluence was corrected for sample absorption according to the method of Youngs and Smith [25].

After exposure to UV radiation, the cell suspension was held at 37° C in a shaking water bath for 24 h (LH procedure). Cells with or without LH were assayed for viability after diluting with DTM and plating 0.1 ml on yeast extract—nutrient agar (YENB) (23 g Difco Nutrient Agar, and 7.5 g Difco Yeast Extract per liter). The plates were counted after a 24–48 h incubation at 37° C.

All the experiments were performed under General Electric "gold" fluorescent lights to prevent photoreactivation. The surviving fraction (SF) was determined by the formula $N_r(t)/N_0(t)$, where N_r is the number of colony-forming units (CFU) per milliliter of cell suspension in the irradiated population, N_0 is

the number of CFU/ml in the unirradiated population, and (t) is the time of LH after exposure of the irradiated population to UV radiation.

Photoreactivation. Samples of cell suspensions, both unirradiated and irradiated, with or without LH, were plced in an uncovered glass petri dish on a rotary shaker and exposed to 2 General Electric 15-W daylight fluorescent lamps. The lamps were 3.4 cm above the surface of the cell suspension; the temperature was maintained at about 23° C with an electric fan. The viability after photoreactivation was determined by $M_r(t)/M_0(t)$, where M_r is the number of CFU/ml of UV-irradiated cells, M_0 is the number of CFU/ml of unirradiated cells, and (t) is the time of exposure to white light.

Measurement of DNA degradation. Cells that were prelabeled with [*Me*-^{3}H]-thymine (1 μCi/ml, 30 Ci/mmole, Amersham) during approx. 5 generations of growth were used to measure the degradation of DNA during LH. After labeling with radioactive thymine for several generations, unincorporated radioactive thymine was removed by filtering (Millipore filter; HA, 0.45-μm pore size) and washing the cells 3 times with ~10 ml of prewarmed medium. The cells were resuspended in prewarmed medium and incubated for 1—2 h before harvesting and resuspending in DTM. At various times during LH, 0.5 ml of the cell suspension was mixed with 2 ml of cold 10% trichloroacetic acid (TCA). The TCA precipitate was collected on a Millipore filter (EH, 0.5-μm pore size) that had been pretreated with thymine at 1 mg/ml, and the precipitate was washed 3 times with ~5 ml of cold 0.1% TCA. The filters were dried under a heat lamp, placed in 5 ml of scintillation solution [4 g Omnifluor (New England Nuclear) in 1 l of toluene], and assayed for ^{3}H in a liquid-scintillation spectrometer.

Neutral surcose-gradient sedimentation. The cells were prelabeled with [*Me*-^{3}H]thymine (1 or 5 μCi/ml, 30 Ci/mmole, Amersham) during approx. 5 generations of growth. The gradient method used was a modification of the methods of Bonura and Smith [2] and Krasin and Hutchinson [12]. A 0.2-ml sample of a cell suspension was added to an equal volume of ice-cold 0.07 M tris(hydroxymethyl)aminomethane (Tris)—0.17 M ethylenediaminetetraacetic acid (EDTA) at pH 7.6, containing 400 μg/ml of freshly dissolved lysozyme (Sigma). After 15 min on ice, 0.2 ml of the spheroplast suspension was layered on a 4.8-ml neutral sucrose gradient (5--20%, w/v) containing 0.5% sodium dodecyl sulfate, 5×10^{-3} M Tris, 10^{-3} M sodium citrate, 10^{-2} M sodium chloride (the stock solutions were saturated with chloroform), and pronase at 0.1 mg/ml. Siliclad-treated cellulose nitrate tubes (0.5 × 2 inch) were used. After 90 min at room temperature, the gradients were centrifuged for 40 h at 3400 rpm in a SW 50.1 rotor in a Beckman Model L or L265B ultracentrifuge at 20° C. After centrifugation, 33 fractions were pumped from the bottom of each punctured tube. Each gradient fraction was mixed with 1 ml of cold 10% TCA, collected on EH filters, and washed 3 times with ~5 ml of cold 0.1% TCA. The dried filters were counted as described above.

