R Plasmids which Alter Ultraviolet Light-sensitivity and Enhance Ultraviolet Light-induced Mutability in *Pseudomonas aeruginosa*

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R plasmids pMG1, R2, R931 and pMG15 increased the survival of *Pseudomonas aeruginosa* exposed to ultraviolet radiation (u.v.) in the wild type, and *uvr* and *polA* mutants but did not alter the u.v.-response of a *recA* mutant. The R plasmid RPL11 reduced u.v.-survival in the wild type, and *uvr* and *polA* mutants but did not alter the u.v.-response of a *recA* host. All the plasmids enhanced the level of spontaneous and u.v.-induced back mutation (Trp⁺) in a *trpB1* strain.

The effect of a sublethal concentration of sodium arsenite following u.v.-irradiation was examined. It was concluded that in strains *trpB1*(pMG1) and *trpB1*(R931), u.v.-protection is determined by a *recA*⁺-dependent, arsenite-sensitive repair pathway, whereas in strains *trpB1*(R2) and *trpB1*(pMG15), u.v.-protection is determined by a *recA*⁺-dependent, arsenite-insensitive step in DNA repair.

INTRODUCTION

Certain R plasmids of *Pseudomonas aeruginosa* (Krishnapillai, 1975; Lehrbach *et al.*, 1977*a*) alter the ultraviolet light (u.v.) sensitivity of host bacteria and increase their susceptibility to the mutagenic effects of u.v. light. These properties suggest that the plasmids may contribute to some step or steps involved in the DNA repair pathways of *P. aeruginosa*. Analysis of the u.v.-protective plasmid pMG2 (Lehrbach *et al.*, 1977*a*) suggests that a plasmid-determined repair resynthesis function may be responsible for increased u.v.-survival and enhanced u.v.-mutability of bacteria containing pMG2.

In this study we report the effects on u.v.-survival and u.v.-mutagenesis of several independently isolated R plasmids which alter the u.v.-sensitivity of wild-type host bacteria (G. A. Jacoby, personal communication). We have previously suggested that an arsenite-sensitive, *recA*⁺-dependent step in DNA repair may be responsible for u.v.-protection and enhanced u.v.-induced mutability by the R plasmid pMG2 (Lehrbach *et al.*, 1977*b*). The effect of a sublethal concentration of sodium arsenite on u.v.-survival is reported for the R plasmids used in this study.

METHODS

Bacteria. The R plasmids used are described in Table 1; the bacterial strains were described previously (Lehrbach *et al.*, 1977*a, b*). The *trp* allele (*trpB1*) is designated according to the nomenclature used by Crawford (1975) (formerly *trpF1*; Calhoun *et al.*, 1973).

General culture conditions and transfer of R plasmids. These techniques have been previously described (Kung & Lee, 1973; Lehrbach *et al.*, 1977*a*). Antibiotics were added at the following concentrations (µg ml⁻¹): gentamicin, 20; streptomycin, 200; carbenicillin, 500; tetracycline, 250.

Measurement of u.v. survival and u.v. mutagenesis. These techniques were described by Kung & Lee (1973) and Lehrbach *et al.* (1977*b*). Three plates were used to determine the spontaneous levels of back mutation (Trp⁺). The minimum number of revertants scored per plate was 30 before calculations were made of both the spontaneous and induced frequencies of back mutation.

Table 1. *R* plasmids used in this study

Plasmid	Incom- patibility group	Resistance pattern*	Plasmid origin	Reference
pMG1	P-2	Gm Sm Su Hg UV	South Africa	Jacoby (1974)
R931	P-2	Sm Tc Hg UV	Canada	Tseng & Bryan (1973)
RPL11	P-2	Cb Cm Gm Sm Su Tc Hg	U.S.A.	Korfhagen & Loper (1975)
pMG15	?	Hg UV	Ireland	Falkiner <i>et al.</i> (1977)
				Jacoby (personal communication)
R2	P-9	Cb Sm Su UV	Japan	Kawakami <i>et al.</i> (1972)

* Antibiotic resistances: Cb, carbenicillin; Cm, chloramphenicol; Gm, gentamicin; Sm, streptomycin; Su, sulphonamide; Tc, tetracycline. Hg, Resistance to mercuric ions. UV, U.v.-protection or resistance.

Fig. 1. U.v.-survival curves of strains (a) PAO1, (b) GMA918, (c) GMB112 and (d) PAO2003, without plasmids (●) or with *R* plasmids pMG1 (□), R931 (▽), R2 (△), pMG15 (◇) or RPL11 (○).

RESULTS AND DISCUSSION

U.v.-survival curves for the wild-type strain (PAO1) and three u.v.-radiation-sensitive mutants with and without the *R* plasmids are shown in Fig. 1. These strains were selected to define, as far as possible in this organism, the major repair pathways: they were GMA918, a *uvr*-type mutant (Kung & Lee, 1973); GMB112, a *polA*-type mutant (Lehrbach *et al.*, 1976); and PAO2003, a *recA*-type mutant (Chandler & Krishnapillai, 1974). The *R* plasmids pMG1,

Table 2. U.v.-induction of Trp⁺ revertants in strains *trpB1*, *trpB1*(pMG1), *trpB1*(R931), *trpB1*(R2), *trpB1*(pMG15) and *trpB1*(RPL11)

Strain	Spontaneous frequency per 10 ⁷ cells plated	Induced frequency per 10 ⁷ survivors* at u.v. dose (J m ⁻²):		
		5	10	20
<i>trpB1</i>	3	3 (58)	7 (21)	14 (4.5)
<i>trpB1</i> (pMG1)	7	79 (87)	134 (86)	204 (65)
<i>trpB1</i> (R931)	9	56 (85)	92 (68)	193 (62)
<i>trpB1</i> (R2)	9	38 (85)	73 (70)	123 (50)
<i>trpB1</i> (pMG15)	15	36 (65)	55 (60)	120 (38)
<i>trpB1</i> (RPL11)	11	54 (57)	95 (13)	—† (0.6)

* Numbers show the mean frequency of Trp⁺ revertants per 10⁷ survivors, corrected for spontaneous mutations by subtracting the mean frequency of revertants on the non-irradiated plates (Lehrbach *et al.*, 1977b); numbers in parentheses show the percentage survival.

