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# Survival, DNA-breakdown and induction of prophage lambda in a *Escherichia coli* K12 *recA uvrB* double mutant

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## 1. INTRODUCTION

Recombination deficient mutants of *Escherichia coli* K12 have been isolated by Clark & Margulies (1965), Howard-Flanders & Theriot (1966), as well as by other workers (Fuerst & Siminovitch, 1965; Rörsch, van de Putte, Nattern & Zwenk, 1966). The site of the mutation in these mutants is at a locus designated *recA*, situated on the genetic map of *E. coli* between *pheA* and *cysC* (Willets, Clark & Low, 1969). Other types of recombination deficient mutants have been reported recently (Emerson & Howard-Flanders, 1967).

The *recA* mutation alters several cell functions. The *recA* strains are extremely sensitive to ultraviolet light, although they can excise pyrimidine dimers; they exhibit breakdown of cellular DNA during normal growth and excessive degradation of cellular DNA after exposure to ultraviolet (Clark, Chamberlin, Boyce & Howard-Flanders, 1966). Also, *recA* lysogens release no phage either spontaneously or after exposure to inducing doses of ultraviolet light (Brooks & Clark, 1967; Hertman & Luria, 1967).

Another type of ultraviolet sensitive mutant of *E. coli* K12 was isolated and characterized (Howard-Flanders, Boyce & Theriot, 1966). These mutations map on three widely spaced genetic loci, designated *uvrA*, *uvrB* and *uvrC*. Mutations in any one of the three *uvr* loci cause the loss of the ability to excise pyrimidine dimers. In excision-defective mutants, less DNA breakdown occurs after exposure to ultraviolet light than in wild-type cells (Boyce & Howard-Flanders, 1964). Exposure to mitomycin C induces no DNA breakdown in these mutants (Boyce & Howard-Flanders, 1964).

A *recA uvrA* double mutant was first constructed by Howard-Flanders & Boyce (1966). The ultraviolet dose for 37 % survival of the double mutant is 0·2 erg/mm$^2$, corresponding approximately to one dimer per bacterial genome (Howard-Flanders, 1968). It was reported that excessive DNA degradation is induced in the double mutant after exposure to ultraviolet light, but not after treatment with mitomycin C (Howard-Flanders & Boyce, 1966).

It was suggested in a preceding publication (Hertman & Luria, 1967) that in ultraviolet-irradiated *recA* lysogens, because of extensive DNA degradation, there is an accumulation of one or more substances, which prevent in some way the inactivation of the immunity repressor. The present experiments, originally undertaken to test this hypothesis by studying mitomycin C induction of prophage $\lambda$ in

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a *recA uvrB* strain, demonstrate that while little DNA breakdown is induced by mitomycin C in the double mutant, induction of prophage λ does not take place in the treated cells.

It will be shown, by comparing the growth characteristics of the double mutant to those of the *recA13* or *uvrB5* single mutants and 'wild type' bacteria, that the *uvrB* function is involved in the promotion of cell survival during normal growth. This function of the *uvrB* gene product can be detected only in the absence of a working genetic recombination machinery (*recA*).

## 2. MATERIALS AND METHODS

*Bacterial strains.* The bacterial strains used and their relevant properties are listed in Table 1. The *recA13uvrB5* double mutant was constructed by the following method: Hfr 12 *recA13 thy*$^-$ *his*$^+$ (Hertman, 1967) was crossed with AB1885 F$^-$ *uvrB5*, and *thy*$^+$ *his*$^-$ and *his*$^+$ *thy*$^+$ recombinants were isolated and purified by repeated restreaking on selective plates. The recombinants were tested for fertility with suitable Hfr strains, for sensitivity to ultraviolet light and for reactivation of ultraviolet-irradiated phage T1 and λcI (Howard-Flanders & Theriot, 1966). A recombinant deficient in recombination, extremely sensitive to ultraviolet and unable to reactivate irradiate phage, was isolated and designated IH5528. Isogenic *recA13* and *uvrB5* single mutants and *rec*$^+$ *uvr*$^+$ 'wild type' strain were derived from the recombinant IH5528 by P1-mediated transduction of the *uvrB*$^+$ and *recA*$^+$ genes. Cotransduction of *gal*$^+$ and *uvrB*$^+$ genes was used for selecting *uvrB*$^+$ transductants. AB1885 was used as the *recA*$^+$ donor in transduction experiments. *E. coli* K12 W4032 was used as the *gal*$^+$ *uvrB*$^+$ donor for the transduction of *uvrB*$^+$. Several lysogenic derivatives of the listed strains and their derivatives resistant to λ*vir* were used in part of the experiments. Thymine-requiring strains were obtained by the method of Stacey & Simson (1965). *Escherichia coli* C600/P1 *pen-r* a P1- and penicillin-resistant (Ogawa & Tomizawa, 1967) derivative of *E. coli* C600 (Appleyard, 1954) was used to assay λ-infective centres. *Shigella dysenteriae Sh str-r* was used to assay phage P1.