Results

The effects of lexA101, recB21, recF143 and uvrD3 mutations on UV-radiation sensitivity and LHR in the recA56 background

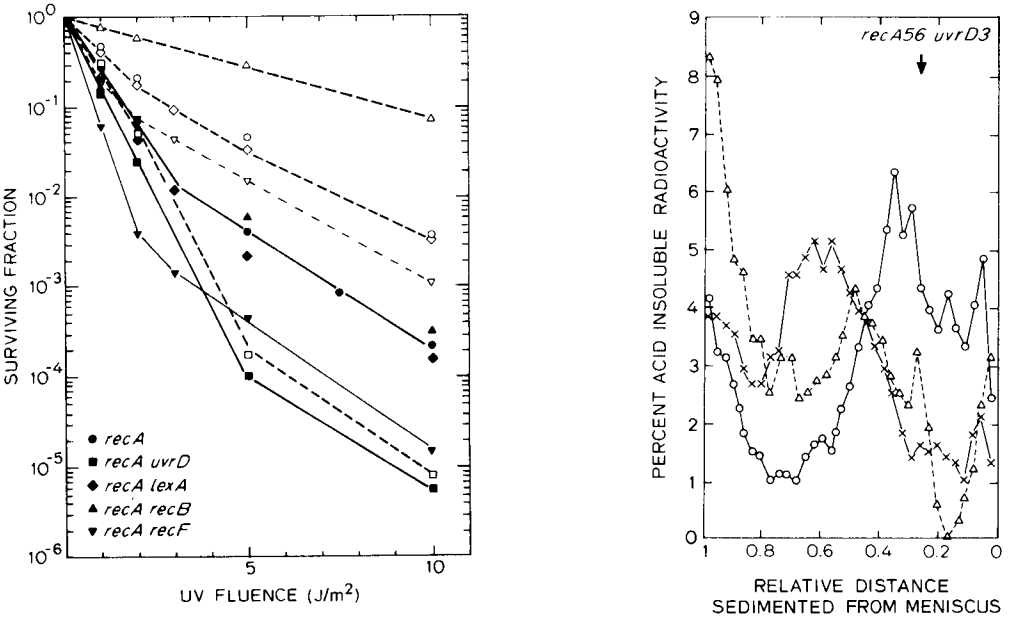

Fig. 1. UV-radiation survival curves for *E. coli recA56* (SR755), *recA56 recB21* (SR756), *recA56 lexA101* (SR757), *recA56 recF143* (SR321), and *recA56 uvrD3* (SR754) strains with or wothout LH (24 h) treatment. Solid lines (closed symbols) represent the viability of cells without LH, and dashed lines (open symbols) represent cells with 24 h of LH. Symbols: □, ■, *recA uvrD*; ◇, ◆, *recA lexA*; △, ▲, *recA recB*; ▽, ▼, *recA recF*; and ○, ●, *recA*. Data points for 5 and 10 J/m^2 are the average of at least 2 Expts.

Fig. 2. Neutral sucrose-gradient sedimentation profile of [^{3}H]thymine prelabelled *E. coli* K12 *recA56 uvrD3* (SR754) DNA. Symbols: X, 10 J/m^2 UV, no LH (this profile is the same as for unirradiated control cells without LH); ○, 10 J/m^2 UV, 24 h LH; △, no UV, 24 h LH. Centrifugation speed and duration are described in the text. The arrow indicates the position of a T2 DNA marker.

The effects of additional mutations on UV-radiation sensitivity and LHR in a *recA* strain (Fig. 1) can be summarized as follows: (1) neither UV-radiation sensitivity nor LHR was changed in *recA lexA* cells; (2) both UV-radiation sensitivity and LHR were increased in *recA recF* cells; (3) in *recA recB* cells, the UV-radiation sensitivity was unchanged, but LHR was greatly increased; (4) in *recA uvrD* cells, the UV-radiation sensitivity was increased, but LHR was greatly reduced.

UV-radiation-induced DNA degradation and DNA double-strand breaks in recA56, recA56 uvrD3, and recA56 recB21 cells during LH

In an attempt to explain the absence of LHR in the *recA uvrD* strain, DNA degradation and DNA double-strand breaks were measured after LH. The reults for DNA degradation in UV-irradiated *recA*, *recA recB* and *recA uvrD* cells held in nongrowth medium are shown in Table 2. UV irradiation induced little or no DNA degradation in either *recA* or *recA recB* cells during LH, but greatly increased the amount occurring in *recA uvrD* cells.

