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Repression of induction by u.v. of λ phage by *exrA* mutations in *Escherichia coli*

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SUMMARY

The effect of ultraviolet radiation (u.v.) on λ lysogens of *exrA* strains of *Escherichia coli* was studied. *exrA* strains could be lysogenized with, as well as support the vegetative reproduction of, λ. However, though spontaneous induction of λ occurred in *exrA* (λ) strain at 10% the frequency of *exrA*$^+$(λ) strain, *exrA* (λ) strains were not induced by u.v. Because λ was not induced in *exrA* (λ) strains, lysogens of these strains were no more sensitive to u.v. than were non-lysogens.

The heat-inducible mutant λ*hcI857* could be induced in *exrA* strains at elevated temperatures. Furthermore, u.v. irradiation of *exrA* (λ*hcI857*) strain did not prevent the heat induction of this λ mutant. The *exrA* mutation appeared to interfere only with the inactivation of λ repressor by u.v.

Among the *exrA* strains studied was strain *Bs*1 (*exrA uvrB*). Whereas the λ lysogen of strain Bs1 could not be induced by u.v. and was no more sensitive to u.v. than its non-lysogen, the *exrA*$^+$*uvrB* (λ) derivative of strain Bs1 could be induced by u.v. and was more sensitive to u.v. than its non-lysogen.

1. INTRODUCTION

It is known that strains of *Escherichia coli* lysogenic for an ultraviolet radiation (u.v.) inducible temperate phage such as λ are more sensitive to u.v. than their non-lysogenic counterparts (Harm, 1966; Mattern, Van Winden & Rörsch, 1965; Markovich, 1956). A reasonable explanation for the increased u.v. sensitivity of lysogens is that the population that is induced by a given dose of u.v. overlaps but is not coincidental with the population that is otherwise lethally injured. However, not all strains of *E. coli* are more sensitive to u.v. when lysogenized with temperate phage. As will be shown in this report, lysogeny does not increase the sensitivity of *exrA** strains to u.v. irradiation. It occurred to us that the reason that lysogens of *exrA* strains are not more sensitive to u.v. than non-lysogens is that the *exrA* gene suppresses prophage induction.

In this report we will show that λ is not induced by u.v. in *exrA* (λ) strains and that this is because λ repressor is not inactivated by u.v. in *exrA* strains.

* In this paper we shall refer to the *malB* linked *exr* gene (Donch & Greenberg, 1968*a*) as *exrA* to distinguish it from *exr* mutations at sites other than *malB* (Mattern *et al.* 1965) which have not been examined by us.

2. MATERIALS AND METHODS

(i) *Bacterial strains.* Bacterial strains used are shown in Table 1.

(ii) *Phage.* λ^+ was isolated from the lysogenic strain W1895. λ^+.K12 and λ^+.B were grown in strain K12 or B of *E. coli* respectively. It will be understood that λ^+.K was used in all experiments involving K12 strains and λ^+.B in strains involving B strains. We shall refer to the phages as λ. λvir was obtained from Dr A. D. Kaiser.

Table 1. *Relevant markers*

	malB	*metA*	*exrA*	*lon*	*hcr*	Source
B (CSH)	−	+	+	−	+	R. Hill
B251	+	+	+	−	+	W. Arber
B/r	−	+	+	−	+	E. Witkin
Bs1	−	+	−	−	−	R. Hill
Bs2	−	+	−	−	+	R. Hill
Bs12	−	+	+	−	−	R. Hill
AB1899	+	+	+	−	+	P. Howard-Flanders
AB1911	+	−	+	+	+	E. Adelberg
PAM 23	+	+	−	−	+	Transduction of strain Bs2
PAM 9911	+	+	−	−	+	Transduction of strain AB1899
PAM 231	+	+	−	−	+	Transduction of strain B/r
PAM 2301	+	+	−	−	+	Transduction of strain B
PAM 47	+	+	−	+	+	Transduction of strain AB1911
PAM 2512	+	+	+	−	+	Spontaneous str^r mutant of B251
PAM 1261	+	+	+	−	−	Transduction of strain Bs12

Abbreviations are as recommended by Demerec, Adelberg, Clark & Hartman (1966). All transductions were done with P1*vir*.

