Novel Patterns of Ultraviolet Mutagenesis and Weigle Reactivation in *Staphylococcus aureus* and Phage ϕ 11

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The effects of u.v. irradiation on the survival of *Staphylococcus aureus* and its phage ϕ 11 were studied. The *recA* and *uvr* mutations affected their survival in a similar way to synonymous mutations in *Escherichia coli*. Weigle reactivation (W-reactivation) of ϕ 11 occurred in wild-type *S. aureus* and in a *uvr* mutant but to a lesser extent than has been found for phage λ in *E. coli*. Reactivation was *recA*-dependent and was accompanied by u.v.-induced mutagenesis in a temperature-sensitive mutant of ϕ 11. Bacterial mutation to streptomycin resistance was induced by u.v. and was also *recA*-dependent. In *S. aureus*, as in *E. coli*, u.v. was a more effective mutagen in the *uvr* genetic background. However, a dose-squared response for u.v.-induced mutation of wild-type and *uvr* strains of *S. aureus* to streptomycin resistance, and of a *trp* auxotroph to tryptophan independence, was found only with u.v. doses below 1 J m^{-2} . We suggest that, in relation to the Uvr mechanism of DNA repair, u.v. mutagenesis in *S. aureus* involves both repairable and non-repairable lesions. As in *E. coli*, the *uvr* genetic background reduced the u.v. dose required for maximal W-reactivation of u.v.-irradiated phage. However, there was no enhancement of W-reactivation by post-irradiation broth incubation of *S. aureus*. Our results are compatible with a non-inducible mechanism for this phenomenon.

INTRODUCTION

Numerous effects of u.v. irradiation in *Escherichia coli* are thought to be due to the induction of 'SOS functions' (Witkin, 1974; Radman, 1974). They include mutagenic Weigle reactivation (W-reactivation) of phage λ (Weigle, 1953; Defais *et al.*, 1971), λ prophage induction (Brooks & Clark, 1967), bacterial mutagenesis (Witkin, 1974), enhanced synthesis of protein X (Gudas & Pardee, 1975) and several others controlled by the *recA* and *lexA* gene products of *E. coli* (for review, see Witkin, 1976). Among these effects, the mechanism of λ prophage induction is best understood. It involves the eventual proteolytic cleavage of the lambda repressor by the *recA* gene product protein X (Roberts *et al.*, 1978). The signal for protein X induction is not known but the similar kinetics of induction of λ prophage and the other functions suggest that they have mechanisms in common (Defais *et al.*, 1976).

Much less is known about SOS functions in other bacteria, although the absence of u.v.-inducible error-prone repair can be inferred from an inability to sustain u.v.-induced mutations. By this criterion, error-prone repair is absent in *Micrococcus radiodurans* (Sweet & Moseley, 1974, 1976), in *Haemophilus influenzae* Rd (Kimball *et al.*, 1977) and in *Proteus mirabilis* (Hutchinson & Medill, 1954; Böhme, 1963). Pollard & Snipes (1977) found no evidence of u.v.-inducible radioresistance or inhibition of post-irradiation DNA degradation in *Pseudomonas* BAL-31, of marine origin. However, Hofemeister & Böhme (1975) demonstrated a low level of W-reactivation of a temperate phage correlated with relatively weak *recA lexA* dependent DNA repair in *P. mirabilis*. Hofemeister (1977) established that post-irradiation DNA degradation in this organism was an inducible *rec* function. In *Bacillus*

subtilis a full range of SOS functions has been found; these resemble those of *E. coli*, although some differences in the kinetics of W-reactivation were noted (Yasbin, 1977*a, b*). U.v. mutability has been shown in myxobacteria (Grimm, 1978) but there was no DNA repair in the *Mycoplasma* species studied by Ghosh *et al.* (1977). Clearly different patterns of u.v.-associated functions exist.

U.v. induction of prophage in *Staphylococcus aureus* is well known (Wyman *et al.*, 1974; Smith & Dunican, 1974; Smith *et al.*, 1975). In addition to several mutants defective in host cell reactivation (Goering & Pattee, 1971), *rec* strains with properties similar to those of *E. coli recA* (Inoue *et al.*, 1972; Wyman *et al.*, 1974) and *E. coli recB, C* (Goering & Pattee, 1971) have been isolated.