*Phages.* P1*kc* (Lennox, 1955); λ reference (Kaiser, 1957); λ*ind*$^-$*c1857* (Sussman & Jacob, 1962); λcI*t*1 (Lieb, 1966); λ*cI26*; λ*vir*, an immunity-insensitive mutant of λ.

*Media.* Tryptone agar and soft agar contained the following ingredients per litre: 10 g Bacto-Tryptone (Difco); 5 g NaCl; 25 mg thymine; 10 g and 6 g Bacto-Agar (Difco), respectively. Tris-glucose-Casamino acids-thymidine (TGAT) medium; 0·08 M-NaCl; 0·02 M-KCl; 0·02 M-$NH_4Cl$; $10^{-3}$ M-$MgCl_2$; $10^{-4}$ M-$CaCl_2$; $2 \times 10^{-6}$ M-$FeCl_3$; $2 \times 10^{-3}$ M-$Na_2SO_4$; 0·12 M-Tris (pH 7·5). 0·4% glucose; 0·25% Casamino acids (Difco); 5 μg $KH_2PO_4$/ml; 0·5 μg thiamine hydrochloride/ml and 2 μg/ml thymidine were added separately. P1-adsorption medium: TGAT with $2{\cdot}5 \times 10^{-3}$ M-$CaCl_2$ added.

*Bacterial counts.* Viable counts were made by spreading 0·2 ml samples of appropriate dilutions on Tryptone agar plates or by plating in Tryptone soft agar.

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*Phage assays and phage stocks.* The methods for growing and assaying phage P1 were described previously (Hertman & Luria, 1967). Assays for infective centres were done by conventional pour plating using Tryptone agar and soft agar with 200 units per ml penicillin G (RAFA lab., Jerusalem) added, using *E. coli* C600/P1 *pen-r* as indicator. Stocks of phage λ were obtained by ultraviolet or heat induction of appropriate lysogens.

*Optical density (OD).* OD of bacterial cultures was measured with Beckman DU spectrophotometer at 615 mμ wavelength. The measurements were made in cuvettes of 1 cm light path.

*Isolation of λ-lysogens.* Phage λ at a concentration of $10^8$ plaque-forming units (pfu) per ml was spotted on soft agar layer seeded with bacteria to be lysogenized. The plates were incubated overnight at the desired temperature. Bacteria from the lytic spots were isolated, purified by repeated restreaking and tested for phage production and immunity to phage λcI.

*Transduction of the* recA$^+$ *gene* was performed according to the procedure described previously (Hertman & Luria, 1967).

*Mitomycin C treatment.* Mitomycin C (MC) (Calbiochem, Los Angeles, U.S.A.) at the desired concentration was added to young cultures ($OD_{615}$ = 0·3 units) kept at 37 °C. After 30 min incubation with shaking, the cells were collected on membrane filters (pore size 0·45 μ; Gelman Instrument Company, Ann Arbor, Michigan, U.S.A.), washed with several volumes of prewarmed TGAT medium and resuspended to the original volume in the same medium.

