Inducible UV repair potential of *Pseudomonas aeruginosa* PAO

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Pseudomonas aeruginosa PAO lacks UV-inducible Weigle reactivation and Weigle mutagenesis of UV-damaged bacteriophages. This lack of UV-inducible, error-prone DNA repair appears to be due to the absence of efficiently expressed *umuDC*-like genes in this species. When the *P. aeruginosa* *recA* gene is introduced into a *recA*(Def) mutant of *Escherichia coli* K12, the *P. aeruginosa* *recA* gene product is capable of mediating UV-induced mutagenesis, indicating that it could participate in a *recA*-*lexA*-like regulatory network and function in inducible DNA repair pathways if such existed in *P. aeruginosa*. The presence of the IncP9, UV-resistance plasmid R2 in *RecA*⁺ strains of *P. aeruginosa* PAO allows UV-inducible, mutagenic DNA repair of UV-irradiated bacteriophages. R2 also greatly stimulates the ability of UV radiation to induce mutagenesis of the bacterial chromosome. When R2 is introduced into *P. aeruginosa* strains containing either the *recA908* or *recA102* mutation, plasmid-mediated UV resistance and Weigle reactivation are not observed. These observations suggest that the increased protection afforded to *P. aeruginosa* by R2 is derived from a *RecA*-mediated, DNA-damage-inducible, error-prone DNA repair system which complements the lack of a chromosomally encoded *umuDC*-like operon.

Introduction

All living organisms must respond to stresses in their environment which challenge the integrity of their genetic material. One of the most pervasive of the stresses with which free-living organisms, particularly those in aquatic environments, must deal is DNA damage of the type produced by UV radiation. To date, the most studied and best understood system of responses to this stress has been that of the enteric bacterium *Escherichia coli* (Walker, 1984). *E. coli* K12 responds to UV challenge by inducing a repertoire of DNA repair systems which are collectively part of the SOS regulatory network (Little & Mount, 1982; Walker, 1984). The expression of these responses is negatively regulated by the product of the *lexA* gene and positively regulated by stress-induced activation of the product of the *recA* gene. The most characteristic of the SOS-controlled repair systems is an error-prone system which requires the gene products of the chromosomal operon *umuDC* (Walker, 1984).

Very little is known about the physiological response

of autochthonous soil and aquatic micro-organisms such as *Pseudomonas aeruginosa* to UV irradiation. The molecular basis for the repair of damage done to DNA by this type of stress has not been explored in these species. *P. aeruginosa* has been observed to be relatively sensitive to (Kung & Lee, 1973; Krishnapillai, 1975; Kokjohn & Miller, 1985; McBeth, 1989) and non-mutable by (Lehrbach *et al.*, 1979; McBeth, 1989) UV radiation. Mutations in various loci, including *recA* (Kokjohn & Miller, 1987, 1988; Horn & Ohman, 1988), which increase the UV sensitivity of this species have been isolated (Krishnapillai, 1975).

In addition to chromosomally-encoded DNA repair systems, various plasmids, such as R46, increase the UV resistance of several enteric species including *E. coli* and *Salmonella typhimurium* (Langer *et al.*, 1981; Walker, 1984; Sedgwick & Goodwin, 1985). This plasmid contains genes (*mucA* and *mucB*) which code for a mutagenic UV-repair system analogous to the *umuDC*-encoded system of the *E. coli* chromosome (Elledge & Walker, 1983). Many aquatic species of bacteria including *P. aeruginosa* harbour plasmids that encode functions that afford increased protection of the host to UV irradiation (Krishnapillai, 1975; Lehrbach *et al.*, 1978; Jacoby *et al.*, 1983; Bale *et al.*, 1988).

In this study, we investigated the ability of UV radiation to induce increased DNA repair potential in *P.*

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Abbreviation: Hcr, host-cell reactivation.