We have studied repair functions in a wild-type *S. aureus* strain and its u.v.-sensitive mutants. Our results on the survival of u.v.-irradiated *S. aureus* and host cell reactivation of u.v.-damaged bacteriophage $\phi 11$ suggest resemblances with the Uvr and Rec mechanisms in *E. coli* and phage λ . U.v. mutagenesis occurs for two mutations investigated and mutations to streptomycin resistance depend on the *recA1* gene product. This is correlated with *recA1*-dependent W-reactivation and mutagenesis in a temperature-sensitive mutant of the temperate phage $\phi 11$. Although error-prone repair functions are found in *S. aureus*, they do not conform to the model of inducible SOS repair developed for *E. coli*.

METHODS

Bacteria and bacteriophages. These are listed in Table 1. The effects of the *uvr* and *recA* mutations on the u.v. survival of host bacteria and phage, and on W-reactivation, were compared with those of their wild-type alleles in strain RN1349. In all other experiments comparisons were made with the original wild-type strain RN450.

Media. Bacteria were cultured in nutrient broth consisting of 1.5% (w/v) tryptose, 0.25% (w/v) soy peptone, 0.5% (w/v) yeast extract and 0.5% (w/v) NaCl. The medium was solidified with 1.2% (w/v) agar when required. Ingredients were obtained from London Analytical & Bacteriological Media, Pendleton, Salford, Lancs. Soft nutrient agar was 2.0% (w/v) blood agar base no. 2 (Oxoid). For phage propagation 4 mM-CaCl₂ was added after autoclaving. Streptomycin media contained 500 µg streptomycin sulphate ml⁻¹ (Glaxo). The tryptophan auxotroph was grown on the modified casein hydrolysate minimal medium (CHM) of Lindberg *et al.* (1972) with the addition of 20 µg L-tryptophan ml⁻¹. Novick buffer was as described by Novick & Brodsky (1972). Phosphate buffer (pH 7.0) contained, per litre, 3.0 g KH₂PO₄, 7.0 g Na₂HPO₄, 4.0 g NaCl and 0.2 g MgSO₄ · 7H₂O.

N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) mutagenesis. For bacterial mutagenesis the method of Adelberg *et al.* (1965) was used. Mutants of phage $\phi 11$ were obtained by the method of Cohen *et al.* (1977).

Ultraviolet irradiation. A Hanovia Chromatolite portable ultraviolet lamp delivering 0.55 J m⁻² s⁻¹ at a distance of 9.5 cm or 0.034 J m⁻² s⁻¹ at 38 cm was used. Output was measured with a Blak-Ray short wave meter

Table 1. *Staphylococcus aureus* bacterial and phage strains

Strain	Characteristics and derivation	Source
Bacteria		
RN450 (8325-4)	Wild-type; strain NCTC 8325 cured of three prophages	R. Novick*
RN1349 (8325-4 <i>his-7</i>)	Histidine-requiring mutant of RN450	R. Novick
RN972 (8325-4 <i>his-7 uvr-420</i>)	Ultraviolet-sensitive derivative of RN1349	R. Novick
RN981 (8325-4 <i>his-7 uvr-461</i>)	Ultraviolet-sensitive derivative of RN1349; also deficient in generalized recombination (Wyman <i>et al.</i> , 1974)	R. Novick
RN450 <i>trp-1</i>	MNNG-induced tryptophan-requiring mutant of RN450	This laboratory
Phage		
$\phi 11$	Wild-type; isolated as a prophage from NCTC 8325	R. Novick
$\phi 11m15$	Clear-plaque mutant of $\phi 11$	R. Novick
$\phi 11ts4$	MNNG-induced temperature-sensitive mutant of $\phi 11$	This laboratory

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(J 225; Ultra-Violet Products, San Gabriel, Calif., U.S.A.). Operations were performed in sodium light to avoid photoreactivation.

Ultraviolet survival curves. Overnight broth cultures were diluted 10-fold in prewarmed nutrient broth and incubated with aeration to a cell density of 2×10^8 to 5×10^8 colony-forming units (c.f.u.) ml⁻¹. The bacteria were resuspended in chilled phosphate buffer and subjected to mild sonication (for 60 s at amplitude 1 using a Dawe Soniprobe type 7532A; Dawe Instruments, London W3 0SD) to disperse clusters. This treatment yielded 80% of colony-forming units as single cells and gave the maximum plate count; it had no effect on the u.v. sensitivity of the bacteria. Sonicated bacterial suspensions were diluted 100-fold in phosphate buffer and 5 ml amounts were irradiated in 9 cm diam. glass Petri dishes with constant agitation. Survivors were counted in soft nutrient agar overlays after 48 h incubation at 37 °C. Phage suspensions at a concentration of about 10^8 plaque-forming units (p.f.u.) ml⁻¹ were irradiated in Novick buffer and survivors were counted in soft nutrient agar overlays containing indicator bacteria after overnight incubation at 30 °C.

