The Effect of a Drug-resistance Factor on Recombination and Repair of DNA in *Escherichia coli* K12

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The presence in recipient strains of *Escherichia coli* K12 of the plasmid R46 greatly reduced the yield of recombinants from crosses with several Hfr strains and virtually abolished the formation of recombinants by P1 transduction without, however, significantly affecting the transfer of the F prime from a strain carrying *Fgal*. The R46 plasmid had paradoxical effects on mutability: it appeared to enhance the yield of mutants following irradiation with ultraviolet light but it reduced the number of mutants detectable in un-irradiated cultures. The effects of this plasmid on ultraviolet light survival of the wild type and several mutants defective for recombination and repair have been measured and the results, in the main, confirm similar observations by Tweats *et al.* (1976). Not only is the survival of the strain harbouring R46 greater than that of the parent strain in all the cases studied, but the survival of ultraviolet irradiated bacteriophage λ is also greater.

INTRODUCTION

A number of enzymic mechanisms have been suggested for the processes of repair and recombination of DNA in the bacterium *Escherichia coli* K12 (for reviews see Grossman *et al.*, 1975; Clark, 1973). In addition to the chromosomal gene functions involved, identified by *rec*, *uvr*, *lex* and *sbc* mutations, a number of extrachromosomal elements appear to be able to influence the repair and recombination of DNA. The increased survival after ultraviolet (u.v.) irradiation of strains carrying ColI compared with their non-colicinogenic parent strains was first described by Howarth (1965) and the effects of other bacterial plasmids which confer on their hosts an increased resistance to DNA damaging agents have been discussed (Drabble & Stocker, 1968; Marsh & Smith, 1969; Siccardi, 1969). With the increased survival there is a concomitant increase in the induced mutation rate (Krishnapillai, 1975) and in *Salmonella typhimurium* the spontaneous mutation rate is also increased (Mortelmans & Stocker, personal communication). However, the mechanism(s) responsible for these phenomena are unknown although one plasmid, R-Utrecht, has been shown to code for a new DNA polymerase (MacPhee, 1974). As certain of the gene products that are involved in the repair of DNA are also required for recombination, the effect of R46 (= R1818 = R-Brighton), a plasmid which increases the resistance of *S. typhimurium* to u.v. light, was investigated. In this paper the effects of this plasmid on u.v. survival and recombination in wild-type strains and in certain mutant strains are reported. With the exception of the results relating to *recA* strains, the data confirm and extend the observations concerned with the u.v. survival of R46⁺ strains reported by Tweats *et al.*

Table 1. *Bacteria, plasmids and bacteriophage*

Strain designation	Relevant genotype	Source
<i>E. coli</i> K12 F ⁻ strains		
s491	<i>pro his udp</i> λ ⁻	R. G. Lloyd
ZF ⁻	<i>met</i> λ ^R λ ⁻ Sm ^S	Laboratory stock strain
24	<i>thr leu thi</i>	E. Meynell
S100	K12 prototroph	R. G. Lloyd
AB1886	<i>thr leu thi pro his arg uvrA</i>	P. Howard-Flanders
KL403	<i>leu pro his thyA polA1</i>	K. B. Low
PO102	as s491, except <i>recA56 his</i> ⁺	Exconjugant from s491 × KL16-99 mating
AT3021	<i>his leu pdxA met thi strA ara</i>	A. Taylor
<i>E. coli</i> K12 F ⁺ strains		
HfrH	<i>met</i> Sm ^S	Laboratory stock strain
KL16	Sm ^S	K. B. Low
KL98	Sm ^S	K. B. Low
w4520	<i>gal</i> Sm ^S <i>Fgal</i>	P. Howard-Flanders
Plasmids		
R46	Amp ^R Tc ^R Sm ^R Pc ^R Su ^R	E. Meynell
<i>Fgal</i>	<i>gal</i> ⁺	(in strain w4520)
Bacteriophage		
P1 kc		Laboratory stock strain

(1976). This work was initiated because R46 had been shown to affect the phenotype of a novel mutant of *E. coli* K12 which, in the absence of R46, could not be transduced using phage P1 (Stacey & Oliver, 1977): these results will be reported later.