Table 1. *Bacterial strains and plasmids used in this study*

Strain or plasmid	Relevant genotype or phenotype	Source
<i>P. aeruginosa</i>		
PAO1	Prototrophic	Miller & Ku (1978)
PAO25	<i>argF10 leu-10</i>	Kokjohn & Miller (1987)
PAO303	<i>argB21</i>	Kokjohn & Miller (1988)
RM8	<i>argB21 recA908</i>	Kokjohn & Miller (1988)
RM265	<i>leu-10 recA102</i>	Kokjohn & Miller (1987)
<i>E. coli</i>		
AB1157	<i>argE3 his-4 leu-6 proA2 trp-1 thi-1 supE44</i>	Kokjohn & Miller (1987)
JC2926	As AB1157 but <i>recA13</i>	Kokjohn & Miller (1987)
<i>Plasmids</i>		
R2	IncP9, Cb ^R Sm ^R Su ^R UV ^R	Jacoby (1977); Korfhagen <i>et al.</i> (1978)
pCP13	Tc ^R Km ^R , broad-host-range cosmid	Darzens & Chakrabarty (1984)
pJC859	pBR322 containing the <i>E. coli recA</i> gene	Kokjohn & Miller (1987)
pKML3001	pCP13 containing the <i>P. aeruginosa recA</i> gene	Kokjohn & Miller (1987)
pKML3002	pCP13 containing the <i>E. coli recA</i> gene from pJC859	This study

aeruginosa PAO in the form of Weigle reactivation and Weigle mutagenesis (Weigle, 1953) of UV-damaged *P. aeruginosa* bacteriophage D3 and F116 DNA. In addition, the inducible nature of the UV-resistance system encoded by the *P. aeruginosa* IncP9 UV-resistance (UV^R) plasmid R2 (Jacoby, 1977; Korfhagen *et al.*, 1978) has been explored. In the absence of R2, significant levels of Weigle reactivation and Weigle mutagenesis could not be detected. R2 was found to encode a true UV-inducible, mutagenic DNA repair system which allows both Weigle reactivation and Weigle mutagenesis of UV-damaged phage DNAs.

Methods

Bacteria, bacteriophages, plasmids and media. Bacterial strains and plasmids used in this study are listed in Table 1. Bacteriophages D3 and F116L have been described (Miller & Ku, 1978). Bacteria were maintained in Luria Broth (LB) (Miller & Ku, 1978). Antibiotics were routinely used for selection at the following concentrations: 300 µg carbenicillin ml⁻¹, 75 µg rifampicin ml⁻¹ and 750 µg streptomycin ml⁻¹. Tetracycline was used at a concentration of 15 µg ml⁻¹ for *E. coli* and 250 µg ml⁻¹ for *P. aeruginosa*. Plating of bacterial strains was done on LBA (LB containing 1.3%, w/v, agar). Lysates of bacteriophages were prepared by the method of Miller & Ku (1978). Strains containing plasmid R2 were constructed by conjugal transfer of the plasmid using the method of Miller & Ku (1978).

UV sensitivity. Cells were grown to early exponential phase (approximately 10⁸ c.f.u. ml⁻¹) in LB at 37 °C. For strains containing R2, carbenicillin was present during this growth. Cultures were harvested by centrifugation at 5000 g in a Sorvall GLC-2B centrifuge. The cells were suspended in 0.85% NaCl and 1.0 ml of this cell suspension was placed in a Petri dish (10 cm diameter) and exposed to various doses of UV radiation from a General Electric germicidal lamp. The intensity of the UV radiation was determined using a UVX radiometer (Ultra-Violet Products). Survivors were determined by plating appropriate dilutions of cells on LBA and incubating overnight at 37 °C. All manipulations and incubations subsequent to UV

irradiation were carried out under conditions designed to minimize photoreactivation.

The UV-sensitivity coefficient (S_{UV}) was calculated from:

$$S_{UV} = \ln[(c.f.u.)_d / (c.f.u.)_0] / d$$

where (c.f.u.)₀ is the concentration of c.f.u. in unexposed samples and (c.f.u.)_d is the recoverable concentration after exposure to dose d of UV radiation. The dose (d) is expressed in J m⁻².

Host-cell reactivation. Host-cell reactivation (Hcr) was measured as described by Miller & Ku (1978), using D3c, a clear-plaque morphology mutant of bacteriophage D3 (Miller & Ku, 1978). Hcr was quantified by calculating the phage sensitivity coefficient (PS_{UV}) from a formula similar to that for S_{UV} .