Ultraviolet reactivation experiments. Exponential-phase cultures were resuspended in 0.1 vol. Novick buffer and sonicated. Samples (about 10^9 c.f.u. ml⁻¹) were irradiated and 0.2 ml sub-samples were adsorbed (15 min at 37 °C) to an equal volume of phage previously irradiated to a survival of about 10^{-3} . The multiplicity of infection was never greater than 10^{-2} and preliminary experiments showed that 70 to 90% of the phage were adsorbed during this period. After adsorption, molten soft nutrient agar containing the indicator bacterium was added to the tubes and the contents were rapidly mixed and poured over the agar base. Plaques were counted after incubation at 30 °C.

Phage mutagenesis associated with *W*-reactivation. Reversion frequencies of the temperature-sensitive mutant phage ϕ 11ts4 were used as a measure of u.v.-induced phage mutagenesis. Phage irradiated to a survival of 10^{-2} were adsorbed at 37 °C to irradiated or unirradiated host cells and plated as described above. The plates were held at 30 °C for precisely 10 min to allow solidification of the agar overlay and then incubated at 41.5 °C. Revertant plaques were counted after 24 h.

Post-irradiation broth incubation. Exponential-phase bacteria were irradiated with a u.v. dose of 12 J m^{-2} , resuspended in fresh nutrient broth at a density of 10^9 c.f.u. ml⁻¹ and incubated at 37 °C with aeration. At intervals samples were withdrawn, adsorbed to irradiated phage ϕ 11m15 or ϕ 11ts4 and plated as described above.

Mutation frequency response (MFR) for u.v.-induced streptomycin resistance. Exponential-phase bacteria were resuspended in phosphate buffer at a density of 10^8 to 10^9 c.f.u. ml⁻¹ and sonicated. Samples were irradiated, plated in thin soft nutrient agar overlays and incubated at 37 °C to allow phenotypic expression. A preliminary experiment had shown no significant increase in the number of mutants after 2.5 h for strain RN450 (wt) and 3.5 h for strain RN972 (*uvr*) and these periods were used for phenotypic expression. A thin layer of soft nutrient agar containing streptomycin sulphate was then added to each plate to give a final concentration of $500 \mu\text{g ml}^{-1}$ and incubation was continued for a further 48 h at 37 °C before streptomycin-resistant colonies were counted.

Reversion of tryptophan auxotrophs. Bacteria were grown in nutrient broth to mid-exponential phase (2×10^8 to 5×10^8 c.f.u. ml⁻¹), washed twice in 3 vol. buffer and sonicated. Irradiated samples were plated directly on unsupplemented CHM to enumerate prototrophs. Unsupplemented medium allowed residual growth and expression of u.v.-induced mutations; supplemented medium was not used because it increased the background levels of spontaneous mutants and reduced the accuracy of the determinations. Survivors were estimated by plating on CHM with tryptophan, and also on nutrient agar.

RESULTS

Ultraviolet survival and host cell reactivation in S. aureus wild-type, recA1 and uvr mutants

The survival curves for strain RN1349 and its two u.v.-sensitive derivatives RN972 and RN981 are shown in Fig. 1. Strain RN981 was originally selected as a u.v.-sensitive mutant also defective in generalized recombination and has been well characterized as an *E. coli* *recA* analogue (Wyman *et al.*, 1974). Strain RN972 was selected from a series of 12 other u.v.-sensitive derivatives of RN1349 because of its extreme u.v. sensitivity and uniformity of colonial morphology.

Strain RN981 (*recA1*) gave a survival curve similar in shape to those of *E. coli* *recA* mutants (Howard-Flanders & Boyce, 1966; Howard-Flanders & Theriot, 1966): the bacteria were extremely sensitive to low doses ($<0.5 \text{ J m}^{-2}$) with a dose for 37% survival (D_{37}) of 0.08 J m^{-2} but the fraction surviving at higher doses was more resistant. Strains RN450 (wt), RN1349 (wt) and RN972 (*uvr*) have shouldered survival curves (see Figs 1 and 5). D_{37} values, calculated from the initial portion of the curves, were 21.0 J m^{-2} for the wild-type and 0.15 J m^{-2} for the *uvr* mutant (RN972).

