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-
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>
<thead>
<tr>
<th>Recipient strain</th>
<th>Genotype</th>
<th>Recombination deficiency index</th>
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<tbody>
<tr>
<td></td>
<td>tsl</td>
<td>recA</td>
</tr>
<tr>
<td>DM937</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DM935</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>DM938</td>
<td>+</td>
<td>1</td>
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<tr>
<td>DM936</td>
<td>1</td>
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</table>

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+] 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.
and the were diluted containing either incubation 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>
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<tr>
<th>Strain no. and genotype</th>
<th>Ratio of no. of colonies on plates with mitomycin C to no. on plates without mitomycin C *</th>
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<tr>
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<tr>
<td>DM936 tsl- recA-</td>
<td>0.05</td>
</tr>
<tr>
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</tr>
<tr>
<td>DM938 tsl+ recA-</td>
<td>3 x 10^-1</td>
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* 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. 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

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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 1H-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. 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.
Exponential cultures doubling time of were scored to a 10-fold into N 100-fold into

saline, -1

and again, centrifuged, were

experiment in incubated at was culture into 100-fold buffer. Exponential-phase

of phosphate-buffered saline, phosphate-buffered saline. Exposed cultures

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.

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

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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 propose this 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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