Repair of DNA double-strand breaks in UV-irradiated
Escherichia coli uwrB recF cells is inhibited by rich growth medium

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Summary

Ultraviolet (UV)-irradiated uwrB recF and uwrB recB cells of Escherichia coli K-12 showed similar radiation sensitivities when plated on minimal growth medium (MM), however, the uwrB recF cells were much more UV radiation-sensitive than the uwrB recB cells when plated on rich growth medium. Sedimentation analysis of the DNA from UV-irradiated uwrB recF cells suggests that the rich medium killing of uwrB recF cells is due to the inhibition of the repair of UV-radiation-induced DNA double-strand breaks, i.e., the killing is due to the inhibition of the recB-dependent pathway of postreplication repair. Furthermore, we demonstrated that the DNA double-strand breaks that were formed in UV-irradiated uwrB recA200(Ts) cells incubated at 42°C in rich growth medium were not repaired whether the medium during subsequent repair incubation at 30°C was MM or rich growth medium, while DNA double-strand breaks that were formed in MM at 42°C could be repaired in MM or in rich growth medium at 30°C. How the absence of an abrupt slowing of DNA synthesis when UV-irradiated cells are held in rich growth medium (Sharma and Smith, 1985b) may prevent the repair of these DNA double-strand breaks is discussed.

UV-irradiated Escherichia coli K-12 uwrA, uwrB and uwrC cells show higher survival if plated on minimal growth medium (MM) rather than on rich growth medium [e.g., yeast extract-nutrient broth (Ganesan and Smith, 1968a; Sharma et al., 1982), or MM plus Casamino Acids (Sharma et al., 1982)]. This phenomenon has been referred to as ‘minimal medium recovery’ (MMR) (Ganesan and Smith, 1968b; Smith, 1971). The lethal effect of rich growth medium on UV-irradiated uwrA(B) cells has been correlated with the inhibition of the repair of DNA daughter-strand gaps (Sharma et al., 1982, 1983). The repair process involved in MMR is largely inducible (Sharma and Smith, 1983), and requires functional recA, lexA and recB genes; the recF gene plays a minor role in MMR (Sharma et al., 1982). However, all of these genes play major roles in postreplication repair (Wang and Smith, 1983).

breaks that are assumed to be formed at unrepaired DNA daughter-strand gaps (Wang and Smith, 1983).

The recB and recF pathways appear to be equally important for cell survival if UV-irradiated cells are plated on MM (Rothman et al., 1975; Wang and Smith, 1983), however, after UV irradiation, uwrB recF cells are much more sensitive to rich medium killing than are uwrB recB cells, i.e., the uwrB recF strain shows much more MMR than does the uwrB recB strain. These results suggest that rich growth medium inhibits the recB-dependent pathway of postreplication repair much more than it inhibits the recF-dependent pathway of postreplication repair. This notion was tested in this work.

Materials and methods

Bacterial strains, media and cultures

The strains of *E. coli* K-12 used in this study are listed in Table 1.

The minimal growth medium (MM), salts buffer (DTM), and minimal plating medium have been described (Sharma and Smith, 1985a). The rich plating medium (YENB plates) was Difco yeast extract (0.75%) plus Difco nutrient agar (2.3%). Liquid YENB was Difco yeast extract (0.75%) and Difco nutrient broth (0.8%). Phosphate buffer (PB) was \( \text{Na}_2\text{HPO}_4 \) (5.83 g/l) and \( \text{KH}_2\text{PO}_4 \) (3.53 g/l), pH 7.0.

Logarithmic-phase cultures were obtained by diluting (100-fold) a fresh overnight culture into MM, and incubating the diluted cultures in a shaking waterbath at 37°C [or at 30°C for the uwrB recA200(Ts) strain] until they reached an optical density at 650 nm (OD\(_{650}\)) of 0.4 (Zeiss PMQ II spectrophotometer). The cultures were diluted with MM to an OD\(_{650}\) of 0.1, and UV irradiated while in MM.