Table 2. *Spontaneous production of λ phage*

	Titre of supernatant	
Bacterial strain	Exponential	Stationary
B251 (λ)	$6{\cdot}5 \times 10^5$	$1{\cdot}5 \times 10^6$
PAM 23 (λ)	$4{\cdot}1 \times 10^4$	$1{\cdot}1 \times 10^5$

Exponentially growing and overnight stationary cultures were centrifuged and the supernatant treated with chloroform and assayed on B251.

Lysogens of λ were prepared as follows: λ phage were plated on to homologous host strains by the overlay method at a concentration that would give 100–200 p.f.u./ml. Plates were incubated for 18 h. The centre of a turbid plaque was picked with a sterile needle and streaked on to the surface of an agar plate. The colonies which arose after incubation for 18 h were purified twice by single colony isolation. Lysogeny was assumed on the basis of immunity to homologous λ phage, sensitivity to homologous λvir and the production of λ from overnight growth in broth.

P1b*vir*, a virulent mutant of P1*kc* previously described by Donch & Greenberg (1968*a*), was used in all transductions.

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λhcI857 is a λ mutant obtained from Dr D. Mount and described by Brooks & Clark (1967) and Sussman & Jacob (1962). It is inducible at elevated temperatures, forms clear plaques at temperatures above 35 °C and turbid plaques at temperatures below 35 °C.

(iii) *Media.* The media and suspension fluids used have been previously described (Donch & Greenberg, 1968*b*) with the exception that complete medium used in these experiments contains 0·5 % yeast extract and is referred to as JN medium.

All incubations unless otherwise stated were done at 37 °C.

(iv) *Ultraviolet survival curves.* Ultraviolet survival curves were performed as previously described (Donch & Greenberg, 1968*b*). Irradiation of 1·5 ergs/mm²/sec

Table 3. *Effect of u.v. irradiation of PAM 23 (exrA) on the capacity to support the growth of λ phage*

u.v. dose (ergs/mm²)	No. of plaques/0·1 ml
0	271
15	283
45	276
200	201
300	184

PAM 23 was grown as described for u.v. survival curves. *λvir* was added at a M.O.I. of five. Unadsorbed phage were removed with anti-λ serum. PAM 2512 was the indicator.

Table 4. *Superinfection of u.v. irradiated* exrA⁺(λ) *and* exrA(λ) *with* λhcI857

Phage marker examined	Phage yield/infected cell	
	exrA⁺(λ)	*exrA*(λ)
C⁺	19	0
C	24	0

The u.v. dose was 300 ergs/mm² for *exr*⁺*A*(λ) and 30 ergs/mm² for *exrA*(λ). Assays were done on PAM 2512. On PAM 2512 C⁺ refers to plaques produced by wild-type λ prophage. C refers to clear plaques produced by *λhcI857* at 37 °C.

was achieved by use of a voltage regulator coupled with a rheostat and a dial setting to produce this dose. A u.v. dose of less than 1·5 ergs/mm²/sec was obtained by filtering the radiation through Saran Wrap which was found to reduce the dose level by a factor of approximately 10.

(v) *Prophage induction.* Ultraviolet induction of λ lysogens was determined by measurement of induced cells by the streptomycin overlay method. Cells were grown overnight with aeration in JN broth containing 0·1 % glucose and 0·01 M-$MgSO_4$. The following day cells were diluted into fresh broth of the same composition and aerated until the viable count was 2–5 × 10^8/ml. Cells were centrifuged at approximately 5000 rev/min for 15 min. The cells were resuspended to the original volume in PBS and then diluted to 2–5 × 10^6/ml. Samples (1·0 ml) were irradiated with u.v. in 60 mm Petri plates. Irradiated samples were appropriately diluted into

soft agar containing a streptomycin-resistant λ_1-sensitive indicator. The soft agar was poured over the surface of JN agar, allowed to set for 5 min and then incubated for up to 3 h. At this time a second soft agar layer was overlaid containing the same indicator and streptomycin to give a final concentration in the plate of 500 μg/ml after diffusion. The addition of indicator bacteria to the second soft agar layer was needed to obtain well-developed plaques. Plates were then incubated again for an additional 16–18 h.