Fig. 1. Survival of u.v.-irradiated *S. aureus*. Exponential-phase cells of strains RN1349 [wt] (○), RN972 [*uvr*] (△) and RN981 [*recA*] (□) were resuspended in buffer (about 10^6 c.f.u. ml^{-1}), sonicated and u.v.-irradiated. Survivors were enumerated in soft nutrient agar overlays after incubation at 37°C for 48 h.

Fig. 2. Survival of u.v.-irradiated phage $\phi 11$ plated on different *S. aureus* host strains. Phage suspensions (about 10^8 p.f.u. ml^{-1}) were u.v.-irradiated and plated with strains RN1349 [wt] (○), RN972 [*uvr*] (△) or RN981 [*recA*] (□) in soft nutrient agar overlays. Survivors were counted after incubation at 30°C for 24 h.

Figure 2 shows the survival of phage $\phi 11$ plated on three different strains. Strain RN972 shows the *hcr* phenotype (D_{37} 2.0 J m^{-2}) typical of excision-repair-deficient mutants of *E. coli* reactivating phage λ (Harm, 1963). As previously reported by Wyman *et al.* (1974) using phage $\phi 11\text{m}15$, strain RN981 was able to reactivate phage $\phi 11$ to almost the same extent as the wild-type. The D_{37} values were 20.5 J m^{-2} and 15.5 J m^{-2} , respectively, when phage $\phi 11$ was plated on RN1349 (wt) and RN981 (*recA1*).

Ultraviolet reactivation experiments

Weigle reactivation and mutagenesis. Figure 3(a) shows the results of W-reactivation experiments with u.v.-irradiated phage $\phi 11$. The wild-type strain (RN1349) was able to reactivate the damaged phage giving a fourfold increase in the number of phages recovered when the host bacteria were irradiated at a dose of 12 J m^{-2} . Strain RN972 (*uvr*) showed reactivation of phage $\phi 11$ only when the phage was heavily irradiated (to 5×10^{-5} survival). However, W-reactivation could be obtained in strain RN972 by using the clear-plaque mutant $\phi 11\text{m}15$ (Fig. 3b). In neither of the strains was reactivation as extensive as in the corresponding *E. coli* strains reactivating phage λ (Hart & Ellison, 1970; Radman & Devoret, 1971). No increased reactivation was detectable in irradiated strain RN981 (*recA1*) with either phage (Figs 3a, b).

Fig. 3. Weigle reactivation and mutagenesis of phage $\phi 11$ by different *S. aureus* hosts. (a) Phage $\phi 11$ was u.v.-irradiated and adsorbed to irradiated strains RN1349 [wt] (○), RN972 [*uvr*] (△) or RN981 [*recA*] (□). After adsorption the mixture was plated in soft nutrient agar overlays using a *her* strain (RN972) as indicator bacterium. Plaques were counted after 24 h at 30 °C. (b) U.v.-irradiated phage $\phi 11\text{m15}$ (a clear-plaque mutant of $\phi 11$) was adsorbed to irradiated strain RN972 [*uvr*] (▲) or RN981 [*recA*] (■) and plated as described above. (c) Temperature-sensitive phage $\phi 11\text{ts4}$, u.v.-irradiated to a survival of 10^{-2} , was adsorbed to irradiated strain RN450 [wt] (●) for 15 min at 37 °C. After plating, incubation was continued at the restrictive temperature (41.5 °C). Revertant plaques were counted after 24 h.

Fig. 4. Effect of post-irradiation nutrient broth incubation of wild-type *S. aureus* strain RN450 on (a) W-reactivation of phage $\phi 11\text{m15}$ and (b) mutagenesis of the temperature-sensitive mutant $\phi 11\text{ts4}$. Exponential-phase cells of strain RN450 were resuspended in Novick buffer and irradiated to give maximum W-reactivation (12 J m^{-2}). A sample was plated immediately with u.v.-irradiated $\phi 11\text{m15}$ (survival 10^{-3} , ●) or u.v.-irradiated $\phi 11\text{ts4}$ (survival 10^{-2} , ▼) as described in Methods. The remaining cells were resuspended in fresh nutrient broth at 37 °C and incubated with shaking. At intervals, samples were withdrawn and plated with the u.v.-irradiated phage. Controls using unirradiated $\phi 11\text{m15}$ (○) and unirradiated $\phi 11\text{ts4}$ (▽) were also included. Plaques were counted after incubation at 30 °C for $\phi 11\text{m15}$ or 41.5 °C for $\phi 11\text{ts4}$.