Weigle reactivation and Weigle mutagenesis of bacteriophages. Lysates of phage D3c (2×10^8 p.f.u. ml⁻¹) in TNM buffer (Cavenagh & Miller, 1986) were irradiated with a dose of UV radiation which gave approximately 0.01% survival in an unirradiated host (200 or 250 J m⁻² depending upon the host strain) and diluted in TNM buffer. Bacteria were grown in LB to early exponential phase (approximately 2×10^8 c.f.u. ml⁻¹), centrifuged, suspended in an equal volume of 0.85% NaCl, and exposed to various doses of UV radiation as described above. One-tenth millilitre samples of irradiated bacteria were mixed with the various dilutions of unirradiated or irradiated phage lysates and absorption was allowed to occur at 37 °C for 10 min in the dark. Lambda top agar (Kokjohn & Miller, 1985) was added, and the mixture was poured onto LBA plates and incubated overnight at 37 °C in the dark. Due to the high UV sensitivity of RecA⁻ strains, approximately 10⁶ c.f.u. of unirradiated cells of the same strain were added at the time of plating of mixtures containing cells exposed to high doses of UV radiation (> 6 J m⁻²). This allowed formation of a confluent lawn and ready detection of phage plaques.

The Weigle reactivation factor (WRF) was calculated from the formula:

$$WRF = \frac{\{[(p.f.u.)_{UV\phi} / (p.f.u.)_{0\phi}]_{\text{on irradiated host}}\}}{\{[(p.f.u.)_{UV\phi} / (p.f.u.)_{0\phi}]_{\text{on unirradiated host}}\}}$$

where (p.f.u.)_{UV ϕ} is the titre of irradiated phages and (p.f.u.)_{0 ϕ} is the titre of the unirradiated lysate.

To determine UV-induced mutagenesis of phage DNA (Weigle mutagenesis), cell-free lysates of wild-type D3 were irradiated as

described above and infected into UV-irradiated or unirradiated hosts. After overnight incubation in the dark, the frequency of clear-plaque mutants among surviving plaques was determined.

Chromosomal mutagenesis. (i) *P. aeruginosa*. Cells were grown in LB at 37 °C to approximately 40 Klett₆₆₀ units. Cultures were harvested by centrifugation, suspended in an equal volume of 0.85% NaCl, and exposed to UV radiation. Samples from irradiated cultures were diluted in 0.85% NaCl and plated on LBA to determine the percentage survival. Samples of the undiluted culture were also plated on LBA and incubated at 37 °C for approximately 20 h. At the end of this time, plates were replicated onto LBA containing rifampicin to enumerate rifampicin-resistant (Rif^R) cells.

(ii) *E. coli*. Selection of mutants was by a modification of the method of Kato *et al.* (1977). Cells were grown at 37 °C to mid-exponential phase in *Pseudomonas* Minimal Medium (PMM) (Miller & Ku, 1978) containing 0.5% vitamin-free Casamino acids (Difco) and 0.4% glucose. Cultures were harvested by centrifugation, suspended in an equal volume of 0.85% NaCl, and exposed to UV radiation. Reversion to histidine prototrophy was assayed by plating samples on Low-His plates which contained PMM, 0.4% glucose, and sufficient histidine to allow some doublings to take place before selection was exerted (Kato *et al.*, 1977). Other required amino acids were present at 20 µg ml⁻¹. The percentage survival was determined by plating dilutions of the irradiated culture on Low-His plates. At high dilution, His⁻ colonies will grow to a sufficient size to be countable.

Results

Dark repair potential

We assessed the potential of isogenic Rec⁺ and Rec⁻ strains of *P. aeruginosa* with and without the UV^R plasmid R2 to survive UV irradiation (Fig. 1, Table 2). Rec⁺ (AB1157) and *recA13* (JC2926) strains of *E. coli* were used as a comparison. Comparison of UV-sensitivity coefficients revealed that Rec⁺ strains of *P. aeruginosa* were approximately ten times more sensitive to UV irradiation than wild-type *E. coli* (Table 2). As has been previously reported (Kokjohn & Miller, 1987, 1988), *recA* mutants of *P. aeruginosa* were more sensitive to UV radiation than their isogenic Rec⁺ parents. However, the increase in sensitivity of *recA* mutants of *P. aeruginosa* was not as dramatic as observed in the Rec⁺ and RecA⁻ isogenic strains of *E. coli*.

