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Effect of *tsl* Mutations in Decreasing Radiation Sensitivity of a *recA*⁻ Strain of *Escherichia coli* K-12

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It has been shown previously that the radiation sensitivity of *lexA*⁻ strains of *Escherichia coli* K-12 can be suppressed by thermosensitive mutations (designated *tsl*) that are closely linked to the *lexA* locus and are thought to be intragenic suppressors of *lexA* mutations (Mount et al., 1973). When a *recA* mutation is crossed into a suppressed *tsl*⁻ strain, the extreme radiation sensitivity usually conferred by a *recA* mutation is decreased, but there is no detectable change in genetic recombination deficiency. Increased resistance to UV in the *tsl*⁻*recA*⁻ strains depends upon ability to synthesize active *uvrA*⁺ product.

recA⁻ strains of *Escherichia coli* K-12 are deficient in genetic recombination and in repair of a variety of deoxyribonucleic acid (DNA) lesions including pyrimidine dimers produced by irradiation with ultraviolet (UV) light (for review, see Clark [7]). They also respond abnormally to UV irradiation in certain other respects that have been reviewed recently (17). The prevailing view is that they are defective in repair of gaps left opposite pyrimidine dimers (19) when UV-damaged DNA is replicated (21). Genetic exchanges between newly formed sister chromosomes each containing gaps is thought to be a mechanism for the repair of these gaps (19).

Essentially all the mutant properties of *recA*⁻ strains are also observed in *lexA*⁻ strains of *E. coli* K-12 and the identical *exrA*⁻ strains (8) of *E. coli* B (3, 13, 17, 20) with the exceptions that *lexA*⁻ strains are recombination proficient, and secondly, they are not as sensitive as *recA*⁻ strains to agents that damage DNA (13). Moody et al. (13) have provided evidence that the *recA*⁺ and *lexA*⁺ products act in a common pathway of DNA repair by showing that *lexA*⁻ *recA*⁻ strains are no more sensitive to UV and ionizing radiation than *lexA*⁺ *recA*⁻ strains. These observations have led to the hypothesis that the *recA*⁺ product acts in two pathways of DNA repair: one is acted upon by the *lexA*⁺ product and is not a genetic recombination pathway and a second is not affected by *lexA* (13).

Other experiments have shown that the LexA⁻ phenotype results from the synthesis of a diffusible product (4, 14). A major class of UV-resistant derivatives of *lexA*⁻ strains grows normally at 30 C but at 42.5 C is defective in cell division and grows into multinucleate filaments without septa (16). The thermosensitive mutations are located to within 0.04 min of the site of the *lex* mutation and could lie within the same gene. This result, in addition to genetic complementation studies, supports the idea that *tsl* mutations alter the diffusible product that gives rise to the Lex⁻ phenotype.

Since the *lexA*⁺ and *recA*⁺ products appear to act in a common DNA repair pathway (13), strains were prepared to test the effect of *tsl* mutations on the properties of a *recA*⁻ strain. A *uvrA* mutation known to block the excision of pyrimidine dimers (2, 12) was also introduced into some of the strains. All strains carrying *tsl* mutations are defective in cell division at 42.5 C.

The survivals of UV-irradiated cultures grown and tested at 30 C, the permissive temperature for growth of *tsl*⁻ strains, are shown in Fig. 1. It is clear that *tsl*⁻ increases the survivals of a *uvrA*⁺ *recA*⁻ strain to a large extent but has little or no effect when present in *uvrA*⁻ *recA*⁺ and *uvrA*⁻ *recA*⁻ strains. These results demonstrate that *tsl* mutations increase the resistance of *recA*⁻ strain to UV, and that the increase depends upon the ability of the strain to make active *uvrA*⁺ product. In other experiments, we have demonstrated a similar effect on the UV sensitivity of a strain with a different *recA*⁻ allele, and have shown that several *tsl*⁻ alleles produce this same result (data not shown). Furthermore, we have shown that *tsl*⁻ also makes a *lon*⁻ strain (1, 11) of *E. coli* K-12 more resistant to UV (A. Walker, unpublished observations).

