The Effect of Plasmid R391 and Other IncJ Plasmids on the Survival of *Escherichia coli* After UV Irradiation

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The presence of the IncJ plasmids R391, R997, R705, R706, R748 and R749 was shown to sensitize *Escherichia coli* AB1157 and both its *uvrA* and *lexA* derivatives to UV irradiation. No alteration in post-irradiation survival was observed in a *recA* mutant containing these plasmids, compared with the non-plasmid-containing *recA* strain. Analysis of recombination frequency in Hfr crosses to *recA*⁺ cells containing plasmid R391 indicated a reduction in recombination frequency compared with that obtained in similar crosses to a non-plasmid-containing strain. This effect was not due to plasmid-encoded restriction or entry exclusion systems and therefore must be considered as a real block in recombination. When cells containing plasmid R391 were irradiated and allowed to photoreactivate, an increase in survival was observed which was comparable to that observed in the non-plasmid-containing derivative. This indicated that post-irradiation processing of UV-induced damage, or lack of such processing, by mechanisms other than photoreactivation was responsible for the UV sensitivity associated with plasmid R391.

INTRODUCTION

Many plasmids derived from various genera have been shown to affect post-UV irradiation survival of *Escherichia coli* (Molina *et al.*, 1979; Pinney, 1980; Ando & Arai, 1980; Chernin & Miroyan, 1981), *Pseudomonas aeruginosa* (Lehrbach *et al.*, 1977), *Streptococcus faecalis* (Miehl *et al.*, 1980) and *Salmonella typhimurium* (Walker, 1978). Of the plasmids which affect post-irradiation survival in *E. coli*, most reports have centred on plasmids which enhance the survival of the host (Chernin & Miroyan, 1981). One of the best-studied plasmids of this type, pKM101, has been shown to encode an analogue of *umuC*, a gene involved directly in error-prone repair (Walker & Dobson, 1979). It has been proposed that the enhanced survival associated with the presence of pKM101 is due to an enhancement of the cells' error-prone repair system (Walker & Dobson, 1979).

There have also been reports of plasmids which decrease the survival of their host after UV irradiation (March & Smith, 1969; Siccardi, 1969; Khmel *et al.*, 1981), and although it has been postulated that such an effect may reflect lack of repair capacity, such effects have not been further characterized.

We report here an initial characterization of the effect of plasmid R391 and other plasmids of the incompatibility group J on the post-irradiation survival of *E. coli* K12.

METHODS

Bacterial strains and plasmids. These are listed in Table 1. Plasmids were transferred from strain JTP686 as described by Miller (1972). Antibiotics (Sigma grade) were used at the following concentrations: kanamycin (Kan) 30 µg ml⁻¹, ampicillin (Amp) 50 µg ml⁻¹, rifampicin (Rif) 100 µg ml⁻¹, streptomycin (Str) 100 µg ml⁻¹ and mercuric chloride (Hg) 20 µg ml⁻¹.

UV survival. Overnight cultures in Luria broth (Miller, 1972) were grown with gentle shaking at 37 °C and reinoculated into the same medium until viable counts between 2 and 3 × 10⁸ were reached (*A*₅₄₀ = 0.1 on a

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Table 1. *Bacterial strains and plasmids*

Strain	Genotype	Source
AB1157	<i>thr-1 leu-6 thi-1 argE3 his-4 proA2 lacY1 galK2 mtl-1 xyl-5 ara-14 rpsL tsx33 supE44</i>	B. Bachman, <i>E. coli</i> Genetic Stock Center, Yale University
AB1886	as AB1157 but <i>uvrA6</i>	
AB2463	as AB1157 but <i>recA13</i>	
AB2494	as AB1157 but <i>lexA1 metB1 arg⁺ leu⁺</i>	
JTP686	<i>rpoB λ^r</i>	This laboratory
JC158	<i>serA6 HfrPO1 lacI22 thi-1 rel-1</i>	A. J. Clark, Dept of Molecular Biology, University of California, Berkeley
Plasmid	Characteristics	Source
R391	Hg ^r Tra ⁺ Km ^r	R. W. Hedges, Royal Postgraduate Medical School, London
R706	as R391	
R705	as R391	
R748	as R391	
R749	as R391	
R391-3b-1	Sm ^r Sp ^r Hg ^r	
R997	Ap ^r Sm ^r Su ^r	

Vitatron spectrophotometer). Cells were collected by centrifugation, washed twice with 0.85% (w/v) saline and resuspended in 0.85% saline, or in M9 medium without growth requirements (Miller, 1972) in the case of photoreactivation experiments. After starvation by shaking for 30 min, 9 ml samples were irradiated using a Griffin and George UV source with dose rate determined by actinometry (Jagger, 1976) or using a UV radiometer (UV Products Inc., San Gabriel, Calif., USA). After irradiation, cells were plated on Luria broth agar with or without antibiotic under yellow lighting and incubated in the dark at 37 °C.