Consistent with these results on DNA degradation is our observation of an increase in the number of DNA double-strand breaks in UV-irradiated *recA uvrD* cells after 24 h of LH, relative to unirradiated control cells (Fig. 2), with

TABLE 2

DNA DEGRADATION DURING LIQUID HOLDING (LH) IN *E. coli* K12 *recA*, *recA uvrD* AND *recA recB* CELLS

Strains	UV fluence (J/m^2)	[^{3}H]Thymine in TCA-insoluble fraction of DNA		
		LH 0 h	LH 6 h	LH 24 h
recA56 (SR755)	0	1.0	0.99	0.92
	5	1.0	0.92	0.91
	10	1.0	0.93	0.93
recA56 uvrD3 (SR754)	0	1.0	1.02	0.99
	5	1.0	0.95	0.73
	10	1.0	0.86	0.65
recA56 recB21 (SR756)	0	1.0	0.99	0.93
	5	1.0	1.01	0.90
	10	1.0	0.96	0.88

no increase in the number of UV-radiation-induced DNA double-strand breaks during LH in *recA* and *recA recB* cells (data not shown).

Kinetics of LHR in UV-irradiated recA56 and recA56 recB21 cells

Since no difference in DNA degradation was observed during LH between *recA* and *recA recB* cells, and UV irradiation did not induce DNA double-strand

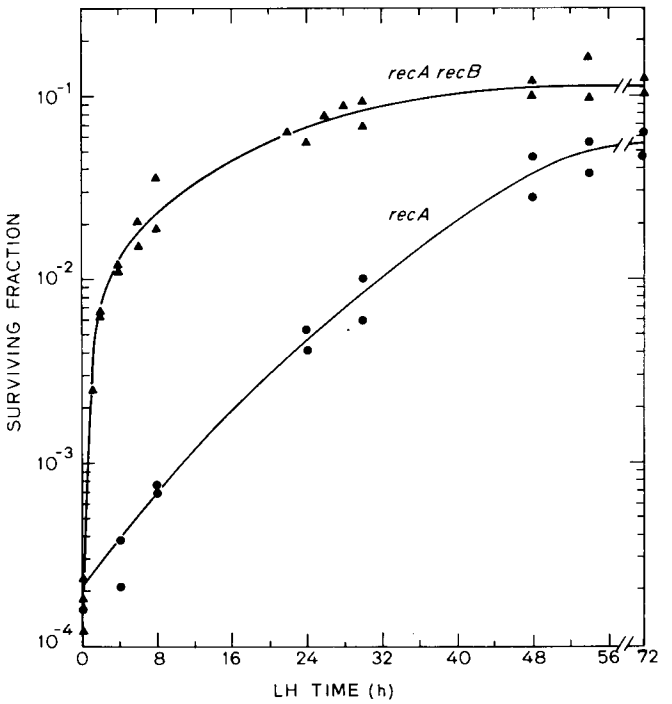

Fig. 3. Survival kinetics of *E. coli* K12 *recA56* (SR755) and *recA56 recB21* (SR756) cells UV-irradiated with 10 J/m^2 as a function of liquid-holding time. Symbols: ●, *redA*; ▲, *recA recB*.