(vi) *Superinfection experiments.* Lysogenic cells were grown overnight with aeration in JN broth containing 0·01 M-$MgSO_4$. They were centrifuged once at 5000 rev/min, resuspended in warm (37 °C) JN broth containing 0·01 M-$MgSO_4$ and irradiated. *λhcI857* was added at a multiplicity of infection of 1. Adsorption was allowed to proceed at 37 °C for 10 min, at which time the phage-cell complex was centrifuged for 5 min at 5000 rev/min. The supernatant was removed and used for determination of percentage adsorption. The infected cells were resuspended to original volume in warm JN broth, diluted by a factor of 10^6 into warm JN broth containing 0·01 M-$MgSO_4$, distributed in 0·5 ml volumes into 100 small tubes and incubated for 90 min. At this time chloroform was added and the broth assayed for free phage. Alternatively, assays were done on plates containing 200 μg streptomycin/ml.

One-step growth and single-burst experiments were done as described in Adams (1959).

3. RESULTS

(i) *U.v. sensitivity of lysogenic* exrA *strains.* Strain B and its derivatives are naturally *lon, malB* and resistant to λ (Chung & Greenberg, 1968). When made *malB*+ by transduction or conjugation strain B also becomes sensitive to λ. PAM 23 is a *malB*+ λ-sensitive transductant of strain Bs2(*exrA*) in which the *exrA* gene is conserved. The λ lysogen of this strain PAM 23(λ) showed little significant increase in sensitivity to u.v. irradiation over PAM 23 (Fig. 1). These experiments were repeated with *malB*+λ^s derivatives of Bs4, 5 and 7, all *exrA* mutants of strain B (Donch & Greenberg, 1968*a*), and their λ lysogens, with the same results.

The *exrA* gene of strain PAM 23 was cotransduced with *malB*+ (or *metA*+ with K12 strains) into strains of *E. coli* with different genetic backgrounds. PAM 2301 is the *malB*+*exrA* transductant of strain B and was used to determine that it was the *exrA* gene and not some second mutation which accounted for the behaviour of PAM 23. PAM 231 is strain B/r made *malB*+ *exrA* by transduction. PAM 9911 is the *exrA*-containing derivative of the K12*lon* mutant AB1899 (Howard-Flanders, Simson & Theriot, 1964) and PAM 47 is the *exrA* derivative of the K12 strain AB1911 which is *lon*+ and is wild type in its u.v. sensitivity. Figure 2(*a*) and (*b*) shows the u.v. survival curves of the lysogens and non-lysogens of each of these strains. In none of these strains was the lysogen more sensitive to u.v. than the non-lysogen. However, when B251(λ)(*exrA*+*lon*) was tested for u.v. sensitivity, it was found to be more sensitive to u.v. than strain B251 (Fig. 3).

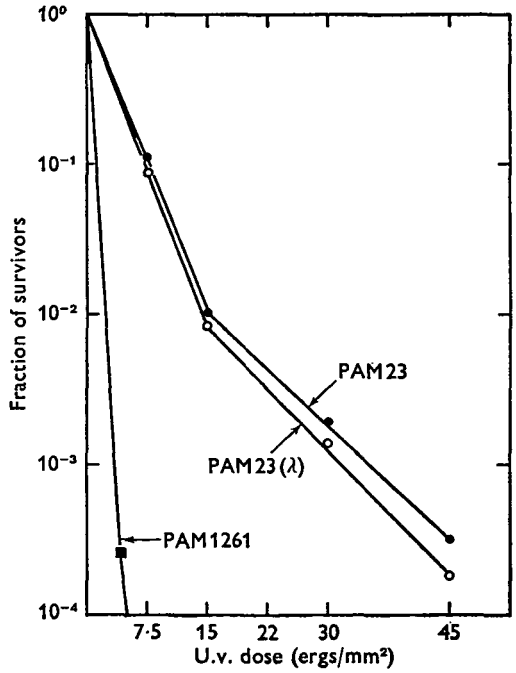

Fig. 1. U.v. survival curves of the *exrA* strain PAM23 and PAM23 (λ), and PAM1261.