The data in Fig. 3(c) show the increase in frequency of revertants of the temperature-sensitive phage mutant $\phi 11\text{ts4}$ during similar W-reactivation experiments in the wild-type host bacterium. The reversion frequency was maximal at a dose of 12 J m^{-2} , the same as for W-reactivation. Plaques of phage $\phi 11$ on the mutant host bacteria are indistinct at the restrictive temperature and it was therefore not possible to determine whether u.v. mutagenesis occurred in the *rec* and *uvr* genetic backgrounds.

Effect of post-irradiation incubation on Weigle reactivation and mutagenesis. Figure 4 shows the extent of W-reactivation of phage $\phi 11\text{m15}$ and number of u.v.-induced revertants

Fig. 5. Mutation frequency response for streptomycin resistance in u.v.-irradiated *S. aureus* RN450 and RN972. Exponential-phase cells of strains RN450 [wt] (a) and RN972 [uvr] (b) were irradiated and about 10^8 c.f.u. were plated in soft nutrient agar overlays. After incubation at 37°C to allow phenotypic expression, an additional thin layer of soft nutrient agar containing streptomycin sulphate (final concn $500 \mu\text{g ml}^{-1}$) was added to each plate. Streptomycin-resistant (Str^R) colonies were counted after a further 48 h incubation at 37°C . Points represented on the graph are corrected for the spontaneous mutation frequency ($<4 \times 10^{-7}$) and are the means for three separate experiments. Dashed lines represent the surviving fraction determined in soft nutrient agar overlays. (c) Log-log plot of the same data: strain RN450 [wt] (■), strain RN972 [uvr] (▼). Theoretical (slope = 2) lines are shown.

of $\phi 11\text{ts4}$ obtained when bacteria irradiated at a dose of 12 J m^{-2} were incubated at 37°C in broth for various periods before infection with the irradiated phage. No improvement in reactivation or increase in mutation occurred and there was a steady decline in both during the incubation. These results differ from those obtained for *E. coli*, in which W-reactivation and mutagenesis were both enhanced by post-irradiation incubation (Defais *et al.*, 1976).

Ultraviolet mutagenesis of S. aureus

Forward mutation to streptomycin resistance. The mutation frequency responses (MFR) of strains RN450 (wt) and RN972 (*uvr*) to u.v. irradiation in relation to their survival are shown in Fig. 5. An absolute increase in number of streptomycin-resistant mutants occurred in both strains. Considerably more mutants were induced per unit dose for the *uvr* strain than the wild-type strain and the maximum yield was greater. A similar result has been described for

Fig. 6. (a) Mutation frequency response for u.v.-induced reversion to prototrophy of *S. aureus* strain RN450 *trp-1*. Washed exponential-phase cells of RN450 *trp-1* were u.v.-irradiated and samples were spread on CHM agar at a density of 3×10^7 to 4×10^8 ml $^{-1}$. Prototrophs were counted after incubation for 48 h at 37 °C. Survivors were determined on CHM agar containing 20 µg L-tryptophan ml $^{-1}$. Points are the means for at least three separate experiments and are corrected for the spontaneous mutation frequency (6.1×10^{-7}). The dashed line represents the surviving fraction. (b) Log-log plot of the same data. Theoretical (slope = 1 and slope = 2) lines are shown.

u.v.-induced streptomycin resistance in analogous mutants of *E. coli* (Witkin, 1966). A log-log plot of the data (Fig. 5c) shows that there was a dose-squared relationship between u.v. dose and frequency of streptomycin-resistant mutants in the low (<1 J m $^{-2}$) dose range for both strains. No streptomycin-resistant derivatives of strain RN981 (*recA1*) were obtained using the methods described and plating out 10^9 c.f.u., confirming its u.v. stability and similarity with *recA* mutants of *E. coli*.

