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

By D. J. TWEATS*, MARGARET J. THOMPSON†,
R. J. PINNEY AND J. T. SMITH

*Microbiology Section, Department of Pharmaceutics, The School of Pharmacy,
University of London, London WC1N 1AX*

(Received 4 July 1975)

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*), repolymerizing (*pol*) and joining (*lig*) stages of the excision repair process. Recombination deficient 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 chloramphenicol before u.v. irradiation, but did not protect cultures treated with chloramphenicol 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.*, 1974*b*).

* Present address: Genetics Department, University College of Swansea, Singleton Park, Swansea.

† Present address: Distillers Company Ltd, Glenochil Technical Centre, Menstrie, Clackmannanshire.

Table 1. *Properties of E. coli strains used*

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

* 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 (Tweats, 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 × 10⁻⁶ J/mm²/s, or at 60 cm above the sample (dose rate of 1.4 × 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 *lig2* 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

Fig. 2

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

Fig. 4

Fig. 3. Ultraviolet protection conferred by R46 on (a) *E. coli* strain JG138 *polA1* and (b) the ligase-defective *E. coli* strain N1252 *lop-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.

Fig. 5

Fig. 5. Ultraviolet protection mediated by R46 on (a) *E. coli* strain AB2470 *recB21* and (b) *E. coli* strain NH4021 *recC22*. (a) ○, AB2470 *recB21*; ●, AB2470 *recB21* (R46). (b) ○, NH4021 *recC22*; ●, NH4021 *recC22* (R46). --□--, As in Fig. 1.

Fig. 6

Fig. 6. Effect of R46 on sensitivity of (a) *E. coli* strain AB2463 *recA13* and (b) *E. coli* strain JC5495 *recA13 recB21*. (a) ○, AB2463; ●, AB2463 (R46). (b) ○, JC5495; ●, JC5495 (R46). Broken line is part of the survival curve for the wild-type strain N1071 (see 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 *recA* 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.

Fig. 7

Fig. 7. Effect of R46 on the survival of *E. coli* strain N1071 treated with chloramphenicol (Cm) after u.v. irradiation. Washed exponential cultures of strain N1071 (○) and N1071 (R46) (●) were irradiated as described in Methods. They were then resuspended in nutrient broth containing 10 μg Cm/ml. The suspensions were incubated for 3 h at 37 °C before plating. --□--, Survival of strain N1071 without Cm treatment.

Fig. 8

Fig. 8. R46-mediated u.v. resistance in *E. coli* strain N1071 treated with chloramphenicol (Cm) before irradiation. Exponential cultures of strain N1071 (○) and N1071 (R46) (●) were incubated for 2 h in nutrient broth containing 10 μg Cm/ml. They were then washed and irradiated as described in Methods. --□--, Survival of strain N1071 without Cm treatment.

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.

Tweats *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 nuclease 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