*Ultraviolet irradiation.* Young cultures in TGAT medium at $OD_{615}$ = 0·3 units were irradiated in thin layers on a rotating platform at 2·2 m distance from a 15W Westinghouse Sterilamp G15T8. A dose of 1 sec corresponded to about 1 erg/mm$^2$. All manipulations following the irradiation were done in dim light.

*Measurement of DNA degradation.* DNA degradation was tested by the release of labelled thymidine in acid-soluble form from DNA. Overnight cultures were diluted 30-fold in prewarmed TGAT medium with 5 μc [$^3$H]methyl thymidine (specific activity 25·2 c/mM; The Radiochemical Centre, Amersham, England) added. The cultures were grown for 3–4 generations at 37 °C with aeration. To remove the label, the cells were then collected on a membrane filter, washed with prewarmed TGAT medium and resuspended in the medium to the original volume. The washed cells were irradiated with ultraviolet light and incubated at 37 °C. Unirradiated controls were run simultaneously. When MC-induced degradation was measured, the labelled cells were first treated with MC and then collected on the membrane filter to remove the label and the drug. Duplicate samples (0·4 ml) were withdrawn between 0 and 100 min incubation and added to 0·2 ml of 20% TCA (trichloroacetic acid) kept in an ice bath; 0·02 ml of a 0·2% solution of calf thymus DNA was added to increase the bulk of the precipitate. The samples were centrifuged (10000 rev/min, 15 min) and the pellet was resuspended in 0·5 ml of cold 5% TCA and centrifuged. Both supernatants were pooled and extracted with 4 volumes of ether and the residual ether was evaporated. The pellet was washed twice with ethyl alcohol, dried, resuspended in 0·2 ml of trifluoroacetic acid and

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Table 1. *Strains of* Escherichia coli K12 *used*

| Strain no. | Designation in this work | Sex | Genetic loci relevant to this work* | | | Derivation | Reference |
|---|---|---|---|---|---|---|---|
| | | | *rec* | *uvr* | *thy*† | | |
| AB1885 | | F⁻ | + | *uvrB5* | + | NG mutation from AB1157 | Howard-Flanders *et al.* 1966. |
| Hfr no. 12 | Hfr*recA* | Hfr | *recA13* | + | *thy*⁻ | Recombinant from cross P4X6 × AB 2463*his*⁺ by P1 transduction | Hertman, 1966 |
| IH5528 | *E. coli recA uvrB* | F⁻ | *recA13* | *uvrB5* | *thy*⁻ | Recombinant from cross Hfr no. 12 × AB1885 | This work |
| IH5529 | *E. coli uvrB* | F⁻ | + | *uvrB5* | *thy*⁻ | *recA*⁺ by P1 transduction to IH5528 | This work |
| IH5530 | *E. coli recA* | F⁻ | *recA13* | + | *thy*⁻ | *uvr*⁺ by P1 transduction to IH5528 | This work |
| IH5531 | *E. coli w.t.* | F⁻ | + | + | *thy*⁻ | *recA*⁺*uvrB*⁺ by P1 transduction to IH5528 | This work |

* Abbreviations: *rec*, recombination deficient; *uvr*, excision defective; *thy*, thymine biosynthesis; *his*, histidine bisynthesis; NG, *N*-methyl-*N'*-nitro-nitrosoguanidine.

† Thymine requirement in all strains: 20 μg/ml thymine or 2 μg/ml thymidine.

hydrolysed in sealed tubes (140 °C, 30 min). The acid was then evaporated and the residue dissolved in 1 ml water. The cold acid-soluble and the hydrolysed cold acid-insoluble fraction, each in 1 ml water, were introduced into separate scintillation vials, and 10 ml dioxane-based scintillation mixture was added. The radioactivity was determined in a Packard Tri-Carb liquid scintillation counter.

## 3. RESULTS

*Growth characteristics.* Under normal conditions of culture, *recA* mutants grow more slowly than their *recA*$^+$ counterparts. The ratio of optical density (OD) to the number of colony formers is greater in *recA* cultures than in cultures of their *recA*$^+$ ancestors (Wing, Levine & Smith, 1968). Wing *et al.* (1968) have also shown that this increase in the ratio is due, at least in part, to cells which grow in size, but cannot divide and form colonies.