Ultraviolet-induced reversion of RN450 *trp-1* to prototrophy. The MFR of the tryptophan auxotroph to increasing u.v. dose is shown in Fig. 6(a). The survival of the irradiated bacteria was poorer when plated on nutrient agar than on CHM with added tryptophan; the frequency of revertants was calculated using the latter figures. The results were similar to those obtained with mutation to streptomycin resistance. Mutations to prototrophy were induced at u.v. fluences which had little effect on bacterial viability. The MFR curve shows an apparent linear relationship between u.v. fluence and induction of prototrophs. A log-log plot of the data is shown in Fig. 6(b). As with the experiments on mutation to streptomycin resistance in strain RN450, the dose-squared relationship is probably confined to the low dose range (<1 J m $^{-2}$). However, data obtained in the low dose range are less reliable owing to the increased significance of spontaneous background mutations.

DISCUSSION

The u.v. survival and host cell reactivating characteristics of the *S. aureus* mutants RN972 and RN981 confirm their resemblance, respectively, to the excisionless (*uvr*) and recombination-deficient (*recA*) mutants of *E. coli* (Wyman *et al.*, 1974). Table 2 shows the approximate number of dimers estimated to constitute a single lethal hit for u.v. irradiation of *S. aureus*, phage $\phi 11$ and *E. coli*. The u.v. dose for maximal W-reactivation in wild-type *S. aureus* (12 J m $^{-2}$) was lower than in wild-type *E. coli* (30 J m $^{-2}$) (Defais *et al.*, 1971). However, the bacterial survival level for maximal W-reactivation in *S. aureus* ($>80\%$) was similar to that in *E. coli* (Witkin, 1976), and also *B. subtilis* (Yasbin, 1977a), but differed from that in *P. mirabilis*, in which maximal reactivation occurs in wild-type bacteria at a survival level of only 0.1% (Hofemeister, 1977). In the *S. aureus* *uvr* mutant the maximal reactivating dose was 1 J m $^{-2}$; at this dose the level of bacterial survival was much lower (about 0.1%), as in *E. coli* *uvr* mutants (Witkin, 1976). For both wild-type and *uvr* strains,

Table 2. *Estimated pyrimidine dimers per genome per lethal hit for u.v. irradiation of S. aureus, phage ϕ 11 and E. coli, obtained from u.v. survival curves*

The *S. aureus* data were estimated from the D_{37} values of the survival curves in Figs 1 and 2, assuming 60 dimers per genome for each $J\ m^{-2}$ (Witkin, 1976), and correcting for the higher A + T content of *S. aureus* (65–68%; Garrity *et al.*, 1969) and phage ϕ 11 (62.5–64.8%; Brown *et al.*, 1972). It is assumed that the *S. aureus* genome is of similar size and has a similar distribution of thymines to that of *E. coli*. The mol. wt of phage ϕ 11 DNA is 3.27×10^7 (Brown *et al.*, 1972). Dimer formation in phage DNA is from Radman *et al.* (1970). The *E. coli* data are from Howard-Flanders & Boyce (1966); the figures in brackets are recalculated on the basis of 60 dimers per genome for each $J\ m^{-2}$.

	Bacterial genotype		
	wild-type	<i>uvr</i>	<i>recA</i>
<i>S. aureus</i>	2230	16	8
Phage ϕ 11 on <i>S. aureus</i>	13.3	1.3	10.0
<i>E. coli</i>	3700	60	22
	(2960)	(48)	(18)

maximum W-reactivation was more pronounced for the clear-plaque mutant phage ϕ 11m15 than for the wild-type phage ϕ 11.

Prophage reactivation – the repair of a damaged superinfecting phage by recombination with a related prophage – could be a complication in W-reactivation experiments. Strain RN450 was cured of three prophages by u.v. induction (Novick, 1967), but Rudin & Lindberg (1975) have indicated that defective prophages may still be present. The survival of u.v.-damaged phage ϕ 11 was almost identical on strain RN450 and its *recA* derivative, strain RN981. Since a functional *recA* gene is necessary for prophage reactivation in *E. coli* (Hart & Ellison, 1970) it is unlikely that prophage reactivation was a factor in our experiments.

