R Factor-mediated Resistance to Ultraviolet Light in Strains of 
Escherichia coli Deficient in Known Repair Functions

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SUMMARY

The expression of resistance to u.v. irradiation mediated by R factor R46 has 
been studied in strains deficient in excision repair and recombination repair. The 
R factor protected wild-type bacteria and also wild-type cells in which repair had 
been inhibited by the substitution of bromouracil for chromosomal thymine. It 
increased the survival of strains defective in the endonucleolytic (uvr), repolymeriz-
ing (pol) and joining (lig) stages of the excision repair process. Recombination de-
cicient bacteria mutant at the recB or recC loci were protected by R46, but the 
R factor had little effect on the survival of a recA strain or a recA recB double 
mutant. R46 increased the survival of cells that had been treated with chloram-
phenicol before u.v. irradiation, but did not protect cultures treated with chloram-
phenicol after irradiation. It is concluded that R46 confers resistance to the lethal 
effects of u.v. irradiation by a mechanism that is independent of excision repair. 
Resistance appears to be mediated by an inducible gene product, which is possibly 
a nuclease and dependent on a functional host recA gene for expression.

INTRODUCTION

A number of plasmids confer resistance to the lethal effects of u.v. irradiation on wild-type 
bacteria (Howarth, 1965; Drabble & Stocker, 1968; Marsh & Smith, 1969). These include 
the fi- N group R factor R46, which has also been designated R-Brighton (Anderson & 
Datta, 1965), R1818 (Datta & Kontomichalou, 1965) and TP120 (Grindley, Grindley & 
Anderson, 1972). It has been suggested that this u.v. protection may result from the participation 
of plasmid-borne gene products in the repair of the damaged host genome (Howarth, 
1965; Marsh & Smith, 1969; Siccardi, 1969). To investigate possible interactions of such 
genes with host DNA repair pathways, we studied the effects of R46 on the u.v. sensitivity 
of Escherichia coli strains that are deficient in excision repair (Setlow & Carrier, 1964; 
Boyce & Howard-Flanders, 1964) and recombination repair (Rupp & Howard-Flanders, 
1968).

A preliminary report of some of this work has been published (Tweats et al., 1974b).

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Table 1. Properties of E. coli strains used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1071*</td>
<td>lac galE galK trp tss</td>
<td>M. Gellert</td>
<td>Gellert &amp; Bullock (1970)</td>
</tr>
<tr>
<td>AB1886</td>
<td>pro ara arg his leu thr lac thi tss gal xyl mtl uvrA6</td>
<td>N. Willetts</td>
<td>Boyce &amp; Howard-Flanders (1964)</td>
</tr>
<tr>
<td>JG138</td>
<td>rha lac thy polA1</td>
<td>Marion Monk</td>
<td>Monk, Peacey &amp; Gross (1971)</td>
</tr>
<tr>
<td>N1252</td>
<td>as N1071, but also bio thi lep-8 lig-2</td>
<td>M. Gellert</td>
<td>Gellert &amp; Bullock (1970)</td>
</tr>
<tr>
<td>AB2463</td>
<td>pro his arg thi thr leu lac gal ara xyl mtl tss recA13</td>
<td>N. Willetts</td>
<td>Howard-Flanders, Boyce &amp; Theriot (1966)</td>
</tr>
<tr>
<td>AB2470</td>
<td>pro his arg thi thr leu lac gal ara xyl mtl tss recB21</td>
<td>N. Willetts</td>
<td>Howard-Flanders et al. (1966)</td>
</tr>
<tr>
<td>JC5495</td>
<td>ara arg his leu thr lac thi tss gal xyl mtl recA13 recB21</td>
<td>N. Willetts</td>
<td>Willetts &amp; Clark (1969)</td>
</tr>
<tr>
<td>NH4021</td>
<td>metA28 arg thi gal tss recC22</td>
<td>P. Barth</td>
<td></td>
</tr>
</tbody>
</table>

* A thymineless (thy) derivative of this strain was isolated by the trimethoprim selection technique of Stacey & Simson (1965).

Methods

Bacteria. All strains used were derivatives of *Escherichia coli* K12. They are listed, together with their genotypes and source, in Table 1.

R factor. R46 is a fi- N group plasmid (Datta & Hedges, 1971) isolated from a strain first characterized by Professor E. S. Anderson. It confers resistance to ampicillin, streptomycin, sulphonamides and tetracycline.

Media. These have been described (Tweets, Pinney & Smith, 1974a).