Photoreactivation. Strains after irradiation at 9 J m⁻² in M9 medium were tested for UV survival as described. Samples were photoreactivated essentially as described by Williams *et al.* (1979) for various times. Control samples unirradiated or irradiated without photoreactivation were also titrated for viable fraction.

Recombination frequencies. Hfr × F⁻ crosses to strains with and without plasmid R391 were carried out as described by Miller (1972), using JC158 as the donor and selecting for recombination of the threonine locus in recipients auxotrophic for threonine. Tests for plasmid-encoded restriction of Hfr DNA were as described by Coetzee *et al.* (1972). To determine if plasmid-encoded entry exclusion was present in R391-containing cells and frequency of transfer of F'*lac* Tn3 to strains with and without plasmid R391 was examined. Transfer was carried out as described by Miller (1972).

RESULTS

Effect of IncJ plasmids on the UV survival of E. coli

Figure 1 illustrates the effect of the IncJ plasmids R391, R997, R705, R706, R748 and R749 on the post-UV survival of *E. coli* strain AB1157. All these plasmids sensitized their host to UV irradiation. The extent of the sensitization appeared to be similar for each of the plasmids over the particular UV dose range examined. The possibility that the decreased survival reflected a lack of repair capacity was examined by analysing the effect of these six IncJ plasmids in repair-deficient backgrounds. It is proposed that if the IncJ plasmids were affecting the operation of a particular repair process then if the plasmid were present in strains deficient in such a process, there should no longer be a plasmid effect. Three main repair processes have been defined in *E. coli* (Witkin, 1976) – photoreactivation, which can be abolished by incubation in the dark or by mutation at the *phr* locus, excision repair, which can be abolished by mutation at one of three loci, *uvrA*, *uvrB* or *uvrC* and finally post-replication repair which can be abolished by mutation at the *recA* locus.

Irradiation of repair-proficient strains containing these six plasmids with post-irradiation incubation in the dark was shown to sensitize such strains (Fig. 1), indicating the ability of these plasmids to sensitize in the absence of photoreactivation. Figure 2 illustrates the effect of the six IncJ plasmids on the survival of strain AB1886 (*uvrA*): all the plasmids increased the sensitivity of this derivative to UV. Figure 3 illustrates the survival of strain AB2463 (*recA*) containing the six IncJ plasmids. No increase in sensitivity could be observed with any of the plasmids tested,

Fig. 1. Effect of six *IncJ* plasmids on the survival of *E. coli* AB1157 after UV irradiation. (a) ●, AB1157; ○, AB1157(R391). (b) ●, AB1157; ○, AB1157(R997). (c) ●, AB1157; ○, AB1157(R705); ▲, AB1157(R706). (d) ●, AB1157; ○, AB1157(R748); ▲, AB1157(R749). Each experiment was done at least five times; the values shown are the means (this also applies to Figs 2–6).

Fig. 2. Effect of *IncJ* plasmids on the survival of *E. coli* AB1886 (*uvrA*) after UV irradiation. (a) ●, AB1886; ○, AB1886(R391). (b) ●, AB1886; ○, AB1886(R997). (c) ●, AB1886; ○, AB1886(R705); ▲, AB1886(R706). (d) ●, AB1886; ○, AB1886(R748); ▲, AB1886(R749).

Fig. 3. Effect of *IncJ* group plasmids on the survival of *E. coli* AB2463 (*recA*) after UV irradiation. (a) ●, AB2463; ○, AB2463(R391). (b) ●, AB2463; ○, AB2463(R997). (c) ●, AB2463; ○, AB2463(R705); ▲, AB2463(R706). (d) ●, AB2463; ○, AB2463(R748); ▲, AB2463(R749).

Fig. 4

Fig. 5

Fig. 4. Effect of plasmid R391 on the survival of *E. coli* AB2494 (*lexA*) after UV irradiation. ■, AB2494; □, AB2494(R391).

Fig. 5. Effect of photoreactivating light on the post-UV irradiation survival of strain AB1886 with and without plasmid R391. ●, AB1886 unirradiated; □, AB1886 irradiated but without photoreactivation; ▲, AB1886 irradiated and photoreactivated; △, AB1886(R391) unirradiated; ■, AB1886(R391) irradiated but without photoreactivation; ○, AB1886(R391) irradiated and photoreactivated.