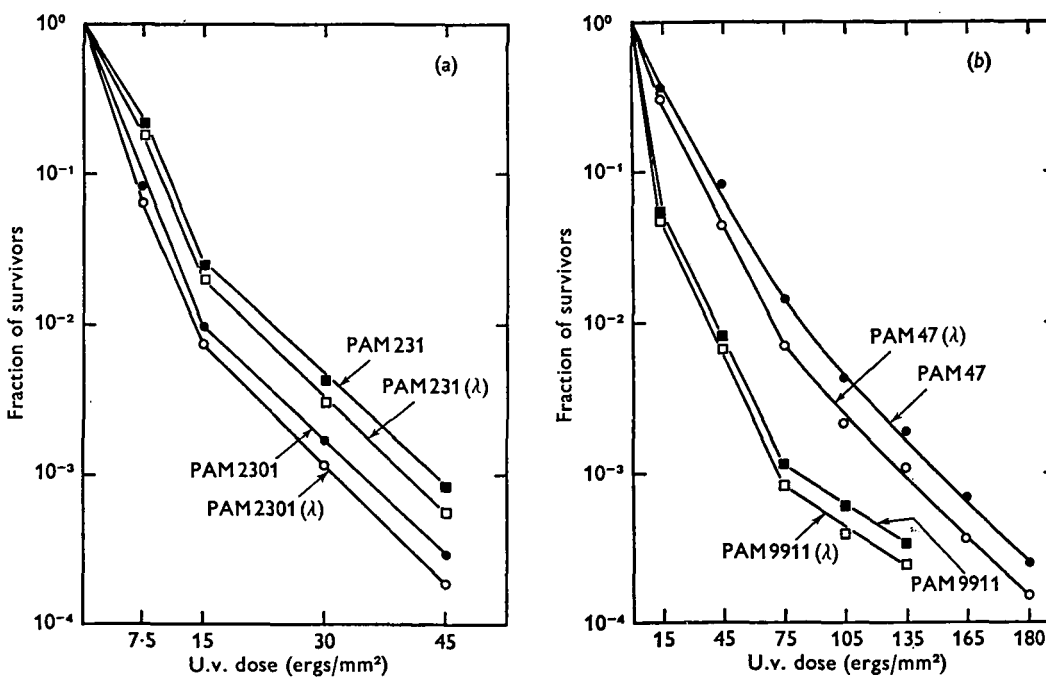

Fig. 2. (*a*) U.v. survival curves of transductants PAM231 and PAM2301 of B/r and B(CSH) respectively. Also shown are λ lysogens of each transductant. (*b*) U.v. survival curves of transductants PAM9911 and PAM47 of AB1899 and AB1911 respectively. Also shown are λ lysogens of each transductant.

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(ii) *Failure to induce prophage in* ExrA *strains.* To explain these observations the possibility was examined that in *exrA* (λ) strains λ was not induced by u.v. Figure 4 shows that strain B251 (λ) was induced to nearly 100%, whereas at equivalent levels of lethality as well as at equal doses of u.v. PAM23 (λ) was not induced.

Further proof of the effect of the *exrA* gene on repression of λ induction is given by strain Bs1 isolated by Hill & Simson (1961). This strain has been shown to contain an *exrA* mutation and also a *uvr* mutation (*uvrB*) linked to *gal* (Mattern,

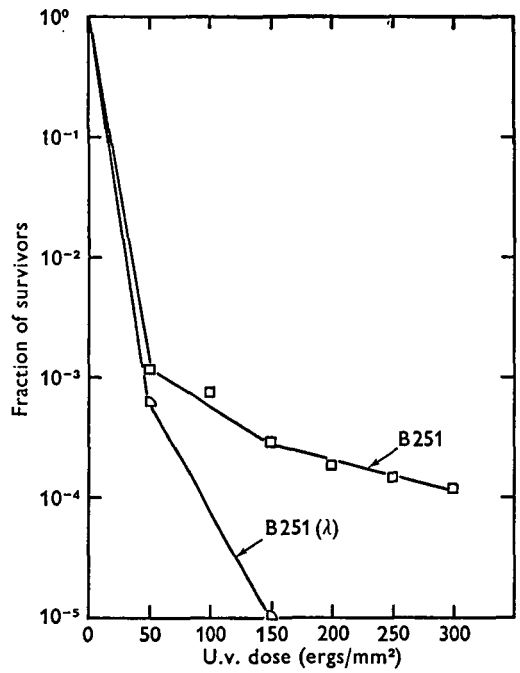

Fig. 3. U.v. survival curves of B251 and B251 (λ).