tsl⁻ does not influence the genetic recombina-

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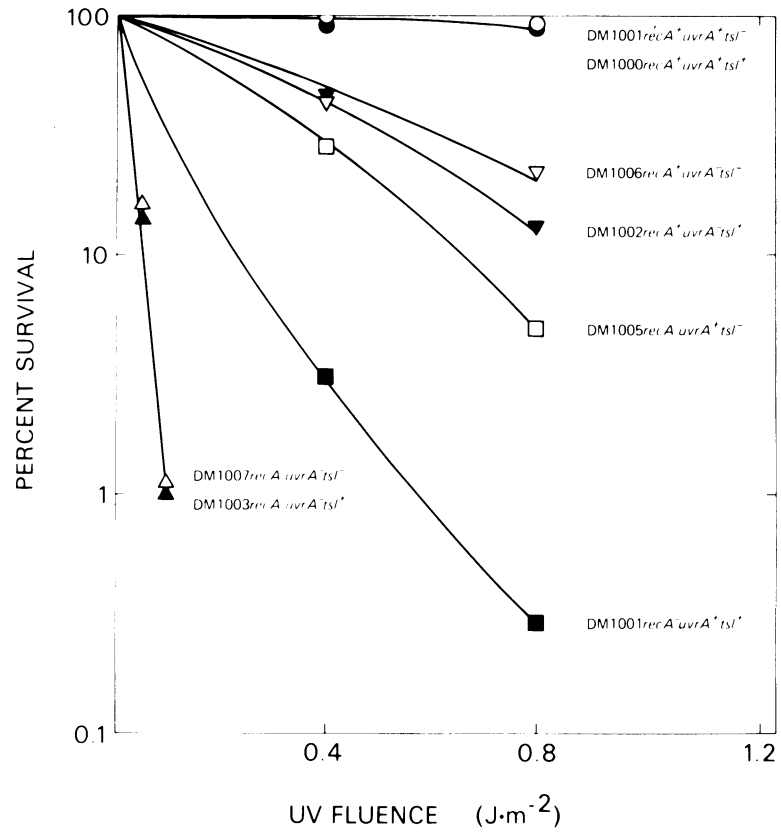

FIG. 1. *UV survival curves of strains. The nomenclature is that of Demerec et al. (7) and Taylor and Trotter (22). lexA refers to a lex mutation at 80.9 min on the E. coli K-12 linkage map. Since these mutations are dominant (5, 15), it is not possible to assign them to a single complementation group. It has not been possible to demonstrate by genetic methods the presence or absence of lexA⁻ in these tsl⁻ strains. Exponential N broth cultures of 30 C were diluted 100-fold into phosphate-buffered saline and irradiated 40 cm from a germicidal lamp at a dose rate of 0.025 J/m²/s. Survivors were inoculated on N agar plates, and colonies were scored after 18 h of incubation at 30 C (14).*

tion deficiency of a *recA*⁻ strain at 30 C (Table 1). It has also been shown that *tsl*⁻ does not influence failure of either spontaneous or UV induction of phage λ in lysogenic *recA*⁻ strains (data not shown). However, it decreases the sensitivity of a *recA*⁻ strain to the alkylating agent methylmethane sulfonate and the cross-linking agent mitomycin C (Fig. 2, Table 2). The effects of *tsl*⁻ on the phenotype of strains with a *recA* mutation thus seem to be specific for DNA repair.

Rates of DNA synthesis in unirradiated *tsl*⁻ *recA*⁻ and *tsl*⁺ *recA*⁻ cultures growing at 30 C are not significantly different (Fig. 3). Rates are not lower in UV-irradiated cultures at a dose of 0.75 J/m², but the amount of DNA synthesized is reduced and is slightly lower in the *tsl*⁺ as compared to the *tsl*⁻ strain (Fig. 3). DNA breakdown measurements shown in Fig. 4 indicate that the extensive breakdown (5) that

TABLE 1. *Recombination deficiency of tsl recA strains*[a]

Recipient strain	Genotype		Recombination deficiency index
	tsl	*recA*	
DM937	+	+	1.0
DM935	1	+	1.0
DM938	+	1	3×10^4
DM936	1	1	$>10^5$

[a] The donor strain is AB259 (Hfr Hayes). Mating mixtures (1 Hfr cell to 10 F cells) were prepared from exponential cultures growing at 30 C, and matings were terminated after 60 min at 30 C. They were inoculated on to plates selective for Thr⁺ Leu⁺ [Str^R] recombinants. The recombination deficiency index is the ratio of the number of recombinants obtained from the mating with strain DM937 to the number obtained with each individual mutant strain. A large value of the index represents decreased ability for recombinant formation relative to the *recA*⁺ strain.