When plasmid R2 was introduced into the Rec⁺ strains of *P. aeruginosa*, UV resistance was increased to levels approaching those of wild-type *E. coli* (Fig. 1, Table 2). No increase in UV resistance was observed after introduction of R2 into either of the *P. aeruginosa* *recA* mutants.

Host-cell reactivation (Hcr) of UV-damaged bacteriophages

The UV resistance of an organism as measured by kill curves potentially includes constitutively expressed and damage-inducible components. To assess the constitu-

Fig. 1. UV sensitivity of various *P. aeruginosa* strains with and without R2. Cultures were grown to early exponential phase and exposed to various doses of UV radiation. The frequency of surviving cells was determined by plating on LB agar. Means and standard errors of at least three experiments are presented. (a) *E. coli* AB1157 (□), *P. aeruginosa* PAO303 (Rec⁺) (○), PAO303(R2) (●), RM8 (*recA908*) (△), RM8(R2) (▲). (b) PAO25 (Rec⁺) (○), PAO25(R2) (●), RM265 (*recA102*) (△), RM265(R2) (▲).

Fig. 2. Hcr of UV irradiated bacteriophage D3c. Bacteriophage lysates were exposed to various doses of UV radiation and used to infect unirradiated host cells. Means and standard errors of at least three experiments are presented. Hosts: (a) PAO303 (Rec⁺) (○), PAO303(R2) (●), RM8 (*recA908*) (△), RM8(R2) (▲). (b) PAO25 (Rec⁺) (○), PAO25(R2) (●), RM265 (*recA102*) (△), RM265(R2) (▲).

tive component of the response of *P. aeruginosa* to UV-imposed DNA damage, a cell-free lysate of bacteriophage D3c was irradiated with various doses of UV radiation and the ability of Rec⁺ and Rec⁻ cells to repair the damage done to the phage genome was evaluated (Fig. 2, Table 2). The potential of each of the *recA* mutants to carry out Hcr was not reduced when compared to their isogenic RecA⁺ parents.

Table 2. *Sensitivity of various strains to UV-induced damage*

To determine the sensitivity of bacteria to UV radiation, cultures were grown to early exponential phase and exposed to various doses of UV radiation. Hcr was determined by exposing cell-free lysates of bacteriophage D3c to various doses of UV radiation and infecting unirradiated hosts. The UV dose for 1% survival of bacteria or bacteriophages was determined from plots like those displayed in Figs 1 and 2. The UV sensitivity coefficient (S_{UV}) and phage sensitivity coefficient (PS_{UV}) were determined from the formulae described in Methods.

Strain	Relevant phenotype or genotype	UV sensitivity		Hcr of phage D3c	
		UV dose for 1% survival ($J\ m^{-2}$)	S_{UV}	UV dose for 1% survival ($J\ m^{-2}$)	PS_{UV}
<i>E. coli</i>					
AB1157	Rec ⁺	152	-0.030	ND	ND
JC2926	<i>recA13</i>	3.0	-1.520	ND	ND
<i>P. aeruginosa</i>					
PAO303	Rec ⁺	12.4	-0.366	114	-0.040
PAO25	Rec ⁺	17.8	-0.268	117	-0.040
RM8	<i>recA908</i>	4.4	-1.176	102	-0.044
RM265	<i>recA102</i>	5.2	-1.136	106	-0.043
R2-containing					
<i>P. aeruginosa</i>					
PAO303 (R2)	Rec ⁺	60	-0.077	183	-0.024
PAO25 (R2)	Rec ⁺	106	-0.044	160	-0.029
RM8(R2)	<i>recA908</i>	4.4	-1.174	95	-0.049
RM265(R2)	<i>recA102</i>	4.2	-1.169	111	-0.042

ND, Not determined.