Ultraviolet irradiation. Washed, exponential-phase cultures were resuspended to 10⁸ viable organisms/ml at 20 °C in Davis & Mingioli (1950) salts solution which lacked a carbon source (DM base). The suspension (2 ml) was transferred to a 5 cm diameter glass Petri dish and irradiated, with stirring, under a Hanovia model 12 low pressure mercury lamp, which emitted light at 254 nm. The lamp was clamped either at 18 cm above the sample, giving a dose rate of 5.2 x 10⁻⁶ J/mm²/s, or at 60 cm above the sample (dose rate of 1.4 x 10⁻⁶ J/mm²/s). Dosimetry was determined using a model J-225 Blak-Ray u.v. meter. Viable counts of irradiated suspensions were estimated by dilution in DM base and spreading 0.1 ml samples on to nutrient agar plates. These were incubated overnight at 37 °C. Exposure of irradiated cells to ambient artificial light was minimized to prevent photoreactivation of u.v.-induced damage (Setlow, 1966), as recommended by Marsh & Smith (1969).

Preparation and u.v. irradiation of cultures grown in bromodeoxyuridine. Thymine-requiring mutants were selected by the method of Stacey & Simson (1965) and grown overnight in DM medium supplemented with 60 μg thymine/ml. These cultures were diluted 1 in 100 into DM medium containing 200 μg bromodeoxyuridine (BUDR)/ml instead of thymine, and incubation was continued for 40 h. The cells were again diluted 1 in 100 into BUDR-containing medium and incubated for a further 3 h before being washed and irradiated as described. Irradiated cultures were diluted in DM base and plated on DM medium containing 60 μg thymine/ml.

Treatment of cultures with chloramphenicol before irradiation. Overnight nutrient broth cultures were diluted 1 in 100 into fresh broth and incubated for 3 h before chloramphenicol
was added at a final concentration of 10 μg/ml. The cells were incubated for a further 2 h in the presence of the drug and were then washed and irradiated.

*Treatment of cultures with chloramphenicol after irradiation.* Exponential-phase cultures were grown and irradiated in the normal manner. Irradiated suspension (1 ml) was filtered through a membrane filter (pore size 0.45 μm) and resuspended in 1 ml nutrient broth containing 10 μg chloramphenicol. Each suspension was incubated for 3 h before dilution in DMbase and plating on nutrient agar.

**RESULTS**

**Ultraviolet protection conferred by R46 on wild-type strains**

R46 confers u.v. resistance on *E. coli* strain N1071, which is wild-type with respect to both excision and recombination repair (Fig. 1). When repair was inhibited by the substitution of bromouracil for chromosomal thymine (Aoki, Boyce & Howard-Flanders, 1966; Hanawalt, 1968), the R factor still increased the survival of this wild-type strain after u.v. irradiation (Fig. 2).

**Effect of R46 on strains deficient in excision repair**

A functional *uvrA* gene is necessary for the first, endonucleolytic step in the excision repair process (Boyce & Howard-Flanders, 1964; Braun & Grossman, 1974) and the *E. coli uvrA* mutant (strain AB1886) was, as expected, much more sensitive to u.v. light than strain N1071, which is wild-type with respect to excision repair activity (Fig. 1). However, R46 conferred u.v. protection on strain AB1886 as well as on strain N1071 (Fig. 1). In strain AB1886, u.v. protection was apparent as a change in the slope of the survival curve rather than as the production of a shoulder.

The *polA1* mutant (De Lucia & Cairns, 1969) is defective in the production of DNA polymerase I, which is necessary for the normal resynthesis of DNA after thymine dimers have been excised (Kornberg, 1969; Glickman, 1974). The u.v. survival curve of the *E. coli polA1* mutant (strain JG138) showed a characteristic loss of shoulder (Fig. 3a), but R46 still increased the survival of this strain. Once again the protective effect of R46 was seen as a change in the slope of the survival curve.

*Escherichia coli* strain N1252 (Gellert & Bullock, 1970), which has the genotype *lop-8 lig-2*, produces excess defective ligase and is therefore deficient in the final step of excision repair (Hanawalt, 1968). R46 also conferred an increased resistance to u.v. light on this strain (Fig. 3b). Yet again the R factor-mediated u.v. resistance was apparent as a change in slope of the survival curve, rather than as the production of a shoulder. Recent examination of strains in which the *lig* mutation has been separated from the Lop phenotype suggests that the original *lop lig* strains have an enhanced u.v. sensitivity due to a second, distant mutation (Gottesman, Hicks & Gellert, 1973). However, results (Fig. 4) using a derivative of strain N1071 into which only the *liga* mutation had been transduced showed that R46 still conferred u.v. protection on this ligase-defective strain.

**Effect of R46 on strains deficient in recombination repair**

Replication of DNA that contains thymine dimers results in the production of a gap in the daughter strand opposite each dimer (Rupp & Howard-Flanders, 1968). These gaps are subsequently repaired by a mechanism involving recombination, which in *E. coli* requires the products of at least three cistrons: *recA*, *recB* and *recC* (Clark & Margulies, 1965; Howard-Flanders, Boyce & Theriot, 1966). The *recA* gene product appears to have several
Fig. 1. Ultraviolet protection mediated by R factor R46 on *E. coli* strain N1071, which is wild-type with respect to u.v. repair, and strain AB1886, which is *uvrA6*. –□--; N1071; ■, N1071 (R46); ○, AB1886; ●, AB1886 (R46). The broken line for the R− strain N1071 is repeated in all figures as a wild-type control.