**UV-irradiation**

The source and method for UV-irradiation (254 nm) have been described (Sharma and Smith, 1983). For survival determination, irradiated and nonirradiated cell suspensions were diluted in PB and plated on MM and YENB plates. Incubation was for 1–3 days at 37°C. All experiments were done under yellow light to prevent photoreactivation.

**Measurement of DNA repair**

The methods used for alkaline and neutral sucrose gradient sedimentation were similar to those previously described (Sharma and Smith, 1983; Wang and Smith, 1983), except that the cells were UV-irradiated while in medium, and were shaken for 120 min at 37°C to allow the completion of repair.

The following protocol was used for the uwrB recA200(Ts) strain. Cells were grown as described above, adjusted to an OD\(_{650}\) of 0.1, and shaken for 60 min at 42°C. Then, the culture was readjusted to an OD\(_{650}\) of 0.1 (the media and dishes were preequilibrated to 42°C), UV-irradiated and shaken for 5 min at 42°C before pulse labeling.

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**TABLE 1**

**LIST OF STRAINS OF *E. coli* K-12**

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Relevant genotype</th>
<th>Other characteristics</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR305</td>
<td>( \Delta(uwrB-chla) ) recF143</td>
<td>( \text{thyA36 deo}(C27?) ) leuB19 lacZ53 mulB45 rha-5 rpsL151</td>
<td>D.A. Youngs</td>
</tr>
<tr>
<td>SR839</td>
<td>( \Delta(uwrB-chla) ) recA56</td>
<td>Same as SR305, except also srlA300::Tn10</td>
<td>M. Tang</td>
</tr>
<tr>
<td>SR840</td>
<td>( \Delta(uwrB-chla) ) recB21 recF143</td>
<td>( \text{thyA} ) deo (C27?) leuB19 lacZ53 mulB45 rha-5 rpsL151</td>
<td>M. Tang</td>
</tr>
<tr>
<td>SR1160</td>
<td>( \Delta(uwrB-chla) ) recB21</td>
<td>Same as SR840</td>
<td>Wang and Smith, 1983</td>
</tr>
<tr>
<td>SR1347</td>
<td>( \Delta(uwrB-chla) ) recA200(Ts)</td>
<td>Same as SR839</td>
<td>T.V. Wang</td>
</tr>
</tbody>
</table>

* Genotype symbols are those used by Bachmann (1983). All strains are F\(^{-}\) and \( \lambda^- \).
with \(^{3}H\) thymidine at 25 \(\mu\)Ci/ml (80.2 Ci/mmole, New England Nuclear) for 10 min at 42°C. After pulse-labeling, the cultures (nonirradiated and UV-irradiated) were collected by filtration, washed with DTM and resuspended in MM. To resuspend the cells in rich growth medium, 1 vol. of 5 \(\times\) YENB was added to 4 vol. of the culture in MM. The cells in MM and in rich growth medium were incubated for 100 min at 42°C to allow the maximum production of DNA double-strand breaks, and then were shifted to 30°C for 120 min to allow repair to occur. The cells were converted to spheroplasts and layered on top of gradients [4.8 ml of 5 to 20% sucrose containing 0.1% (vol./vol.) Triton X-100]. The layered gradients were held for at least 120 min in the dark at room temperature, and then were centrifuged at 3700 rpm for 40 h at 20°C. The number-average molecular weight \((M_n)\) was calculated as described by Bonura et al. (1975) using \(^{14}C\) thymine-labeled bacteriophage T2 DNA as a molecular weight marker. The \(M_n\) values were used to calculate the number of DNA double-strand breaks (DSB) per double-strand genome (DSG) using the equation: DSB/DSG = \((M_{n(0)}/M_{n(uv)} - 1) (2.8 \times 10^9/M_{n(0)})\). Where \(M_{n(0)}\) and \(M_{n(uv)}\) represent the number-average molecular weight of DNA from unirradiated and UV-irradiated cells, respectively. A value of \(2.8 \times 10^9\) dalton was used for the molecular weight of the \(E. coli\) genome (Cairns, 1963).