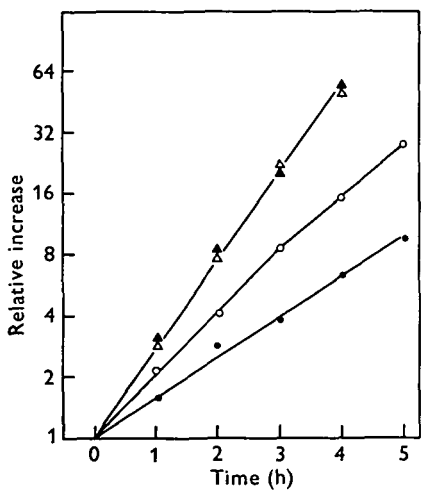


Fig. 1

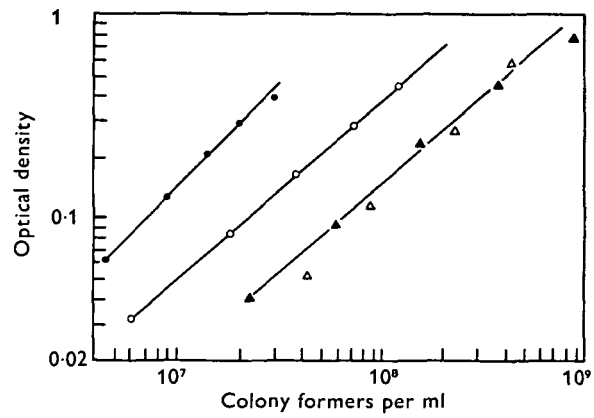


Fig. 2

Fig. 1. Relative increase in the number of colony formers in growing cultures. Overnight cultures were diluted 30-fold in fresh prewarmed medium and incubated at 37 °C. Viable counts were started after 1 h. ●—●, *E. coli recA uvrB*; ○—○, *E coli recA*; △—△, *E. coli uvrB*; ▲—▲, *E. coli w.t.*

Fig. 2. The ratio of optical density to the number of colony formers in growing cultures. Overnight cultures were diluted 30-fold in fresh prewarmed medium. Viable counts and optical density readings were started after 1 h. ●—●, *E. coli recA uvrB*; ○—○, *E. coli recA*; △—△, *E. coli uvrB*; ▲—▲, *E. coli w.t.*

The *E. coli recA uvrB* double mutant grows even more slowly than the *recA* single mutant. The relative increase in viable count in growing cultures of the double mutant, its single mutant counterparts and the 'wild type' bacteria are shown in Fig. 1. While, *uvrB* and *w.t.* bacteria grow at the same rate with a doubling time of about 40 min, the length of the doubling time in *recA* and *recA uvrB* cultures is 60 min and 90 min respectively. This decrease in the rate of growth is



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paralleled by an increase in the ratio of OD to the number of colony formers (Fig. 2). Thus, growing cultures at 0·3 $OD_{615}$ units contain $2 \times 10^8$ *w.t.* or *uvrB*, $7 \times 10^7$ *recA* and $2 \times 10^7$ *recA uvrB* colony formers per ml (Fig. 2).

Microscopic examinations revealed that *recA* and *recA uvrB* cultures contain a large fraction of cells which are up to 5 times longer than cells in comparable *w.t.* or *uvrB* cultures.

The next experiments were aimed at detecting in *recA uvrB* cultures cells unable to form colonies, but still carrying an undamaged heat-inducible prophage. The strains to be tested were lysogenized with λc*I857*, a double mutant of phage λ