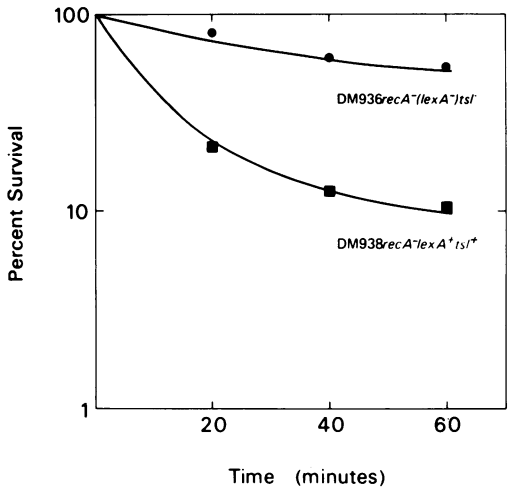

FIG. 2. *Methylmethane sulfonate survival curves of strains. Exponential N broth cultures at 30 C were diluted 100-fold into phosphate-buffered saline and methylmethane sulfonate added to a final concentration of 0.002 M. Samples were diluted 100-fold into N broth and left at 0 C for 30 min to stop the reaction. Survivors were scored as in the experiment in Fig. 1. The survival of cultures of strains DM935 recA⁺ lexA⁺ tsl⁺ and DM937 recA⁺ (lexA3) tsl-1 was 100%.*

TABLE 2. *Sensitivity of tsl⁻ recA⁻ strain to mitomycin C*

Strain no. and genotype	Ratio of no. of colonies on plates with mitomycin C to no. on plates without mitomycin C[a]
DM935 *tsl⁻ recA⁺*	0.29
DM936 *tsl⁻ recA⁻*	0.05
DM937 *tsl⁺ recA⁺*	0.12
DM938 *tsl⁺ recA⁻*	3×10^{-7}

[a] Exponential-phase cultures in N broth at 30 C were diluted in broth and inoculated to N agar plates containing either 0 or 0.6 μg of mitomycin C per ml, and the plates scored for visible colonies after 40 h of incubation at 30 C.

occurs in both unirradiated and UV-irradiated cultures of *recA⁻* strains at 30 C is not affected by *tsl⁻*. An indicator of the effect of UV irradiation on DNA synthesis is the time at which cell division is re-initiated in UV-irradiated cultures. This experiment has the advantage of showing the response of those cells in the culture that recover the ability to undergo divisions and form viable progeny cells. UV-irradiated cultures of both *tsl⁻ recA⁻* and *tsl⁺ recA⁻* strains begin to divide 2.5 h after exposure to a UV dose of 0.75 J/m² (Fig. 5). A substantial delay in DNA synthesis in the survivors of the *tsl⁻ recA⁻* culture would be

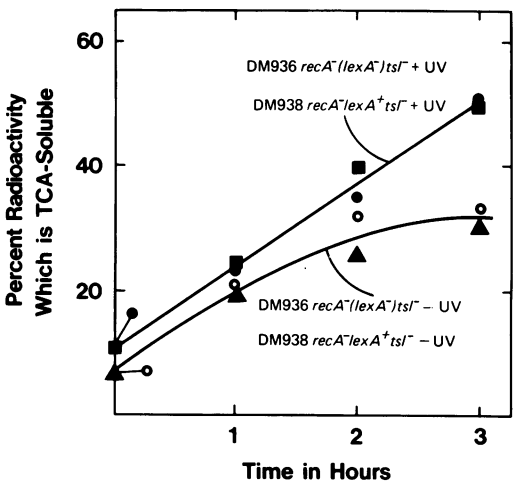

FIG. 3. *DNA degradation in UV-irradiated cultures. The method of Clark et al. (5) for measuring breakdown of DNA label from trichloroacetic acid-insoluble to -soluble material was used. Cells were exposed to a UV dose of 10 J/m².*