Zwenk & Rörsch, 1966; Greenberg, 1967). The *exrA* mutation was eliminated by transduction of the wild-type allele from B251, the resulting strain being filamentous following u.v. irradiation (*exrA* mutations repress filamentation (Donch, Green & Greenberg, 1968)). The λ lysogen of this strain was sensitive to u.v. induction (Fig. 4). Furthermore, this strain was induced by and achieved maximum induction at lower doses of u.v. than the more resistant parental strain B251 (λ), while Bs1 (*exrA uvr*) (λ) was not induced. It was then the *exrA* mutation of strain Bs1 (λ) that suppressed u.v. induction of λ.

U.v. survival curves of Bs1 (*exrA uvr*), Bs1 (*exrA*$^+$*uvr*) and their λ lysogens are shown in Fig. 5. Bs1 (*exrA uvr*) and its lysogen are equally sensitive to u.v. while Bs1 (*exrA*$^+$*uvr*) (λ) is markedly more sensitive than Bs1 (*exrA*$^+$*uvr*). The increased sensitivity to u.v. is the consequence of the u.v. induction of λ in the *exrA*$^+$*uvr* (λ) derivative of strain Bs1.

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Spontaneous production of λ in PAM23(λ) was found to be 10% of the value obtained with B251(λ) (Table 2). When the yield of phage per infected bacteria was examined in either one-step growth experiments or single-burst experiments, it was found that PAM23 yielded an average of 57 λ phage per bacteria whereas B251 yielded 130.

Figure 6 shows the results of one-step growth curves of *λvir* in PAM23 and B251 performed as described in Adams (1959). While there was no significant effect on the latent period due to the *exrA* mutation, there was a decrease in the yield of phage particles as a result of the *exrA* gene. Hence, whereas the *exrA* mutation

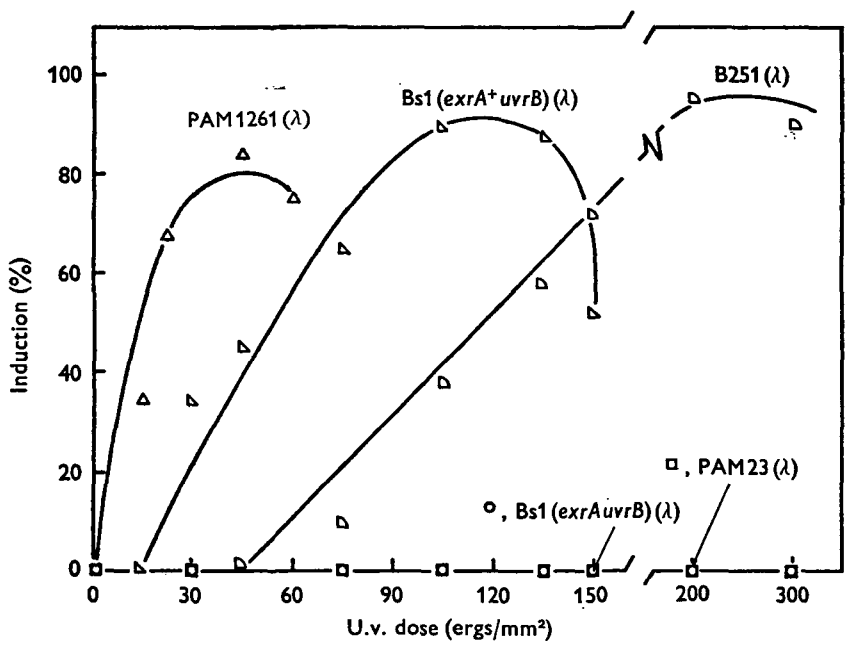

Fig. 4. U.v. induction of PAM1261(λ), Bs1(*exrA uvrB*)(λ), Bs1(*exrA*+*uvrB*)(λ), B251(λ) and PAM23(λ).

completely suppressed u.v. induction of λ it had only a moderate effect on spontaneous induction and burst size of λ.

When λ was plated on PAM23 the number of turbid plaques that were observed was approximately equal to the number that occurred when B251 was used as a host. λ lysogens could be readily isolated from either strain, which would imply no marked disturbance in the recombinational events leading to lysogeny in PAM23.