Table 2. *Comparison of the number of colony formers to the number of heat-induced infective centres in growing cultures of* E. coli recA uvrB, E. coli recA, E. coli uvrB *and* E. coli w.t. *lysogenic for* λcI857

| Experiment no. | Strain genotype | No. of colony* formers per ml at 32 °C | No. of infective* centres per ml (15 min 42 °C) | Inf. centres per colony formers |
|---|---|---|---|---|
| 1 | *recA uvrB* | $2{\cdot}1 \times 10^7$ | $5{\cdot}7 \times 10^7$ | 2·7 |
| | *recA uvr*$^+$ | $3{\cdot}6 \times 10^7$ | $3{\cdot}3 \times 10^7$ | 0·9 |
| | *rec*$^+$*uvrB* | $5{\cdot}5 \times 10^7$ | $5{\cdot}8 \times 10^7$ | 1·0 |
| | *rec*$^+$*uvr*$^+$ | $3{\cdot}7 \times 10^7$ | $3{\cdot}4 \times 10^7$ | 0·9 |
| 2 | *recA uvrB* | $4{\cdot}4 \times 10^7$ | $8{\cdot}0 \times 10^7$ | 1·8 |
| | *recA uvr*$^+$ | $4{\cdot}3 \times 10^7$ | $3{\cdot}8 \times 10^7$ | 0·88 |
| | *rec*$^+$*uvrB* | $5{\cdot}5 \times 10^7$ | $5{\cdot}9 \times 10^7$ | 1·0 |
| | *rec*$^+$*uvr*$^+$ | $5{\cdot}2 \times 10^7$ | $4{\cdot}9 \times 10^7$ | 0·9 |
| 3 | *recA uvrB* | $4{\cdot}1 \times 10^7$ | $7{\cdot}6 \times 10^7$ | 1·8 |
| | *recA uvr*$^+$ | $4{\cdot}5 \times 10^7$ | $4{\cdot}0 \times 10^7$ | 0·88 |

* Cultures growing at 32 °C were diluted into complete medium kept at 42 °C and plated after 15 min for infective centres. The number of infective centres in unheated cultures was determined. Heating for 15 min at 42 °C was found to be the optimum for heat induction.

which produces a repressor insensitive to ultraviolet light, but sensitive to temperature above 39 °C (Sussman & Jacob, 1962). It was shown previously (Brooks & Clark, 1967; Hertman & Luria, 1967) that *recA* bacteria lysogenic for this phage can be induced by heat. Young cultures grown at 32 °C were shifted to 42 °C for 15 min and the number of infective centres induced by heat was determined. The number of cells which produce infective centres was compared to the number of colony formers in unheated controls (Table 2). In cultures of *w.t.*, *uvrB* and *recA* bacteria lysogenic for λc*I857*, the number of infective centres never exceeded the number of colony formers. Cultures of *E. coli* (λc*I857*) *recA uvrB* contained, however, 1·8–2·7 times as many cells capable of producing phage as capable of forming colonies (Table 2).

Thus, in growing *recA uvrB* cultures damaged cells are generated which cannot form colonies but still carry an active prophage. Such cells are found at 32 °C in cultures of the double mutant only and appear to carry lesions repairable by both the excision and the recombination mechanism.

*Spontaneous degradation of cellular DNA.* Spontaneous breakdown of DNA was

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observed in cultures of *recA* mutants (Clark *et al.* 1965). Since it was found that at the same OD cultures of the double mutant contain less colony formers than cultures of the *recA* single mutant (Fig. 2), it was of interest to compare spontaneous DNA degradation in cultures of the single and double mutants. Bacteria were grown in medium containing radioactive thymidine for at least three generations, filtered and resuspended in non-radioactive medium. The cells were then incubated at 37 °C and cold TCA (5 %) soluble and insoluble radioactivity was determined at intervals (Fig. 3). About 50 % of the total label incorporated was found