Results and discussion

The UV-radiation survival of various mutant cells grown to logarithmic-phase in MM and plated on MM and rich growth medium are shown in Fig. 1. In agreement with earlier data (Rothman et al., 1975; Wang and Smith, 1981, 1983). \(uvrB\) \(recB\) and \(uvrB\) \(recF\) cells showed a similar UV-radiation survival when plated on MM, however, the \(uvrB\) \(recF\) strain was much more UV-radiation-sensitive than the \(uvrB\) \(recB\) strain if plated on rich growth medium. The latter result is in agreement with our earlier observation (Sharma et al., 1982) that the \(uvrB\) \(recB\) strain shows only a small amount of MMR.

Since the major repair system operating in \(uvrB\) \(recB\) cells is the \(recF\)-dependent repair of DNA daughter-strand gaps that are formed after UV-irradiation, we studied the effect of rich growth medium on the repair of DNA daughter-strand gaps in \(uvrB\) \(recB\) cells. Consistent with the survival data, rich growth medium showed only a small effect on the amount of DNA daughter-strand gap repair observed in UV-irradiated \(uvrB\) \(recB\) cells (data not shown). Taken together, these data suggest only a small role for the \(recF\) gene in MMR repair.

The UV-radiation survival of the \(uvrB\) \(recF\) strain was \(\sim 6\)-fold higher (based upon a comparison of the \(F_{10}\) values, i.e., the fluence to yield 10% survival) than that for the \(uvrB\) \(recB\) \(recF\) strain when plated on rich growth medium (Fig. 1). When plated on rich growth medium, the \(uvrB\) \(recB\) \(recF\) strain was as UV radiation sensitive as a \(uvrB\) \(recA\) strain (Fig. 1), which shows no MMR and no postreplication repair (Ganesan and Smith, 1970; Sharma and Smith, 1985a; Smith and Meun, 1970). These survival data suggest that, in the \(uvrB\)
recF strain, rich growth medium may inhibit the recB-dependent pathway of postreplication repair. Therefore, we investigated the effect of rich growth medium on the recB-dependent pathway of postreplication repair, i.e., the repair of DNA double-strand breaks that apparently arise at unrepaired DNA daughter-strand gaps (Wang and Smith, 1983).

Based upon our survival studies (Fig. 1), and knowledge of the role of recB in postreplication repair (Wang and Smith, 1983), rich growth medium should inhibit the repair of DNA double-strand breaks in UV-irradiated uvrB recF cells. This inhibition could be due to one or more reasons: (i) more DNA double-strand breaks are produced when UV-irradiated uvrB recF cells are incubated in rich growth medium, (ii) the number of DNA double-strand breaks produced are the same in MM and rich growth medium, but their repair is inhibited by rich growth medium.

To explore these two possibilities, we measured the effect of rich growth medium on the formation and repair of DNA double-strand breaks in UV-irradiated uvrB recF cells. After 2 h of postirradiation incubation, the formation of DNA double-strand breaks was observed for cells suspended either in MM or rich growth medium (Fig. 2). The number of DNA double-strand breaks at 2 h of postirradiation (0.25 J/m²) incubation in MM and rich growth medium were 2 and 4 DNA double-strand breaks per double-strand genome, respectively. On further incubation in MM (4 h at 37°C), most of these DNA double-strand breaks were repaired (Fig. 2A). However, only about half of the [³H]DNA returned to a high molecular weight [i.e., it sedimented under the peak of unirradiated DNA (Fig. 2B)] when the postirradiation incuba-

**TABLE 2**

**EFFECT OF RICH GROWTH MEDIUM ON THE REPAIR OF DNA DOUBLE-STRAND BREAKS IN UV-IRRADIATED E. coli K-12 ΔuvrB recA200(Ts)**