Ability of UV radiation to induce DNA repair in P. aeruginosa

In order to examine the inducible nature of DNA repair in *P. aeruginosa*, Weigle reactivation of UV-damaged bacteriophage D3c was assayed in various strains after exposure of the host cell to low doses of UV radiation. The phages were irradiated at a dose which produced approximately 0.01% survival in an unirradiated host strain, and the UV dose to the host was varied. Both RecA⁺ strains and *recA* mutants showed only the slightest increases in the ability to reactivate UV-damaged phage DNA after exposure of the bacterium to UV radiation (Fig. 3). Similar results were obtained with UV-irradiated bacteriophage F116L (data not shown).

Lack of UV-induced mutagenesis in P. aeruginosa

Weigle mutagenesis of UV-damaged phage D3. Weigle mutagenesis was assayed by determining the frequency of clear-plaque morphology mutants among wild-type D3 phages repaired by host cells which had been exposed to low doses of UV radiation (Fig. 4). The spontaneous frequency of clear-plaque mutants among progeny phages repaired in RecA⁺ and *recA* mutants was the same ($1 \pm 0.5 \times 10^{-2}$) and was consistent with rates

previously reported (Egan & Holloway, 1961). No increase in phage mutability was observed when the host bacterium was exposed to UV radiation.

Chromosomal mutagenesis. The frequency of Rif^R cells was determined after exposure to various doses of UV radiation (Table 3). A slight induction of Rif^R mutants was consistently observed.

Restoration of UV-induced mutagenesis of recA mutants of E. coli by functional P. aeruginosa RecA protein

From the data presented above, it appears that, unlike *E. coli*, *P. aeruginosa* PAO does not possess the ability to induce additional DNA-repair capacity following exposure to UV irradiation. However, there are at least two explanations for this apparent lack of UV-inducible repair. First, the *P. aeruginosa* chromosome may lack functioning structural genes for DNA-damage-inducible repair (e.g. *umuDC* analogues). Second, the genes may be present, but the *P. aeruginosa recA* gene product is incapable of regulating their expression.

We have previously cloned the *P. aeruginosa recA* gene and demonstrated that it restores recombinational proficiency and UV resistance to *recA* mutants of *E. coli* (Kokjohn & Miller, 1985, 1987). To determine if the *P.*

Fig. 3

Fig. 3. Weigle reactivation of UV-irradiated bacteriophage D3c. Irradiated and unirradiated bacteriophages were used to infect host bacteria which had been exposed to various low doses of UV radiation. The Weigle reactivation factor (WRF) was calculated from the formula given in Methods and is a measure of the inducible DNA repair capacity of the host cell. Means of at least three experiments are plotted. Error bars omitted for clarity. The difference in the WRF for PAO303(R2)/PAO303 and for PAO25(R2)/PAO25 at 2 J m^{-2} was significant at the $P < 0.05$ level. Hosts: (a) PAO303 (Rec⁺) (○), PAO303(R2) (●), RM8 (*recA908*) (△), RM8(R2) (▲). (b) PAO25 (Rec⁺) (○), PAO25(R2) (●), RM265 (*recA102*) (△), RM265(R2) (▲).

Fig. 4

Fig. 4. Weigle mutagenesis of UV-irradiated bacteriophage D3. The fraction of progeny phage showing clear-plaque morphology was calculated in experiments conducted as described in Fig. 3. A representative experiment is presented. Data from other experiments were qualitatively the same. PAO25 (Rec⁺) (○), PAO25(R2) (●), RM265 (*recA102*) (□), RM265(R2) (▲).

Table 3. *R2-stimulated, UV-induced mutagenesis of the P. aeruginosa chromosome*

Cultures were grown to early exponential phase and exposed to various doses of UV radiation. Survival rates and numbers of rifampicin-resistant (Rif^R) mutants were determined by differential plating. Two representative experiments are shown.