- ANDERSON, E. S. & DATTA, N. (1965). Resistance to penicillins and its transfer in Enterobacteriaceae. *Lancet* **i**, 407-409.
- AOKI, S., BOYCE, R. P. & HOWARD-FLANDERS, P. (1966). Sensitization of *Escherichia coli* to radiation by bromouracil: excessive post-irradiation breakdown of deoxyribonucleic acid without concomitant synthesis. *Nature, London* **209**, 686-688.
- BARBOUR, S. D. & CLARK, A. J. (1970). Biochemical and genetic studies of recombination proficiency in *Escherichia coli*. I. Enzymatic activity associated with *recB*⁺ and *recC*⁺ genes. *Proceedings of the National Academy of Sciences of the United States of America* **65**, 955-961.
- BIRKS, J. H. & PINNEY, R. J. (1975). Correlation between thymineless elimination and the absence of *hsp* II (*Eco* RII) specificity in N-group R factors. *Journal of Bacteriology* **121**, 1208-1210.
- BOYCE, R. P. & HOWARD-FLANDERS, P. (1964). Release of ultraviolet light-induced thymine dimers from DNA in *E. coli* K-12. *Proceedings of the National Academy of Sciences of the United States of America* **51**, 293-300.
- BRAUN, A. & GROSSMAN, L. (1974). An endonuclease from *Escherichia coli* that acts preferentially on UV-irradiated DNA and is absent from the *uvrA* and *uvrB* mutants. *Proceedings of the National Academy of Sciences of the United States of America* **71**, 1838-1842.
- CLARK, A. J. & MARGULIES, A. D. (1965). Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K12. *Proceedings of the National Academy of Sciences of the United States of America* **53**, 451-459.
- DATTA, N. & HEDGES, R. W. (1971). Compatibility groups among *fi*⁻ R factors. *Nature, London* **234**, 222-223.
- DATTA, N. & KONTOMICHALOU, P. (1965). Penicillinase synthesis controlled by infectious R factors from Enterobacteriaceae. *Nature, London* **208**, 239-241.
- DAVIS, B. D. & MINGIOLI, E. S. (1950). Mutants of *Escherichia coli* requiring methionine or vitamin B₁₂. *Journal of Bacteriology* **60**, 17-28.
- DE LUCIA, P. & CAIRNS, J. (1969). Isolation of an *E. coli* strain with a mutation affecting DNA polymerase. *Nature, London* **224**, 1164-1166.
- DRABBLE, W. T. & STOCKER, B. A. D. (1968). R (transmissible drug resistance) factors in *Salmonella typhimurium*: pattern of transduction by phage P22 and ultraviolet-protection effect. *Journal of General Microbiology* **53**, 109-123.
- GELLERT, M. & BULLOCK, M. L. (1970). DNA ligase mutants of *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America* **67**, 1580-1587.
- GLICKMAN, B. W. (1974). The role of DNA polymerase I in pyrimidine dimer excision and repair replication in *Escherichia coli* K12 following ultraviolet irradiation. *Biochimica et biophysica acta* **335**, 115-122.
- GOTTESMAN, M. M., HICKS, M. L. & GELLERT, M. (1973). Genetics and function of DNA ligase in *Escherichia coli*. *Journal of Molecular Biology* **77**, 531-547.
- GRINDLEY, N. D. F., GRINDLEY, J. N. & ANDERSON, E. S. (1972). R factor compatibility groups. *Molecular and General Genetics* **119**, 287-297.
- HANAWALT, P. C. (1968). Cellular recovery from photochemical damage. In *Photophysiology*, vol. 4, pp. 203-251. Edited by A. C. Giese. New York and London: Academic Press.
- HOWARD-FLANDERS, P., BOYCE, R. P. & THERIOT, L. (1966). Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. *Genetics* **53**, 1119-1136.
- HOWARTH, S. (1965). Resistance to the bactericidal effect of ultraviolet radiation conferred on enterobacteria by the colicine factor *coll*. *Journal of General Microbiology* **40**, 43-55.
- KORNBERG, A. (1969). Active centre of DNA polymerase. *Science, New York* **163**, 1410-1418.
- MACPHEE, D. G. (1973). Effect of *rec* mutations on the ultraviolet protecting and mutation-enhancing properties of the plasmid R-Utrecht in *Salmonella typhimurium*. *Mutation Research* **19**, 357-359.
- MACPHEE, D. G. (1974). DNA polymerase activity determined by the ultraviolet-protecting plasmid, R-Utrecht. *Nature, London* **251**, 432-434.
- MARSH, E. B. & SMITH, D. H. (1969). R factors improving survival of *Escherichia coli* K-12 after ultraviolet irradiation. *Journal of Bacteriology* **100**, 128-139.
- MONK, M., PEACEY, M. & GROSS, J. D. (1971). Repair of damage induced by ultraviolet light in DNA polymerase-defective *Escherichia coli* cells. *Journal of Molecular Biology* **58**, 623-630.

- OISHI, M. (1969). An ATP-dependent deoxyribonuclease from *Escherichia coli* with a possible role in genetic recombination. *Proceedings of the National Academy of Sciences of the United States of America* **64**, 1292-1299.
- PINNEY, R. J. & SMITH, J. T. (1972). R factor elimination during thymine starvation: effects of inhibition of protein synthesis and readdition of thymine. *Journal of Bacteriology* **111**, 361-367.
- RUPP, W. D. & HOWARD-FLANDERS, P. (1968). Discontinuities in the DNA synthesised in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. *Journal of Molecular Biology* **31**, 291-304.
- SETLOW, R. B. (1966). Cyclobutane-type pyrimidine dimers in polynucleotides. *Science, New York* **153**, 379-386.
- SETLOW, R. B. & CARRIER, W. L. (1964). The disappearance of thymine dimers from DNA: an error-correcting mechanism. *Proceedings of the National Academy of Sciences of the United States of America* **51**, 226-231.
- SICCARDI, A. G. (1969). Effects of R factors and other plasmids on ultraviolet susceptibility and host cell reactivation property of *Escherichia coli*. *Journal of Bacteriology* **100**, 337-346.
- STACEY, K. A. & SIMSON, E. (1965). Improved method for the isolation of thymine requiring mutants of *Escherichia coli*. *Journal of Bacteriology* **90**, 554-555.
- TWEATS, D. J., PINNEY, R. J. & SMITH, J. T. (1974*a*). R-factor-mediated nuclease activity involved in thymine-less elimination. *Journal of Bacteriology* **118**, 790-795.
- TWEATS, D. J., PINNEY, R. J., THOMPSON, M. & SMITH, J. T. (1974*b*). R-factor mediated resistance to ultraviolet light. *Journal of Pharmacy and Pharmacology* **26**, 97P-98P.
- WILLETTTS, N. S. & CLARK, A. J. (1969). Characteristics of some multiply recombination-deficient strains of *Escherichia coli*. *Journal of Bacteriology* **100**, 231-239.