METHODS

Bacteria and bacteriophage. The strains used in this investigation are listed in Table 1.

Media and genetic manipulations. The composition of the media and the methods used in the experiments were described in detail by Stacey & Oliver (1977).

Ultraviolet irradiation. For the irradiation of *rec*⁺, *polA*, *uvrA* and *lex* mutants, a Hanovia lamp (CHI/1367) which delivered a dose of 3 J m⁻² s⁻¹ was used. For *recA* mutants, a Philips lamp which had one-tenth of the power of the Hanovia lamp and delivered a dose of 0.3 J m⁻² s⁻¹ was used.

Transfer of R46. The drug resistance factor R46 was obtained in a host strain which was lysogenic for bacteriophage λ (strain 24 from Dr E. Meynell). As the products of the *red* genes of λ can effect the survival of the host after irradiation (Trgovcevic & Rupp, 1974) and can increase the yield of recombinants (Signer & Weil, 1968; Lloyd, 1971; Oliver, unpublished results), the plasmid was first transferred to the non-lysogenic λ-resistant strain, ZF⁻. From this strain the plasmid was transferred into the strains used in this investigation.

RESULTS

Survival following ultraviolet irradiation

Strains containing R46 exhibited an increased survival following u.v. irradiation when compared with their parent strains (Fig. 1). The increase in survival was most apparent with the wild-type strain, s491. A possible explanation for the protection afforded by R46 is that the plasmid codes for a 'repair system' which removes damage which might otherwise result in lethality. This system might be analogous to one of the repair systems that have been identified in *E. coli*. To test this suggestion the plasmid was transferred to various strains that were deficient in known repair functions.

The plasmid afforded protection to a *uvrA* mutant which is deficient in the endonucleolytic incision step of the excision repair pathway (Braun & Grossman, 1974). It also gave protection to a *polA* mutant which is deficient in DNA polymerase I, thought to be involved in the excision and resynthesis stages of repair (Fig. 1). Protection of *recB* and *recC* strains by R46 has been reported by Tweats *et al.* (1976), but they did not detect any protection in

Fig. 1

Fig. 2

Fig. 1. Ultraviolet light survival of wild-type, *uvrA* and *polA* strains and their derivatives containing R46: ○, s491; ●, s491(R46); □, AB1886; ■, AB1886(R46); △, KL403; ▲, KL403(R46).

Fig. 2. Ultraviolet light survival of a *recA* mutant and its derivative containing R46: ○, P0102; ●, P0102(R46).

Fig. 3. Survival of ultraviolet light irradiated λ CI on wild-type and *recA* strains and their derivatives containing R46: ○, s491; ●, s491(R46); □, P0102; ■, P0102(R46).

recA strains. Using a u.v. source emitting much less radiation, we have detected protection in *recA* strains (Fig. 2). Moreover irradiated λ CI plated with higher efficiency on *recA*(R46) strains than on *recA* mutants (Fig. 3).

Thus R46 increases the survival of both repair-proficient and repair-deficient bacteria, although in the case of the mutant strains the plasmid does not restore the u.v. survival to that of the wild-type strain. The unrelated drug resistance factor R1-19 had no effect on the survival of these strains, implying that the protection conferred by R46 required genes carried by the plasmid and was not just a physical effect due to an increase in DNA content.

In addition to the increased resistance to irradiation of the host strain the presence of the plasmid increased the plating efficiency of irradiated bacteriophage; when irradiated bacteriophage λ CI was plated on strains s491 and s491(R46) there was an increased survival on the strain bearing the plasmid (Fig. 3).