Strain	UV dose (J m^{-2})	Expt no.	Survival rate (%)	Mutation frequency (Rif ^R mutants per 10^8 survivors)
PAO25	0	1	100	15
		2	100	14
	2	1	100	22
		2	98	17
	4	1	73	31
		2	66	25
	6	1	48	68
		2	35	58
PAO25(R2)	0	1	100	167
		2	100	70
	5	1	74	810
		2	50	770
	10	1	65	1305
		2	50	2600
	20	1	37	2052
		2	29	3206

aeruginosa RecA protein is capable of inducing mutagenic repair in a species known to contain functional *umuDC* genes, we introduced our clone of the gene into *E. coli* JC2926, a *recA13* derivative of AB1157. *E. coli*

recA13 strains are non-mutable by UV irradiation. The reversion frequency of the *his-4* allele in *recA13* mutants is $< 1 \times 10^{-8}$ (Kato *et al.*, 1977).

The presence of the *P. aeruginosa* *recA* gene in the low-copy-number plasmid pKML3001 (Kokjohn & Miller, 1987) restored the UV mutability of the *his-4* allele of JC2926 (Table 4). The levels of induced mutagenesis were equivalent to the levels obtained when the *E. coli* *recA* gene, cloned into the same vector (pKML3002), was introduced into JC2926 and to the levels observed with the Rec⁺ parental strain AB1157.

Plasmid-mediated reactivation of UV-irradiated bacteriophages

Expression of plasmid-encoded UV-inducible repair systems such as the *mucAB* system of R46 is controlled by the SOS regulatory system in *E. coli* (Elledge & Walker, 1983). Since it appeared that the *P. aeruginosa* *recA* gene was capable of regulating gene expression in response to UV exposure, we investigated whether R2-augmented UV resistance in *P. aeruginosa* was inducible and dependent on a functional RecA protein. To determine if R2-dependent UV resistance was due to the augmentation of the constitutively expressed repair capabilities of *P. aeruginosa*, the ability of cells containing plasmid R2 to repair UV-damaged phage genomes was evaluated as

Table 4. Restoration of UV-induced mutagenesis of *E. coli* *recA13* mutants by the cloned *P. aeruginosa* gene

Cultures were grown to early exponential phase and exposed to various doses of UV radiation. Survival rates and numbers of histidine prototrophic revertants of the *his-4* allele were determined by differential plating. pKML3001 contains the *P. aeruginosa* *recA* gene, and pKML3002 contains the *E. coli* *recA* gene. Two representative experiments are shown.

Strain	UV dose (J m ⁻²)	Expt no.	Survival rate (%)	Mutation frequency (His ⁺ mutants per 10 ⁸ survivors)
JC2926(pKML3001) (<i>recA13</i>)	0	1	100	1.2
		2	100	13
	5	1	100	135
		2	90	134
	10	1	73	345
		2	65	355
	20	1	37	1778
		2	48	1292
	0	1	100	3
		2	100	11
JC2926(pKML3002)	5	1	73	45
		2	70	72
	10	1	38	126
		2	30	400
	20	1	27	294
		2	20	588
AB1157 (Rec ⁺)	0	1	100	23
		2	100	30
	5	1	100	50
		2	74	52
	10	1	100	164
		2	43	360
	20	1	40	720
		2	31	741

described above for Hcr (Fig. 2). The presence of R2 increased the potential of RecA⁺ hosts to repair UV-damaged phage DNA only slightly (Table 2). This suggests that the majority of increased UV resistance conferred by R2 is due to a damage-inducible system (or systems) of repair. No increase in plasmid-mediated reactivation was observed in R2-containing *recA* mutants. Similar results were obtained when UV-damaged F116L phages were assayed in the same manner (data not shown).

R2-mediated Weigle reactivation of UV-damaged D3c phages

The presence of R2 in RecA⁺ strains allowed for Weigle reactivation of UV-irradiated D3c phages (Fig. 3). The *recA* mutants did not exhibit Weigle reactivation even when R2 was present. Weigle reactivation of irradiated F116L phages was also observed in RecA⁺ strains containing R2, but not in plasmid-free isogens (data not shown).

Mutagenic nature of R2-mediated UV-repair

Weigle mutagenesis of UV-damaged phage D3. When R2 was introduced into RecA⁺ cells, the level of clear-plaque mutants among the progeny of phages repaired in unirradiated hosts was not affected ($1 \pm 0.5 \times 10^{-2}$). However, increased mutagenesis was observed after exposure of the plasmid-containing host to UV (Fig. 4). No increase in mutation frequency was observed in a *recA* mutant containing R2.