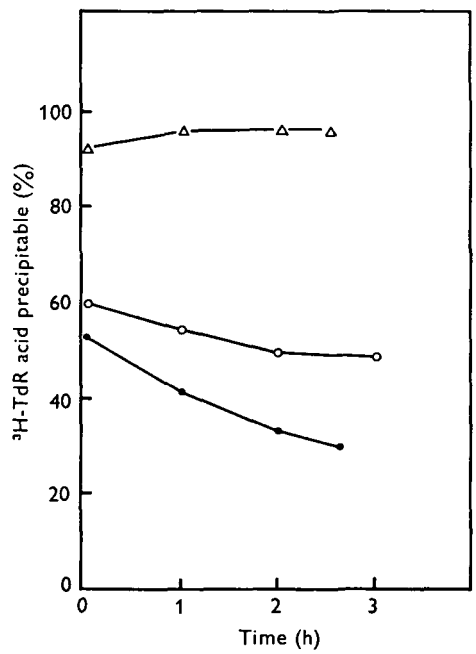


Fig. 3. Release of radioactivity from the acid-precipitable fraction of bacteria during normal growth. The bacteria were labelled by growth in medium supplemented with [³H]thymidine, harvested on a membrane filter, washed and incubated in non-radioactive medium. The percentage of total radioactivity of cells and suspending medium that is precipitable in cold trichloroacetic acid is plotted as a function of time of incubation at 37 °C. ●—●, *E. coli recA uvrB*; ○—○, *E. coli recA*; △—△, *E. coli uvrB*.

in the acid-soluble fraction in both *recA* and *recA uvrB* cells, compared to 10 % found acid-soluble in the *uvrB* mutant; 15–20 % of the initial acid-precipitable label was solubilized in *recA* cultures after 2 h at 37 °C, compared to 20–40 % solubilized during the same incubation interval in cultures of the double mutant.

Thus, the spontaneous DNA breakdown occurring in *recA* mutants during growth appears to be intensified in bacteria carrying both the *recA* and *uvrB* mutations.

*Sensitivity to ultraviolet light and mitomycin C.* Howard-Flanders & Boyce (1966) reported that the *recA uvrA* double mutant is many times more sensitive to ultraviolet light than strains carrying either mutation alone. Extreme sensitivity to



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MC was indicated by the same authors, although quantitative data were not presented.

Like the *rceA uvrA* double mutant, *E. coli recA uvrB* bacteria are also extremely sensitive to ultraviolet light (Fig. 4A). This double mutant is 10 times more u.v.-sensitive than the *recA* and 40 times more u.v.-sensitive than the *uvrB* single mutants. Mitomycin C sensitivity is enhanced, but to a less extent. The double mutant is twice more MC-sensitive than the *recA* and 4 times more MC-sensitive than the *uvrB* single mutants (Fig. 4B).

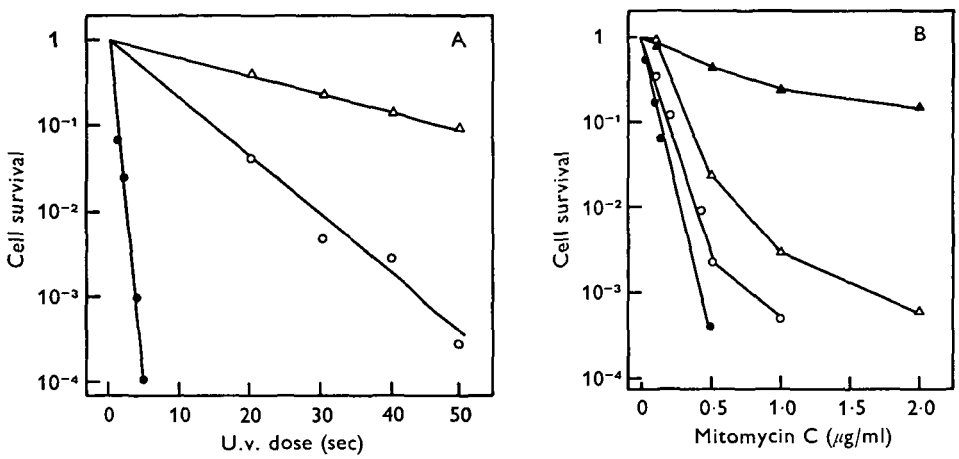


Fig. 4. Effect of various doses of ultraviolet light and mitomycin C on survival. Young cultures at $OD_{615}$ = 0·3 units were exposed to various doses of ultraviolet light or treated with various concentrations of mitomycin C. A, Cell survival as a function of ultraviolet dose. B, Cell survival as a function of mitomycin C concentration. ●—●, *E. coli recA uvrB*; ○—○, *E. coli recA*; △—△, *E. coli uvrB*; ▲—▲, *E. coli w.t.*

*Degradation of cellular DNA after exposure to mitomycin C or ultraviolet light.* Experimental evidence so far indicates (Howard-Flanders *et al.* 1966) that the *uvrA*, *uvrB* and *uvrC* genes are required simultaneously in excision of pyrimidine dimers and some other DNA defects (Boyce & Howard-Flanders, 1964). Little ultraviolet or mitomycin C induced breakdown of DNA occurs in these strains (Boyce & Howard-Flanders, 1964). It was recently reported (Howard-Flanders & Boyce, 1966) that MC induced DNA-breakdown does not occur in *recA uvrA* double mutants; exposure to ultraviolet light, however, induces excessive DNA degradation in such mutants.