Mutation rate

The plasmid R46 exerted an antimutator effect on the spontaneous reversion rates of the auxotrophic markers of strain s491 (Table 2), and the frequency of nalidixic acid resistant

Table 2. *Effect of R46 on mutation rates*

Strain	Mutagen	Frequency of mutation per survivor		
		<i>pro</i> ⁺	<i>his</i> ⁺	Nal ^R
S49I	none	5.3×10^{-7}	3.1×10^{-7}	2.3×10^{-7}
S49I(R46)	none	3.2×10^{-8}	1.2×10^{-8}	3.4×10^{-8}
S49I	u.v.*	8.2×10^{-6}	ND	4.1×10^{-6}
S49I(R46)	u.v.*	9.8×10^{-4}	ND	6.3×10^{-5}

ND, Not determined.

* U.v. dose: 3 J m⁻² s⁻¹. The survival, N/N_0 , was 10 % for S49I and 20 % for S49I(R46).Table 3. *Recombination proficiency of strains containing R46, as measured by conjugal mating*

Recipient	Donor	Selected marker*	Recombinants (ml ⁻¹) (per donor)†	Recombination deficiency‡
S49I	KL16	<i>his</i> ⁺	3.37×10^{-3}	1
S49I(R46)	KL16	<i>his</i> ⁺	3.25×10^{-4}	9.6×10^{-2}
S49I	KL98	<i>his</i> ⁺	1.43×10^{-1}	1
S49I(R46)	KL98	<i>his</i> ⁺	7.5×10^{-6}	5.2×10^{-5}
S49I	HfrH	<i>pro</i> ⁺	4.3×10^{-1}	1
S49I(R46)	HfrH	<i>pro</i> ⁺	1.2×10^{-4}	2.8×10^{-4}
S49I	S100(<i>Fgal</i>)	<i>gal</i> ⁺	9.0×10^{-2}	1
S49I(R46)	S100(<i>Fgal</i>)	<i>gal</i> ⁺	6.8×10^{-2}	7.5×10^{-1}

* Counter-selection of donor for resistance to azauracil.

† Checked by restreaking.

‡ Ratio of the no. of recombinants formed to the no. of recombinants formed with S49I.

mutants was similarly reduced. However, the mutation rates after u.v. irradiation were much enhanced (Table 2).

Effect on recombination

When the plasmid was present in the recipient strain of a conjugation mating there was a dramatic reduction in the yield of recombinants (Table 3). With strain S49I(R46) as recipient the reduction in recombinants was greater for strain HfrH than for KL16. The inability to produce recombinants was not a result of the ampicillin present in the selection plates as no more recombinants were detected when it was omitted or when tetracycline was used in its place to ensure that the recombinants contained R46. There was, however, little difference in the ability of strains S49I and S49I(R46) to receive the *Fgal* factor, which implies that R46 exerts its effect at the level of recombination and not by reducing the ability of the recipient to accept donor DNA. [This experiment is possible because R46 is compatible with F⁺ (Hedges, 1972)]. *recB* mutants, which are defective in recombination, yielded even fewer recombinants when carrying R46. As strain S49I was unsuitable for measuring the linkage of unselected markers, the plasmid was transferred to AT3021 and this strain was used as a recipient in matings with HfrH.AT737 as donor. Although the number of Leu⁺ recombinants was low it was possible to score the unselected *ara* and *pdxA* markers. The linkage of these markers was greatly disturbed (Table 4).

When strains carrying R46 were used as recipients in generalized transduction experiments very few recombinants could be detected (Table 5). This was not due to poor adsorption of the bacteriophage as R46 had no effect on the efficiency of plating of P1 or on the frequency of lysogenization by P1CM (Oliver, unpublished experiments).

The reduction in the yield of recombinants and in the linkage between genetic markers is characteristic of restriction of the transferred DNA. However no new restriction or modification system has been demonstrated in bacteria containing R46 (Hedges, 1972) although other members of the N incompatibility group do code for the EcoRII restriction and modi-

Table 4. *Effect of R46 on the co-inheritance of unselected proximal markers in matings with HfrH*

Exponentially growing cultures of donor (HfrH) and recipient strains were mixed in a ratio of 1:10 donor to recipient cells. Matings were carried out in a static water bath at 37 °C for 30 min.