Chromosomal mutagenesis. The frequency of Rif^R survivors of UV irradiation was determined in R2-containing strains (Table 3). At equivalent levels of survival, chromosomal mutagenesis was strikingly augmented over rates obtained without the plasmid. Both spontaneous and UV-stimulated frequencies were increased.

Discussion

P. aeruginosa is surprisingly sensitive to UV irradiation when compared to *E. coli*. As a natural member of soil

and aquatic microbial communities, *P. aeruginosa* is continually exposed to a variety of physical and chemical agents which cause DNA damage similar to that produced by UV radiation (Peak & Peak, 1989). The lack of DNA repair potential in *P. aeruginosa* would not be predicted since it appears that this species' needs for stress-inducible repair capacity is certainly as great as and possibly greater than that of *E. coli*.

While RecA⁺ strains of *P. aeruginosa* PAO are approximately ten times more sensitive to UV radiation than are RecA⁺ strains of *E. coli*, *recA* mutants of *E. coli* and *P. aeruginosa* display similar levels of sensitivity (Fig. 1, Table 2). At the same time, the potential for RecA⁺ and RecA⁻ isogens of *P. aeruginosa* to host-cell-reactivate UV-damaged bacteriophages is equivalent (Fig. 2, Table 2). These observations suggest that one or more *recA*-dependent, inducible repair systems which exist in *E. coli* are lacking in *P. aeruginosa* PAO.

In *E. coli*, Weigle reactivation and UV-induced mutagenesis are dependent on the products of at least four genes, *recA*, *lexA*, *umuD* and *umuC* (Kato, *et al.*, 1977). The products of the *recA* and *lexA* genes regulate these processes as part of the SOS network. The induction of expression of these systems is mediated by the RecA protein, which stimulates cleavage of the repressor of the network, LexA protein, following exposure of the cell to UV radiation (Little *et al.*, 1980; Little & Mount, 1982; Walker, 1984). *recA* mutants of *E. coli* are incapable of inducing the SOS network (Walker, 1984). The products of the *umuDC* genes are structural components of the molecular processes underlying Weigle reactivation and UV-inducible mutagenesis (Kato & Shinoura, 1977; Kato *et al.*, 1977). In addition, the RecA protein has one or more functional roles in these processes. RecA accelerates the post-translational auto-proteolytic activation of the UmuD protein and may have an additional function as well (Nohmi *et al.*, 1988). Therefore, *recA*(Def) *lexA*(Def) strains are non-mutable despite the absence of functional LexA (Nohmi *et al.*, 1988).

P. aeruginosa PAO appears to lack both Weigle reactivation (Fig. 3) and Weigle mutagenesis (Fig. 4) of UV-damaged bacteriophages. UV-mediated mutagenesis of the bacterial chromosome is, at best, only slightly inducible in this species (Table 3). The lack, or low level of expression, of these processes in *P. aeruginosa* suggests that either (i) this bacterium lacks functional genes necessary for these repair systems (i.e. *umuDC* analogues), (ii) the expression of these systems is not under the control of a *lexA-recA*-like switch, or (iii) these systems are not induced by UV radiation.

Our laboratory has demonstrated that the cloned *P. aeruginosa recA* gene, when introduced into *recA*(Def) mutants of *E. coli*, is capable of regulating several SOS

functions including UV-dependent activation of λ prophages (Kokjohn & Miller, 1985, 1987) and DNA-damage-inducible (*din*) gene expression (Kokjohn & Miller, 1987). In *P. aeruginosa*, UV-dependent prophage activation is also regulated by the RecA protein (Kokjohn & Miller, 1987, 1988), and expression of the *recA* gene itself is autoregulated and induced by DNA-damaging agents (Horn & Ohman, 1988; Miller & Kokjohn, 1988). Furthermore, the *E. coli* and *P. aeruginosa* RecA proteins apparently regulate UV-stimulated activation of λ prophages by a similar molecular mechanism (Kokjohn & Miller, 1985, 1987). When present in *E. coli*, expression of the *P. aeruginosa recA* gene can be regulated by the LexA protein (Miller & Kokjohn, 1988). Here we show that it also restores UV-inducible mutagenesis in *E. coli recA*(Def) mutants (Table 4), suggesting that it is also capable of fulfilling the functional roles of the *E. coli* RecA protein in SOS repair. Therefore, the *P. aeruginosa* RecA protein should be fully capable of participating in a *recA-lexA*-like regulatory switch and functioning in inducible DNA repair pathways if such exist in *P. aeruginosa*.