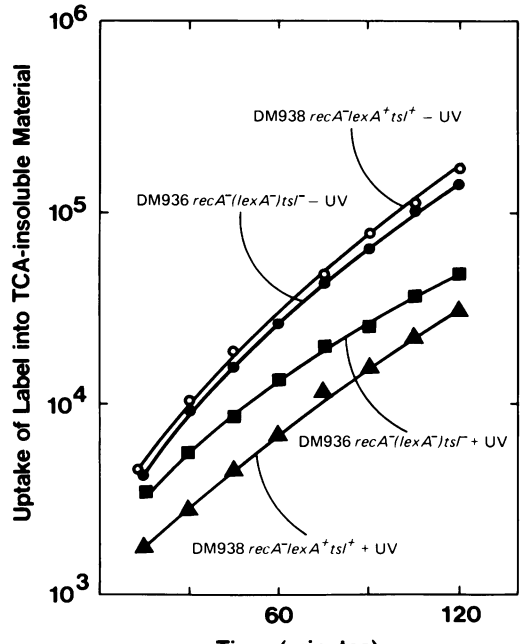

FIG. 4. *Rates of DNA synthesis in UV-irradiated cultures. Exponential cultures in EM9 medium at 30 C were divided into two parts, one of which was treated with a UV dose of 0.75 J/m², and the other was left untreated. Both parts were diluted fourfold into fresh EM9 containing ³H-labeled thymidine (18 Ci/mmol) at a final concentration of 20 μCi/ml and deoxyadenosine at 100 μg/ml. Aliquots (0.1-ml) were removed to Millipore filters that were washed in 5% trichloroacetic acid at 0 C for 30 min. Filters were dried, added to scintillation fluid, and counted.*

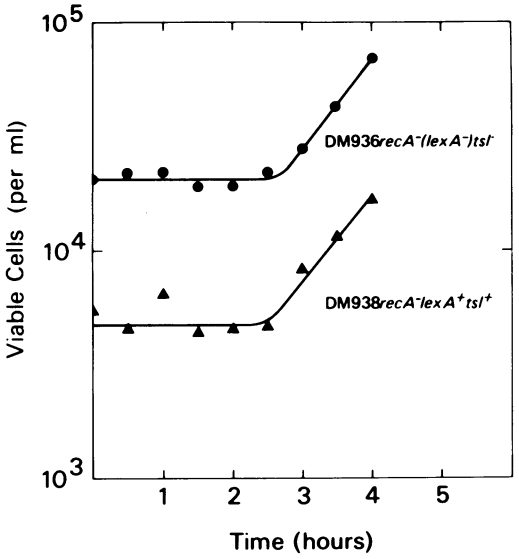

FIG. 5. *Cell division in UV-irradiated cultures. Exponential cultures in N broth at 30 C were diluted 100-fold into phosphate-buffered saline and exposed to a UV fluence of 0.75 J/m². They were then diluted 10-fold into N broth and incubated at 30 C. Survivors were scored as in the experiment in Fig. 1. The doubling time of unirradiated control cultures was 75 min.*

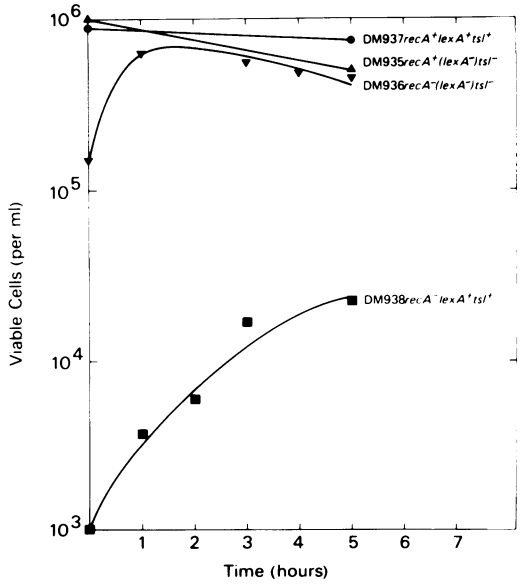

FIG. 6. *Recovery of UV-irradiated cultures in buffer. Exponential-phase N broth cultures at 30 C were centrifuged, washed in the same volume of phosphate-buffered saline, centrifuged and washed again, and the final cell suspension was diluted 100-fold into phosphate-buffered saline. The diluted culture was exposed to a UV fluence of 0.75 J/m² and incubated at 30 C. Survivors were scored as in the experiment in Fig. 1.*

expected to result in a corresponding lag in cell division, and this is not observed.