(iii) *Competence of an irradiated ExrA strain.* It seemed unlikely that the dose of u.v. used destroyed the host-cell reactivation (HCR+) ability of the ExrA strains. Takebe, Ichikawa, Iwo & Kondo (1967) had not only shown that HCR derivatives of K12 strains could be induced by u.v. but that lysogens of HCR strains were more sensitive to u.v. than non-lysogens. Figure 4 shows that PAM1261 (λ), Bs12*malB*+ *uvrA*(λ) could be induced at doses of u.v. as low as those used in attempting to induce PAM23(λ) even though PAM1261 is more sensitive to the lethal effects of

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u.v. than PAM23 (Fig. 1). In view of the fact that an Hcr(λ) strain is induced at very low doses of u.v., it is not likely that PAM23 (λ) was not induced because its Hcr+ properties were destroyed by u.v.

To test the effect of u.v. irradiation of the Hcr+ *exrA* strain on vegetative λ phage development PAM23 was irradiated at the doses of u.v. used to determine the u.v. survival curves (Fig. 1). λ was then added to the irradiated cells. No reduction in plaque forming capacity was observed over the range of doses used and only a moderate reduction was observed at very high doses (Table 3).

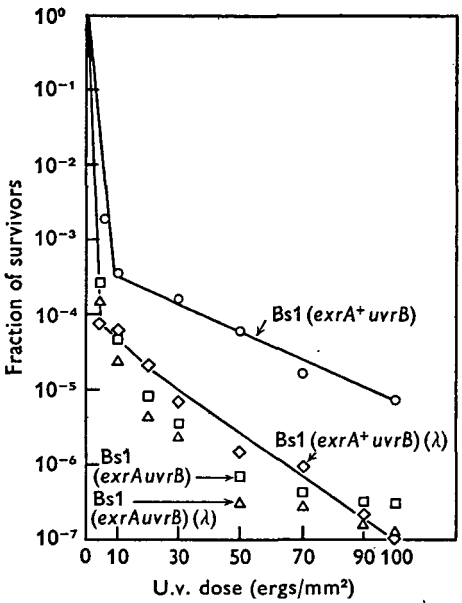

Fig. 5

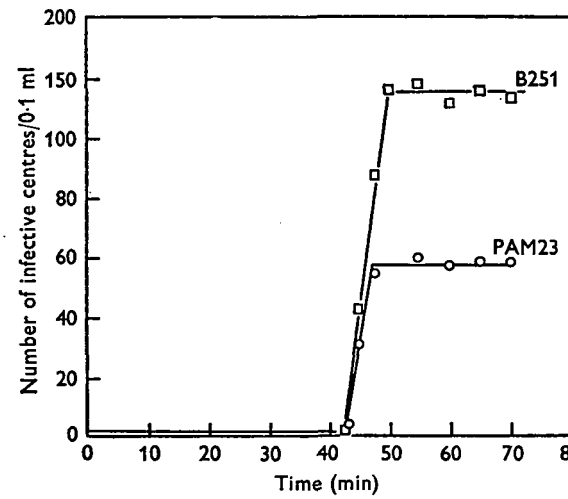

Fig. 6

Fig. 5. U.v. survival curves of Bs1 (*exrA uvrB*), Bs1 (*exrA*+*uvrB*) and λ lysogens of each strain.

Fig. 6. One-step growth curve of λ*vir* in B251 and the *exrA* strain PAM23.

To further test the capacity of an *exrA* strain to support vegetative λ development, PAM23 (λ*hcI857*) was used. λ*hcI857* lysogenizes at 30 °C but can be induced at 42 °C. In three experiments PAM23 (λ*hcI857*) yielded an average of 90 phages/cell at 42 °C, The average yield from *exrA*+ strains lysogenized with this mutant of λ was 117. When PAM23 (λ*hcI857*) was u.v. irradiated and then incubated at 42 °C as many plaques were produced as in an unirradiated sample incubated at 42 °C. This is taken to mean that, when the immunity substance of λ*hcI857* was destroyed by heat, the phage could develop vegetatively in the irradiated *exrA* strain PAM23.

(iv) *Superinfection breakdown.* Since λ*hcI857* is genetically marked for clear plaque production it provided an opportunity to test whether immunity was lifted following u.v. irradiation. When B251 (λ) and PAM23 (λ) were u.v. irradiated then superinfected with λ*hcI857*, B251 (λ) showed a mixture of plaques indicating a

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lifting of immunity, while PAM23(λ) gave no plaques (Table 4). Immunity was not lifted in PAM23(λ), indicating that immunity substance (repressor) was not destroyed.