Recipient	Selected marker <i>leu</i> ⁺	Co-inheritance of unselected proximal markers (%)	
		<i>ara</i> ⁺	<i>pdxA</i> ⁺
AT3021	100	94	93
AT3021(R46)	50	28	49

Table 5. *Effect of R46 on the transduction of chromosomal markers by phage P1*

Donor	Recipient	Transduction frequency per adsorbed phage	
		<i>pro</i> ⁺	<i>his</i> ⁺
P1 (S100)	S491	4.2×10^{-6}	8.3×10^{-6}
P1 (S100)	S491(R46)	$< 1 \times 10^{-9}$	$< 1 \times 10^{-9}$

fication system. Moreover when both donor and recipient cells contain R46 there is still a reduction in the yield of recombinants (Jones & Oliver, unpublished results).

A similar plasmid, R-Utrecht, has also been reported to increase survival of the host bacterium, due to a plasmid-coded DNA polymerase with similarities to the *polA* gene product (MacPhee, 1974). If a similar mechanism is responsible for these experimental results, then R46 should also code for a DNA polymerase. Indeed R46 does partially restore the resistance to u.v. irradiation to a *polA* mutant, but if R46 codes for a DNA polymerase that can replace the *polA* gene product then the presence of R46 should enable a *polA* mutant to support the growth of λ *spi* mutants which are not able to grow in strains lacking polymerase I (Zissler, Signer & Schafer, 1971). There was no difference in the efficiency of plating of λ *bio11* (a deletion mutant of λ which has the *Spi*⁻ phenotype) on KL403, a *polA* mutant, or KL403 (R46). It is likely, therefore, that R46 does not have a polymerase I-like gene product.

DISCUSSION

These experiments demonstrate that the plasmid R46 affects recombination following transduction or conjugation. This is in addition to the mutator effect reported to occur in *Salmonella typhimurium* (Mortelmans & Stocker, personal communication) and the resistance to u.v. light that it confers on both *S. typhimurium* and *E. coli* (Tweats *et al.*, 1976).

The increased survival after u.v. irradiation conferred by ColI and R46 could be explained by a plasmid-coded endonuclease which causes single-strand 'nicks' in double-stranded DNA that supplement, or partially replace in *uvr* strains, the endonucleolytic action of the *uvr*⁺ gene products (Tweats & Smith, 1974). Such nicks have been observed in plasmid DNA in cells subject to thymine starvation (Freifelder, 1969; Pinney, Bremer & Smith, 1974). The presence of a nuclease of this kind might explain the effects reported here of R46 on recombination because it might either diminish the life-time of the recombination intermediate or shorten the length of DNA involved. However, there are a number of observations that imply that single-strand breaks encourage recombination. The recombinogenic effect of u.v. irradiation depends on a functional *uvr* system and is not seen in *uvr* strains. Moreover, the effect of R46 on mutation is paradoxical: it is an anti-mutator in unirradiated strains but it enhances u.v.-induced mutation rates. An explanation for part of this difference may lie in the suggestion of Pinney *et al.* (1974) that the nuclease is induced by thymine starvation, and therefore, by analogy, perhaps by u.v. radiation. If the nuclease is induced its effects may be different when the amount of DNA damage is quite different. The change in the extent of co-inheritance of closely linked but unselected markers caused by the presence of

R46 in the zygotes is consistent with DNA damage. A similar loss of linkage and increase in multiple crossing over has been observed several times in the recombinants obtained after u.v. irradiation of the Hfr donor immediately before conjugation (see Stacey, Evenchik & Hayes, 1969).

Other plasmids that have been reported to affect the survival of bacteria after treatment with DNA damaging agents include ColI (Howarth, 1965), certain drug resistance factors (Marsh & Smith, 1969) and some sex factors of *Pseudomonas* (Krishnapillai, 1975). The drug resistance factor R1-19 has recently been shown to afford protection against ultraviolet light to *recA* strains (Dr J. Holton, personal communication). The closely related N group plasmids, R45 and R48, confer radioresistance on their hosts but do not affect the yield of recombinants after conjugation or transduction. It is, therefore, not yet possible to decide whether the three changes in phenotype that the presence of R46 causes are the result of a single gene or not.

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