<table>
<thead>
<tr>
<th>Medium during the formation of DNA DSB at 42°C b</th>
<th>Medium during the repair of DNA DSB at 30°C c</th>
<th>Did the repair of DSB occur?</th>
<th>Panels in Fig. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM</td>
<td>MM</td>
<td>Yes</td>
<td>A (compare ● and ▲)</td>
</tr>
<tr>
<td>MM</td>
<td>RM</td>
<td>Yes</td>
<td>B (compare ● and △)</td>
</tr>
<tr>
<td>RM</td>
<td>RM</td>
<td>No</td>
<td>C (compare ○ and △)</td>
</tr>
<tr>
<td>RM</td>
<td>MM</td>
<td>No</td>
<td>D (compare ○ and ▲)</td>
</tr>
</tbody>
</table>

a Cells were UV-irradiated (0.5 J/m²) and pulse-labeled with [³H]thymidine in MM at 42°C.
b Incubated for 100 min to observe the maximum production of DNA double-strand breaks (DSB). Rich growth medium (RM) was yeast extract-nutrient broth.
c Incubated for 120 min to observe the maximum repair of DNA DSB.
tion was in rich growth medium, suggesting that the repair of DNA double-strand breaks under these conditions was incomplete.

The accumulation of more DNA double-strand breaks in rich growth medium than in MM after 2 h of postirradiation incubation (Fig. 2) suggests that rich growth medium enhances the formation of DNA double-strand breaks, however, since the kinetics reflect both the formation and repair of DNA double-strand breaks, an inhibition of repair by rich growth medium would yield the same result.

To study separately the formation and repair of DNA double-strand breaks, we have employed a urrB recA200(Ts) strain. This strain is RecA1 at 42°C (nonpermissive temperature) and RecA1 at 30°C (permissive temperature) (Lloyd et al., 1974; After UV irradiation (0.5 J/m²). urrB recA(Ts) cells were pulse-labeled with [³H]thymidine and incubated at 42°C (in MM or rich growth medium) to accumulate DNA double-strand breaks, and then the repair of these DNA double-strand breaks was followed by further incubation in MM or rich growth medium at 30°C. The results shown in Fig. 3, and summarized in Table 2, demonstrate that in urrB recA(Ts) cells: (i) the numbers of DNA double-strand breaks formed during postirradiation incubation in MM or rich growth medium were the same (12 DNA double-strand breaks per genome), (ii) DNA double-strand breaks formed in rich growth medium were not repaired whether the cells were incubated in MM or rich growth medium, and (iii) DNA double-strand breaks formed in MM were repaired whether the cells were incubated in MM or rich growth medium.

Since the DNA double-strand breaks that were formed when the cells were incubated in rich medium were not repairable regardless of the type of medium used during the repair phase, we suggest that the DNA double-strand breaks that were not repaired when the urrB recF cells were switched to rich medium (Fig. 2) were probably the double-strand breaks that were formed after these cells were switched to rich growth medium.

To explain how the presence of rich growth medium may convert repairable DNA double-strand breaks to nonrepairable DNA double-strand breaks, we propose the following mechanism. Recently we have shown that the rich-medium killing of UV-irradiated urrA cells correlates with the lack of an abrupt inhibition of DNA synthesis in rich growth medium. The abrupt inhibition of DNA synthesis that occurs immediately after UV-
irradiation in cells incubated in MM apparently favors the recombinational repair of DNA daughter-strand gaps (Sharma and Smith, 1985b). However, if DNA synthesis is not inhibited, the sister duplex containing a double-strand break may move further away from the adjacent sister duplex needed to initiate recombination in order to generate lesion free DNA, thereby converting repairable DNA double-strand breaks to nonrepairable DNA double-strand breaks without affecting the yield of double-strand breaks.

In summary, a major portion of the rich-medium killing of UV-irradiated *uwrB recF* cells is due to the inhibition of the *recB*-dependent pathway of postreplication repair, i.e., the repair of DNA double-strand breaks that arise at the sites of unrepaired DNA daughter-strand gaps.

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**References**