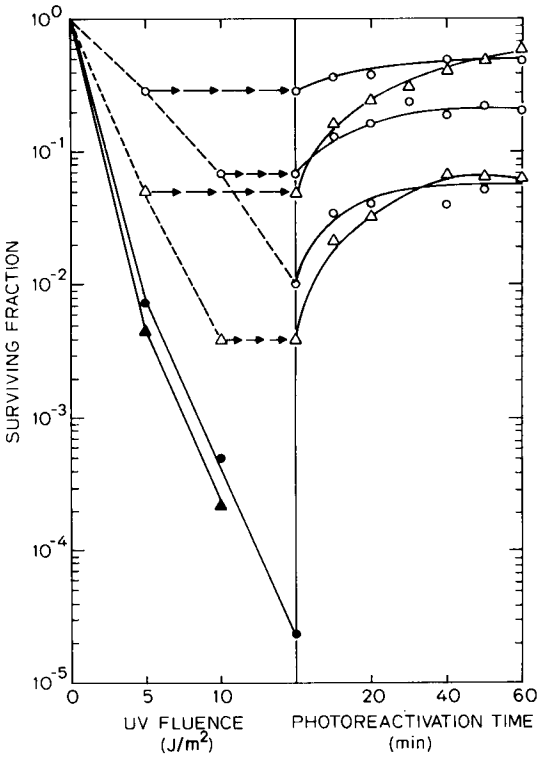

Fig. 4. UV-radiation survival curves of *recA56* (SR755) and *recA56 recB21* (SR756) cells under different experimental conditions. Left panel: curves represent cells with (dashed line, open symbols) or without (solid line, closed symbols) a 24 h LH treatment. Symbols: △, ▲, *recA*; ○, ●, *recA recB*. Right panel: the increased viability after different times of photoreactivation (see text) after 24 h of LH. Symbols are the same as in the left panel. Those survival points that were further irradiated with visible light are indicated by the arrowed lines. Data points are the average of 2 Expts.

breaks in either of these strains, the higher extent of LHR seen in *recA recB* cells may refelect a higher efficiency of pyrimidine dimer removal in these cells during LH. Indeed, we observed that the rate of LHR in the *recA recB* strain was higher than the *recA* strain, and the difference in the extent of LHR between these 2 strains was reduced by prolonging the LH time (Fig. 3).

Photoreactivation of UV irradiated recA56 and recA56 recB21 cells

As a measure of the amount of residual pyrimidine dimers present, we compared the amount of photoreactivation obtained for the *recA* and *recA recB* strains after LH. After the same UV-radiation fluence and LH period, the *recA* cells exhibited more photoreactivation than did the *recA recB* cells (Fig. 4). Moreover, the difference in survival of the UV-irradiated *recA* and *recA recB* cells after 24 h of LH was diminished by the subsequent photoreactivation. Immediately after UV irradiation, the *recA recB* strain showed photoreactivation kinetics that were faster than those for the *recA* strain (Fig. 5).

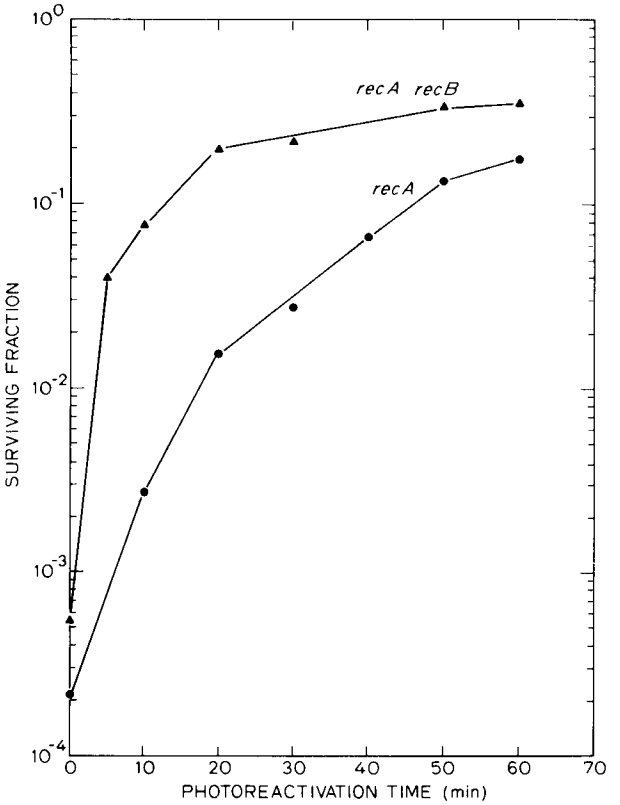

Fig. 5. Photoreactivation curves of *recA56* (SR755) and *recA56 recB21* (SR756) cells after 10 J/m^2 of UV radiation (254 nm). The conditions for photoreactivation were the same as described in Fig. 4. The surviving fraction of the cells held for 70 min without photoreactivation increased to 4.7 X 10^{-3} for *recA recB*, and to 2.4 X 10^{-4} for *recA*. Symbols: ●, *recA*; ▲, *recA recB*. The data points are the average of 2 Expts.

Discussion

The biological effectiveness (i.e., the increase in survival) of LHR (an excision-repair process that can proceed in the absence of growth medium) can ony be detected in cells that are deficient (or partially so) in growth medium dependent repair (e.g., postreplication repair) [20]. Since a *recA* mutation blocks postreplication repair, one might predict that mutations that do not affect the UV-radiation sensitivity of a *recA* strain should have no effect on the extent of LHR observed in a *recA* strain. The observation that an additional *lexA* mutation in a *recA* strain did not affect UV-radiation sensitivity or LHR seemed to fit this notion (Fig. 1). Such a result is also consistent with the suggestion that the *lexA* gene controls the expression of the recA gene [4,7,14].