UV-irradiated cultures of *tsl⁻ recA⁻* and *tsl⁺ recA⁻* strains were incubated in buffer prior to inoculation to nutrient plates. This treatment increased survivals of both strains (Fig. 6). This recovery has been attributed by Ganesan and Smith (9) to a delay in replication of UV-damaged DNA, allowing more time for excision repair. Since recovery is also observed in the *tsl⁻ recA⁻* strain, it is unlikely that the mechanisms of recovery promoted by *tsl* mutations and incubation in buffer are the same.

A further possible explanation for the results that we have observed is that cell division in the *tsl⁻ recA⁻* strain could be abnormal and result in physiological changes that lead to increased tolerance to DNA damage. Cultures of a *recA⁻ tsl⁻* strain growing at 30 C were examined under the microscope, and it was found that the distribution of cell sizes is approximately the

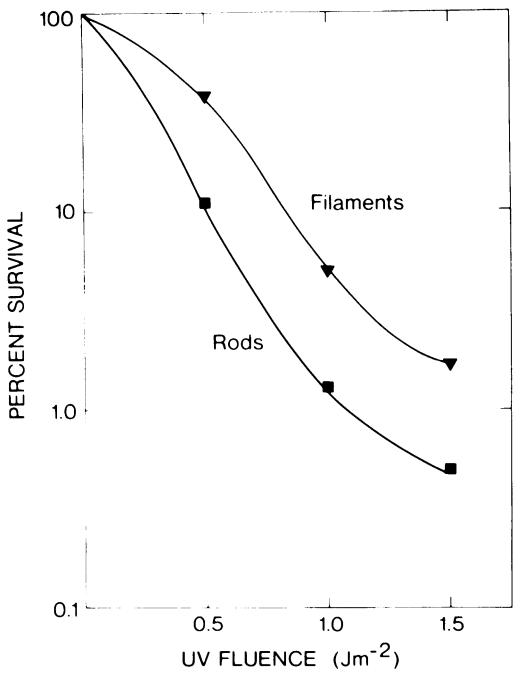

FIG. 7. *Comparison of UV sensitivities of tsl⁻ rods and filaments. Exponential N broth cultures of DM511 at 30 C were diluted 100-fold in N broth and divided into two parts: one part was incubated with aeration at 30 C, the other at 42.5 C for 120 min. Cells in the 42.5 C culture stopped dividing and grew into long filaments while the cells at 30 C divided normally. The cultures were harvested and concentrated 10 times by centrifugation, and adjusted to the same optical density at 500 to 570 nm. The adjusted suspensions were UV-irradiated with a UV fluence of 0.75 J/m², and surviving cells were assayed as in the experiment in Fig. 1.*

same as that of a *recA*$^-$ *tsl*$^+$ strain. Growth into a long filamentous form was induced by incubation of the *tsl*$^-$ *recA*$^-$ strain at 42.5 C for 2 h. These filaments, which grow to be 50 to 100 times the length of normal *E. coli* rods, retain their ability to form colonies on nutrient agar at 30 C and can be tested for their sensitivity to UV irradiation. Filaments of the *tsl*$^-$ *recA*$^-$ strain prepared in this manner are slightly more UV resistant than the rods in 30 C cultures (Fig. 7). These results are interpreted to mean that *tsl*$^-$ *recA*$^-$ cells do not owe their resistance at 30 C to physiological changes in growth and division.

A plausible mechanism for the UV resistance conferred by *tsl* mutations on *recA*$^-$ strains is that they increase the efficiency of excision repair so that replication past pyrimidine dimers and entrance into a postreplication repair pathway that is blocked by *recA* mutations will be less likely to occur. It is interesting to compare this proposed mechanism with their known effect of allowing restoration of postreplication repair of UV damage in *tsl* derivatives of *lex*$^-$ mutants of *E. coli* K-12 (A. K. Ganesan, personal communication).

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