4. DISCUSSION

The results show that in strains of *E. coli* with an *exrA* mutation, λ is not induced by u.v. In the presence of the *exrA* mutation the λ repressor is not inactivated following u.v. irradiation, but the capacity to support vegetative reproduction of λ is not otherwise significantly effected. Thus, in an *exrA* (*λhcI857*) strain when the heat-labile repressor of *λhcI857* is destroyed at an elevated temperature, λ particles are produced; and the capacity to produce λ particles after heat induction is not materially changed by u.v. irradiation. In these properties the *exrA* gene resembles the *recA* gene (Brooks & Clark, 1967).

exrA and *recA* mutations resemble each other also in suppressing u.v. induction of filaments in *lon* strains (Donch *et al.* 1968; Green, Greenberg & Donch (1969)) and u.v.-induced mutations (Witkin, 1969). This suggests that u.v. induction of prophage, of *lon* filaments and of mutations may be related processes and that *recA*$^+$ and *exrA*$^+$ may have related functions. *exrA* and *recA* strains also resemble each other in undergoing abnormally high spontaneous and u.v.-induced degradation of their DNA (Howard-Flanders & Boyce, 1966; Witkin, 1968). Finally both *exrA* and *recA* show reduced heterologous genetic recombination; *recA* shows about 0·01 % of wild-type proficiency, while *exrA* shows about 25 % of wild-type proficiency. According to Witkin (1968) *recA*$^+$ and *exrA*$^+$ may both be concerned with a type of recovery from u.v. irradiation alternative to excision repair of pyrimidine dimers and called post-replicative repair by Rupp & Howard-Flanders (1968). According to these investigators replication of unexcised pyrimidine dimers leads to gaps in the replicated DNA. These gaps are repaired by a process of recombination between strands such that the strand opposite a dimer is reconstructed.

Other mutations have been isolated which are now designated *exr* or *rec* (Mattern *et al.* 1965; Howard-Flanders & Theriot, 1966). These mutations map separately from *recA* and *exrA* and do not have the same phenotypic properties. Mutations such as *recB* and *recC* actually share fewer properties with *recA* than do *exrA* mutations. *recB* and *recC* do not suppress u.v. induction of lysogenic phage (λ) nor do they eliminate the induction of mutations by u.v. although they do reduce the efficiency of heterologous recombinations. Moreover they degrade DNA less than wild type. We do not know how *recB* and *recC* effect u.v. filamentation in *lon* strains.

exr was a designation given by Rörsch *et al.* (1966) and Mattern *et al.* (1966) to a class of u.v.-sensitive mutants of *E. coli*, originally classified as *dar* (dark repair negative), which were sensitive to X-radiation as well as u.v. This subclass served essentially to exclude mutants which are phenotypically Hcr (*uvr*). Mattern, Van Winden & Rörsch (1965) and Van de Putte, Van Sluis, Van Dillewijn & Rörsch (1965) have described and mapped *exr* mutations other than the one we have designated *exrA* (which is very likely the same as *lex*, described by Howard-

Flanders & Boyce (1966). These *exr* mutations do not suppress u.v. induction of prophage and appear to be able to recombine normally. Their properties with regard to u.v. induction of filamentation and mutations are not known.

As to how *exrA* or *recA* mutations suppress the induction of prophage, Hertman & Luria (1967) suggested a model in which the lack of prophage induction in *recA* strains was related to excessive DNA degradation. However a *recA recB* double mutant was found not to degrade DNA excessively yet lysogens of it failed to be induced by u.v. (Willets, 1968). Thus induction of λ does not appear to be directly related to DNA degradation. Nor does there appear to be any correlation between inducibility and recombination proficiency in *recA*, *recB* and *exrA* strains. Moreover, if *recB* and *recC* strains are defective in post-replicative repair this does not seem to be necessary for induction either.

At present all that can be stated with certainty is that both the $exrA^+$ and $recA^+$ gene functions are required for inactivation of λ repressor and that u.v. induction of prophage appears to be related to induction of mutations, filaments in *lon* strains and possibly to the excessive degradation of DNA.

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