There is a similarity between the u.v.-associated SOS functions of *S. aureus* and those of *E. coli*. All occur to some extent independently of the *uvr* gene products but are dependent on *recA* function. U.v. induction of prophage ϕ 11 from lysogens of strain RN450 has been shown by Wyman *et al.* (1974) and by Smith & Dunican (1974), and from strain RN972 (*uvr*) (unpublished results). Strain RN981 (*recA*) lysogens rarely release phage spontaneously and are not u.v.-inducible for phage ϕ 11 (Wyman *et al.*, 1974). Some W-reactivation occurs in both the wild-type and the *uvr* strain and it is accompanied by mutagenesis of the temperature-sensitive mutant phage ϕ 11ts4 in strain RN450. W-reactivation did not occur in the *recA* mutant.

Bacterial mutagenesis to streptomycin resistance takes place in both the wild-type and *uvr* mutant but not in the *recA* mutant, thus indicating that *recA*-dependent error-prone repair of u.v. damage is present in *S. aureus*. We have also obtained u.v.-induced reversion in the tryptophan auxotroph derived from strain RN450.

In *E. coli*, a uniform hypothesis has been devised for the induction of SOS functions including W-reactivation and error-prone DNA repair (Witkin, 1974; Radman, 1974). However, some data do not support fully the concept of uniform coordinate induction. In *lon* strains of *E. coli*, for example, the u.v. dose required to inhibit cell division and produce filaments is much lower than for other SOS functions, but the effect is subject to excision repair (Bridges *et al.*, 1977). Thus, although unexcised pyrimidine dimers signal the division inhibition, fewer are needed than for other SOS functions. There are also aspects of the responses we observed in *S. aureus* which do not conform to the SOS hypothesis.

In *E. coli*, low u.v. doses result in small increases in the mutation frequency but higher doses are relatively more effective (Bridges *et al.*, 1967). This has been interpreted (Doudney, 1976) as being a consequence of the inducibility of error-prone repair, for which low doses must provide both the premutational damage and the inducing signal. In *S. aureus* u.v.-irradiated in the 1 to 12 $J\ m^{-2}$ range, which is associated with SOS induction in wild-type *E. coli*, tryptophan prototrophs accumulated linearly with u.v. dose. Very few new

streptomycin-resistant mutants arose in this range. Thus, in *S. aureus* no evidence has been obtained for a dose-squared relationship in the 1 to 12 J m⁻² range. In the low dose range (0 to 1 J m⁻²) both wild-type strains accumulated mutants exponentially with dose-squared kinetics. The *uvr* strain RN972 also accumulated streptomycin-resistant mutants with dose-squared kinetics in the same low dose range, but it yielded more mutants per unit dose indicating that, as in *E. coli*, the premutational lesions are unexcised dimers. *Staphylococcus aureus* differs from *E. coli* in the important respect that dose-squared kinetics occur only in the low dose range, irrespective of the capability of the bacterium to carry out the excision repair. U.v. mutagenesis, although dependent on unexcised dimers for premutational lesions, must also require a photoproduct which is apparently not subject to excision or acts at the pre-excision stage.

W-reactivation in *S. aureus* follows the *E. coli* pattern, with the *uvr* genetic background reducing the u.v. dose required for maximal reactivation. It is therefore related to the presence of unexcised dimers.

Thus our results suggest that in *S. aureus* there is a difference between the basic mechanisms of bacterial mutagenesis and W-reactivation of phage. Bacterial mutagenesis depends partly on a u.v. photoproduct which is not subject to the Uvr repair mechanism. W-reactivation involves lesions which are repairable by this mechanism.

W-reactivation and its associated mutagenesis in *S. aureus* are at a maximum immediately after irradiation and decline during broth incubation. In *E. coli*, W-reactivation and mutagenesis reach a maximum only after 50 min broth incubation; this is related to a requirement for protein synthesis (Defais *et al.*, 1976). The absence of a broth incubation effect in *S. aureus* suggests that new protein synthesis is not required. However, the requirement may be less, or the rate of synthesis may be greater, so that necessary synthesis is completed before the end of the phage adsorption period.

The *S. aureus* results are compatible with a hypothesis of DNA repair in which *recA* protein is present constitutively at a concentration sufficient for these functions. A related mechanism has been discussed by Bridges (1978), in which deoxyribonucleoside 5'-monophosphates built up by DNA polymerase idling (Villani *et al.*, 1978) act directly to suppress the 'proof-reading' function of the polymerase. The *recA* protein is allocated a direct role in the stabilization of the replicating DNA (Kerr & Hart, 1972; Hofemeister, 1977; Bridges, 1978; Satta *et al.*, 1979) and need not be involved in repressor cleavage or new protein synthesis.

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