The *P. aeruginosa*-specific, IncP9 plasmid R2 dramatically increases the UV resistance of Rec⁺ strains of *P. aeruginosa* (Fig. 1, Table 2) and allows for both Weigle reactivation and UV-induced mutagenesis in this species (Figs 3 and 4, Table 3). The phenotypic expression of these R2-encoded systems is dependent on UV irradiation of the host cell and on a RecA⁺ phenotype. Our experiments demonstrate the potential of *Pseudomonas*-specific UV^R plasmids to mediate a true DNA-damage-inducible mutagenic repair response to UV radiation.

The UV^R plasmid R46 and its derivative pKM101 have been studied in detail in several enteric species (Langer *et al.*, 1981; Elledge & Walker, 1983; Walker, 1984). These plasmids harbour the *mucA* and *mucB* genes (Langer *et al.*, 1981), which are structural and functional analogues of the *E. coli* chromosomal gene *umuD* and *umuC* respectively (Elledge & Walker, 1983). Like *umuDC*, expression of the UV-induced resistance and mutagenic functions encoded by *mucAB* is under the control of the *E. coli lexA-recA* regulatory switch. The dependence of expression of the UV^R phenotype of R2 on a functional *P. aeruginosa* RecA protein suggests that R2 harbours genes which are functional analogues of the *mucAB* genes of R46. This interpretation is supported by the observation that the UV^R phenotype of R46 is also expressed in *P. aeruginosa* PAO after UV irradiation (T. A. Kokjohn & R. V. Miller, unpublished).

Taken together, the observations reported here support the conclusion that *P. aeruginosa* PAO lacks active DNA-damage-inducible repair and mutagenesis pathways. This deficiency may be due either to the absence of structural genes required for these activities (e.g. *umuDC*

analogues) or, if they are present, to their poor expression. In this respect, *P. aeruginosa* PAO may be similar to *S. typhimurium* LT2, which also shows only low levels of UV mutability and Weigle reactivation. It has been demonstrated recently that *S. typhimurium* contains *umuDC*-like sequences which are only marginally functional, probably due to a naturally-occurring defect in the *umuD*-like gene (Smith & Eisenstadt, 1989; Thomas & Sedgwick, 1989).

For free-living soil and aquatic micro-organisms such as *P. aeruginosa* which lack significant levels of UV-inducible DNA repair, UV^R plasmids may be of singular importance to the natural ecology of their host bacteria. In order to survive in their natural habitats, these micro-organisms must cope with exposure to solar UV radiation. Since its lethal effects clearly extend to several metres below the water surface in some environments (Calkins *et al.*, 1976; Bale *et al.*, 1988), UV^R plasmids potentially affect the structure of microbial communities in many aquatic habitats. In addition to the protection they afford the host organism, plasmids such as R2 are often conjugation-proficient (Krishnapillai, 1975) and can be efficiently transferred by transduction as well (Saye *et al.*, 1987). The potential for dissemination of these plasmids through aquatic populations is significant since both conjugation (Bale *et al.*, 1988; O'Morchoe *et al.*, 1988) and transduction (Saye *et al.*, 1987) have been shown to be effective mechanisms of plasmid transmission in natural freshwater populations of *Pseudomonas* and are operative under the conditions found in nature.

Because the repair systems encoded by UV^R plasmids may be mutagenic, their presence may significantly increase the genetic diversity and rate of evolution of natural bacterial communities, at least on an episodic basis. An understanding of the responses of members of the natural aquatic microbiota to DNA-damaging stress and the potential role of UV^R plasmids in moulding natural bacterial populations is fundamental to the development of a true understanding of microbial ecology and the development of realistic models for evolutionary dynamics of free-living bacterial species.

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