† Mean frequency of Trp⁺ revertants appearing on irradiated plates was less than the mean frequency of revertants on the non-irradiated plates.

R931, R2 and pMG15 protected *P. aeruginosa* against the lethal effects of u.v. light in the wild-type strain, the *uvr*-type mutant and the *polA*-type mutant, but had no protective effect in the *recA*-type strain. A *recA*⁺ gene product is therefore necessary for the expression of the u.v.-protective effect of these plasmids. This suggests that the plasmids' gene product(s) contribute to the *recA*⁺-dependent repair of u.v.-damaged host DNA.

The R plasmid RPL11, on the other hand, reduced survival of the wild-type, and the *uvr*-type and the *polA*-type mutants following u.v.-irradiation but did not alter the u.v.-sensitivity of the *recA*-type mutant. The sensitizing effect of RPL11 is thus also dependent on an intact *recA*⁺ gene product.

Since error-prone repair processes are dependent on an intact *recA*⁺ gene in *Escherichia coli* (Witkin, 1976), it was of interest to determine whether these R plasmids had an effect on the yields of u.v.-induced mutations in *P. aeruginosa*. The R plasmids were transferred into the tryptophan auxotroph *trpB1* (Crawford, 1975). Back mutation to prototrophy (Trp⁻ to Trp⁺) was calculated following exposure to various u.v. doses (Table 2). U.v.-induced back mutation was substantially increased with the 'u.v.-protecting' plasmids pMG1, R931, R2 and pMG15. With the R plasmid RPL11, which reduced the survival of strain *trpB1*, a significant increase in u.v.-induced back mutation was observed at low doses. All the plasmids tested increased the spontaneous back mutation rate of strain *trpB1* (Table 2).

The u.v.-protective effect of pMG1, R931, R2 and pMG15 is dependent on the *recA*⁺ gene of *P. aeruginosa* since these plasmids did not alter the u.v.-survival of a *recA*-type mutant of *P. aeruginosa*. Since studies using *E. coli* B have shown that sodium arsenite inhibits a *recA*⁺-dependent step in the repair of u.v.-irradiated DNA (Rossman *et al.*, 1975), the effect of a sublethal concentration of sodium arsenite (1 mM) in the plating medium following u.v.-irradiation was examined in strain *trpB1* containing R plasmids. This would indicate whether arsenite-sensitive or arsenite-insensitive repair pathways were involved in the u.v.-protective effect of these R plasmids. The presence of sodium arsenite eliminated the increased u.v.-survival of *trpB1*(pMG1) and *trpB1*(R931) reducing the survival to that of u.v.-irradiated strain *trpB1* in the presence of sodium arsenite (Fig. 2a). This effect was not observed for *trpB1*(R2) and *trpB1*(pMG15) (Fig. 2b).

Fig. 2. U.v.-survival curves of strains (a) *trpBI* (●, ○), *trpBI*(pMG1) (■, □) and *trpBI*(R931) (▼, ▽) and (b) *trpBI* (●, ○), *trpBI*(R2) (▲, △) and *trpBI*(pMG15) (◆, ◇), in the presence (filled symbols) or absence (open symbols) of 1 mM-sodium arsenite.

Although the u.v.-survival of strain *trpBI* was not significantly affected by the presence of sodium arsenite (unlike *E. coli* B; Rossman *et al.*, 1975), sodium arsenite has been shown to inhibit a *recA*⁺-dependent function which may be responsible for u.v.-protection and enhanced u.v.-mutagenesis by the *Pseudomonas* R plasmid pMG2 (Lehrbach *et al.*, 1977*b*). From these studies the mechanism of action of sodium arsenite is not clear, although further studies in *E. coli* B have shown that the induction of RNA synthesis is the function most sensitive to arsenite inhibition (Rossman *et al.*, 1977). Thus *recA*⁺-dependent functions that require *de novo* protein synthesis for their involvement in repair processes would more likely be affected by the presence of a sublethal concentration of sodium arsenite. The response of the various R plasmids tested in this study to sodium arsenite following u.v.-irradiation indicates that the ability of the P-2 group plasmids R931, pMG1 (and pMG2; Lehrbach *et al.*, 1977*b*) to give enhanced survival of host cells after u.v.-irradiation is determined by a plasmid gene product(s) which participates in a *recA*⁺-dependent, error-prone, arsenite-sensitive pathway of *P. aeruginosa*. However, the R plasmids R2 and pMG15, which are not greatly affected by the presence of sodium arsenite in their protection of host cells against u.v.-damage, may be involved in an arsenite-insensitive step in DNA repair of *P. aeruginosa*, which is also error-prone and *recA*⁺-dependent.

These results, together with the observed ability of the R plasmid RPL11 to increase the u.v.-sensitivity of a wild-type host cell and yet enhance the level of u.v.-induced back mutation in strain *trpBI*, indicate that several mechanisms or gene products may be responsible for these plasmid-mediated alterations in response to u.v.-irradiation.

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