Fig. 6. Effect of plasmid R391 on recombinants recovered in $\text{Hfr} \times \text{F}^-$ crosses. The recombination frequency was calculated as the percentage of threonine prototrophs recovered per recipient. □, JC158 $\times$ AB1157; ■, JC158 $\times$ AB1157(R391).

in fact they all produced a slight but reproducible increase in resistance. The apparent lack of effect of the IncJ plasmids in a *recA* background suggests that a *recA*⁺-dependent process was being affected by the presence of IncJ plasmids.

The effect of the prototype IncJ plasmid R391 (Coetzee *et al.*, 1972) was examined in strain AB2494 (*lexA*) (Fig. 4) and found to sensitize this derivative, indicating a *lexA* independence for the effect.

Effect of photoreactivation on the UV sensitivity associated with plasmid R391

Excision-deficient derivatives held in non-growing conditions were irradiated and survival was monitored after exposure to photoreactivating light for various times after UV irradiation (Fig. 5). Although strains containing plasmid R391 were more sensitive to UV irradiation, allowing photoreactivation to occur could reverse this sensitivity and restore viability to the level of the non-plasmid-containing strain after photoreactivation.

Effect of plasmid R391 on recombination levels in Hfr crosses

The apparent *recA* dependence and *lexA* independence suggests that the effect of plasmid R391 might be at the level of a *recA*-dependent process not related to the inducible *recA*⁺-dependent processes (Witkin, 1976). For this reason the recombination levels in R391-containing cells were examined. Figure 6 illustrates the effect of R391 on threonine recombinants recovered in Hfr crosses between JC158 and the poly-auxotrophic strain AB1157 containing plasmid R391. The presence of plasmid R391 considerably reduced the frequency of recombinants recovered. This apparent block in recombination ability was not due to plasmid-encoded restriction since no reduction in the titre of λ could be detected when titrated on R391-containing strains (data not shown). The frequency of transfer of F'*lac* Tn3 to R391-containing strains was found to be similar to transfer frequencies to the non-plasmid-containing strains, indicating absence of plasmid-encoded entry exclusion of F'-factor DNA.

DISCUSSION

It has been shown that six *IncJ* plasmids, R391, R997, R705, R706, R748 and R749, sensitize *E. coli* K12 to UV irradiation. The nature of this sensitization has been examined. No evidence could be found for phage induction of lytic phenomena (Pembroke, 1982). It was thus proposed that lack of repair capacity was responsible for the UV sensitivity associated with the presence of the *IncJ* plasmids (Pembroke, 1982).

The sensitization appears to be independent of the photoreactivation and excision-repair processes but dependent on the *recA*⁺ genotype as judged by inability of the *IncJ* plasmids to sensitize a *recA* derivative. Since the *recA* genotype is extremely pleiotropic (Witkin, 1976) the effect of plasmid R391 was examined in a *lexA* background. Because of the ability of plasmid R391 to sensitize a *lexA* derivative it is proposed that the lack of repair capacity is not at the level of the *recA*⁺-dependent, inducible functions but rather at the level of some other *recA*⁺-dependent process.

Studies using post-irradiation photoreactivation suggested that if photoreactivation is allowed to take place, a reversal of the initial sensitivity associated with the presence of plasmid R391 can occur. This may be interpreted as suggesting that it is the aberrant processing of UV-induced lesions by processes other than photoreactivation in cells containing plasmid R391 that is responsible for the UV sensitivity. The requirement for the *recA*⁺ genotype suggests that this aberrant processing may be at the level of a *recA*⁺-dependent process.

It has been proposed that the major pathways of *recA*⁺-dependent repair are recombinational (Clark, 1973). Several recombination-deficient mutants have been isolated (Horii & Clark, 1973) and at least two pathways of recombination operate in *E. coli* (Clark, 1973). Analysis of the recombination ability of recombination-proficient strains carrying plasmid R391 suggests that recombination frequencies are reduced significantly in cells containing plasmid R391. This reduced recombination ability appears to be a real block in recombination since no evidence could be found for plasmid-encoded restriction or entry exclusion of incoming Hfr DNA. It is not clear at present whether this recombination deficiency is related to the UV sensitivity. However, studies using the transcriptional probe Mu d(*lac amp*) (Casadaban & Cohen, 1979) suggest that this is the case (J. T. Pembroke, unpublished).

It thus appears that the UV sensitivity associated with the presence of plasmid R391 may be related to a lowering of recombination ability. This lowering in recombination frequency appears to occur in the absence of DNA damage, as recipients in Hfr crosses were unirradiated. The mechanism of UV sensitivity associated with plasmid R391 may be similar to that of all *IncJ* plasmids, although this is as yet uncertain. However, because of the narrow distribution of these plasmids, it would appear that they may all have a common origin (Hedges *et al.*, 1975). Work is in progress to further characterize the effect of plasmid R391.

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