DNA degradation after exposure of the *recA uvrB* double mutant to mitomycin C or ultraviolet light was next investigated. Bacteria were prelabelled with tritiated thymidine for at least three generations and then treated with 2 μg mitomycin C/ml for 30 min at 37 °C. The treated cells were collected on a membrane filter, washed and resuspended in prewarmed TGAT medium. Acid-solubilization of the label was followed in treated cultures incubated at 37 °C. Controls treated the same way, except for the exposure to mitomycin C, were run simultaneously.

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Results of such experiments are shown in Fig. 5. The mitomycin C-treated *recA uvrB* cells solubilized in 2 h 44 % of the initial acid-precipitable label, compared to 40 % solubilized in the untreated controls. *E. coli recA* single mutants treated with mitomycin C lost in 2 h, to the acid soluble fraction, 70 % of the initial acid-precipitable label; the untreated controls lost after 2 h to the acid-soluble fraction 20 % of the label only. No differences were observed between λ-sensitive strains and their lysogenic derivatives. No measurable DNA-breakdown was observed in mitomycin C-treated cultures of *E. coli uvrB* (Fig. 5).

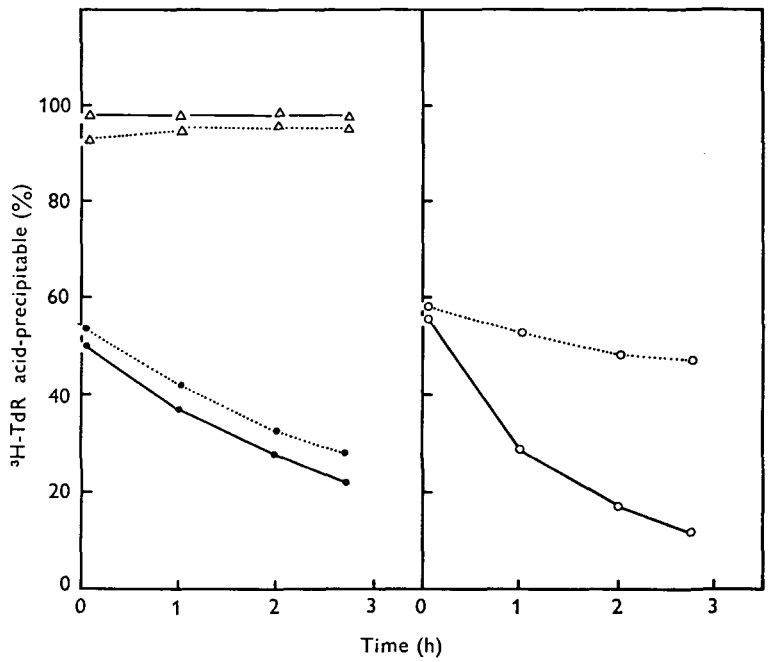


Fig. 5. Release of radioactivity from the acid-insoluble fraction of mitomycin C-treated bacteria. The cells were labelled by growth in [$^3$H]thymidine supplemented medium, treated with 2 μg mitomycin C per ml, harvested, washed and incubated in non-radioactive medium (37 °C). The fraction of total radioactivity of the cells and suspending medium that is precipitable in cold trichloroacetic acid is plotted as a function of the time of incubation at 37 °C. Untreated controls were run simultaneously. ●—●, *E. coli recA uvrB*; ○—○, *E. coli recA*; △—△, *E. coli uvrB*; dotted lines, untreated controls.