Fig. 2. Effect of growth in bromodeoxyuridine (BUDR) on u.v. protection mediated by R46 in a Thy− derivative of *E. coli* strain N1071. Strain N1071 thy was grown for many generations in a minimal medium in which BUDR had replaced thymine. The cells were then washed and u.v.-irradiated as described in Methods. ○, N1071 thy grown in BUDR; ●, N1071 thy (R46) grown in BUDR; –□--; N1071 thy+ grown as in Fig. 1.

Fig. 3. Ultraviolet protection conferred by R46 on (a) *E. coli* strain JG138 polA1 and (b) the ligase-defective *E. coli* strain N1252 tnp-8 lig-2. (a): ○, JG138; ●, JG138 (R46). (b): ○, N1252; ●, N1252 (R46). –□--; As in Fig. 1.

Fig. 4. Ultraviolet protection conferred by R46 on *E. coli* strain N1071 lig-2. ○, N1071 lig-2; ●, N1071 lig-2 (R46). –□--; As in Fig. 1.
functions including control of the synthesis of the ATP-dependent nuclease (exonuclease V) determined by the recB and recC loci (Oishi, 1969; Barbour & Clark, 1970). R46 still produced an increase in the survival of both the E. coli recB mutant (strain AB2470) and the recC mutant (strain NH4021) after u.v. irradiation (Fig. 5). However, the R factor had little effect on the u.v. resistance of the red mutant (strain AB2463) or on the recA recB double mutant (strain JC5495) (Fig. 6).

Effect of inhibition of protein synthesis on R factor-mediated u.v. protection

When the recombination- and excision-proficient strain N1071 was irradiated and then exposed to chloramphenicol, the protective effect of R46 was abolished (Fig. 7). However, the R factor still protected cells that had been treated with chloramphenicol before irradiation (Fig. 8). This suggests that the R factor gene product involved in protection is produced in significant quantities only after irradiation, i.e. it is inducible.

DISCUSSION

R46 protects strain N1071, which has functional excision and recombination repair pathways. Thus even when excision and recombination are working optimally an extra component of resistance is added by R46. The R factor also protects strains in which the excision repair system of the host is defective in any one of its stages, which suggests that the mechanism of u.v. resistance mediated by R46 is totally independent of excision repair.

The interaction of R46 with post-replication recombination repair is more complex. In agreement with the results of similar work by Marsh & Smith (1969) and MacPhee (1973) who used R factors other than R46, it was found that the protective effect was not expressed in a recA host, but since it protected recB and recC strains, it would appear that the u.v.
resistance conferred by the R factor can be expressed in the absence of host-mediated recombination repair. These results suggest that R46 may partially be able to compensate for absence of the recBC nuclease activity.

MacPhee (1974) demonstrated that another N group R factor, R-Utrecht, has an associated polymerizing activity which can functionally complement DNA polymerase I mutants of Salmonella typhimurium in the host cell reactivation of u.v.-damaged phage. This R factor also confers u.v. resistance on its host (Drabble & Stocker, 1968), which MacPhee (1974) suggested could be due in part to its polymerase activity.

It is possible to interpret the protective effect of R46 on a polA1 mutant as being the result of functional complementation between an R factor-mediated DNA polymerase and the defective host enzyme. However, it becomes difficult to imagine how such an enzyme could also confer resistance on hosts that are defective in both the initial endonucleolytic step in excision repair, such as the uvrA mutant, and in the final sealing part of the process, such as that found with the lig-2 strain. Moreover, the polymerizing activity of R-Utrecht was found in untreated cells, whereas our results with cells that had been exposed to chloramphenicol after irradiation suggest that the u.v. repair function of R46 requires induction. Also, if the u.v. protection conferred by R-Utrecht and R46 is mediated solely via the activity of a DNA polymerase similar to polymerase I, it is not clear why these R factors do not protect recA strains or recA recB double mutants.

Tweets et al. (1974a) showed that R46 determines an inducible nuclease activity, which may be involved in the elimination of the R factor under conditions of thymine starvation (Pinney & Smith, 1972; Birks & Pinney, 1975), and which could be responsible for its u.v.
protecting effect. The results presented in this paper are, indeed, more consistent with the hypothesis that R46-mediated u.v. resistance is determined by a nuclelease activity which, in agreement with the results of Marsh & Smith (1969), is dependent on a functional host recA gene for expression. Since, however, it is apparent in recB and recC mutants, we suggest that the R factor gene product may have some positive effect on post-replication sister strand exchange.

REFERENCES