The results for the *recA recB* strain were unexpected. This strain showed the same UV-radiation sensitivity as the *recA* strain, but a much higher survival after 24 h of LH (Fig. 1). The kinetics of LHR were much faster in the *recA recB* strain, suggesting that excision repair is much faster in the *recA recB* strain

than in the *recA* strain during LH. After 72 h of LH, however, the 2 strains showed a similar survival (Fig. 3).

Consistent with this difference in kinetics of LHR (which is equivalent to a difference in the kinetics of excision repair during LH) was the observation that after the same UV-radiation fluence and LH period, the *recA* strain had a larger phororeactivable sector (i.e., more residual pyrimidine dimers were present) than did the *recA recB* strain (Fig. 4). Castellani et al. [3] had shown that there was an overlap between LHR and photoreactivation, i.e., the extents of recovery by these 2 independent processes were similar.

If the difference in LHR between the *recA* and the *recA recB* strains is merely due to the presence of the *recB* gene product [exonuclease V (exoV); 22,23] in the *recA* strain (but its absence in the *recA recB* strain), then the slower rate but similar final extent of LHR (i.e., excision repair) observed in the *recA* strain versus the *recA recB* strain suggests that exoV may bind to pyrimidine dimers, or to the denatured region near a dimer, and thus act as a competitive inhibitor of excision repair during LH.

Experiments in vitro reveal that exoV is able to excise pyrimidine dimers in the presence of ATP [21]. Therefore, it is reasonable to assume that exoV can recognize and bind to pyrimidine dimers during LH, but may be unable to excise them due to the absence of a sufficient energy source. This exoV—DNA complex may then interfere with the *uvrA(B)* endonuclease. A similar overlapping of substrate specificity and mutually interfering reactions has been observed by Patrick and Harm [15] for the photoreactivating enzyme and the *uvrA(B)* endonuclease.

Immediately after UV irradiation, the kinetics of photoreactivation were much reduced in the *recA* strain relative to the *recA recB* strain, although the final extents of photoreactivation were about the same (Fig. 5). These results suggest that, in nongrowth medium, exoV may also interfere with the photoreactivating enzyme by binding at or near pyrimidine dimers.

Ogawa et al. [13] reported that *uvrD* mutants are unable to perform host-cell reactivation, and that the excessive DNA degradation seen in UV-irradiated *uvrD* cells was greatly reduced by an additional *uvrB* mutation. These results implicated the involvement of the *uvrD* gene product in excision repair. Current work in this laboratpry suggests that the involvement of the *uvrD* gene prduct in excision repair is through the control of the size of the patches of repair replication produced by DNA polymerases I and III [K.M. Carlson and K.C. Smith, manuscript in preparation]. Fig. 1 shows that an additional *uvrD* mutation sensitizes *recA* cells. This result is consistent with the observations that the *uvrD* gene product is involved in excision repair. The excessive degradation (Table 2) and production of DNA double-strand breaks (Fig. 2) during LH probably explains the absence of LHR in the *recA uvrD* strain.

A *recF* strain has been reported to be as proficient in excision repair as was a wild-type strain [16], and the *recF* gene product is apparently involved in *recA*-dependent but *recB(C)*-independent genetic recombination and postreplication repair [10,17]. Horii and Clark [10] reported that, in the AB background, an additional *recF* mutation only sensitized a *recA* strain to UV-radiation fluences that produced greater than 99% killing. In the W3110 background, however, our *recA recF* strain showed enhanced UV-radiation sensitivity at all levels of

survival (Fig. 1). In addition to the report that the *recF* gene product functions in a *recA* gene-controlled DNA repair system [17], our observation that a *recF* mutation markedly sensitized *recA* cells to UV radiation and enhances their LHR (Fig. 1) suggests that the *recF* gene product also functions in a *recA*-independent DNA-repair system. Work is in progress to test this hypothesis.

In summary, of the single mutants studied, LHR is highest in *recA* strains of *E. coli* K12 [20]. Adding a *lexA* mutation to a *recA* strain had no additional effect on LHR, adding a *recB* or a *recF* mutation enhanced LHR, but adding a *uvrD* mutation blocked LHR. The molecular basis of these results have been explored.

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