Ultraviolet light induced degradation of cellular DNA occurs in the *recA uvrB* double mutant (Table 3). In bacteria prelabelled with tritiated thymidine, 60 % of the label was acid-solubilized 2 h after exposure to doses from 1–20 min (60–1200 erg/mm$^2$).

Thus little additional DNA degradation is induced by exposure of *recA uvrB* double mutants to mitomycin C, whereas exposure to ultraviolet light causes extensive degradation of cellular DNA.

*Induction of prophage* λ. Lysogens carrying the *recA* mutation are affected in their capability to produce phage either spontaneously or after exposure to ultra-

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violet light and other inducing agents. In u.v.-irradiated *recA* lysogens the prophage and the superinfecting phage are subject to repression (Brooks & Clark, 1967; Hertman & Luria, 1967). It was suggested that the persistence of immunity is connected in some way with the extensive DNA degradation occurring in u.v.-irradiated *recA* lysogens (Hertman & Luria, 1967). It was of interest, therefore, to investigate prophage induction in *recA uvrB* lysogens treated with mitomycin C.

The spontaneous release of phage in growing cultures of *E. coli recA uvrB* ($\lambda$)/$\lambda$ was first measured. Young cultures at about $10^8$ bacteria per ml. contained $4 \times 10^2$–$2 \times 10^3$ plaque forming units (p.f.u.) per ml. About 1 % of the plaques were

Table 3. *Ultraviolet light induced degradation of DNA in* E. coli recA uvrB

| Ultraviolet dose (min) | Percentage* of DNA acid-solubilized after 2 h at 37 °C† |
|---|---|
| 0 | 38 |
| 1 | 67 |
| 2 | 67 |
| 4 | 67 |
| 5 | 67 |
| 10 | 59 |
| 20 | 59 |

* The percentage of radioactivity acid-solubilized after 2 h at 37 °C is divided by the percentage of the total radioactive material which is acid-insoluble at zero time.

† The cells were labelled by growth in the presence of [$^3$H]thymidine, washed free of extracellular radioactive material, irradiated and incubated in complete medium at 37 °C. The radioactivity in both the acid-soluble and the acid-insoluble fractions was determined.

clear. Young cultures of *E. coli recA* ($\lambda$)/$\lambda$ at about $10^8$ cells/ml contained $1 \times 10^1$–$1 \times 10^2$ p.f.u./ml. The concentration of p.f.u. in similar *E. coli uvrB* ($\lambda$)/$\lambda$ cultures was $10^6$/ml Exposure of the *recA uvrB* lysogen to mitomycin C at concentrations of 0·5–10 $\mu$g/ml did not result in phage production. Similar results were obtained with *recA uvrB* lysogens exposed to various doses of ultraviolet light (1–300 erg/mm$^2$).

Thus, while exposure to mitomycin C does not induce DNA-breakdown in *recA uvrB* lysogens, prophage induction is prevented in the treated cells.

*Effect of introducing the recA$^+$ gene into mitomycin C-treated* E. coli recA uvrB ($\lambda$). The next experiments were aimed at answering the following question: Is prophage induction in mitomycin C-treated *recA uvrB* lysogens prevented specifically because of the absence of the *recA* function or because of general cell damage occurring in these sensitive cells? The experiments were done in two ways: (*a*) by studying the transduction of the *recA*$^+$ gene to mitomycin C treated cells, and (*b*) by studying heat induction of a thermosensitive prophage in mitomycin-treated cells.

*E. coli recA uvrB* ($\lambda$) and *E. coli recA* ($\lambda$) were treated with various concentrations of mitomycin C (0·05–10 $\mu$g/ml) for 30 min at 37 °C, filtered, washed and resuspended in MC-free medium. The treated cells were then infected with phage P1

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grown on a *recA*$^+$*uvrB* strain (P1.*recA*$^+$). Allowing 20 min for adsorption, the infected cells were plated for count of λ-infective centres. The production of phage by the *recA*$^+$ transductants as a function of the MC concentration is shown in Fig. 6. The optimum concentration of mitomycin C for both strains was 1–2 μg/ml. The transduction frequency for such cells was $10^{-4}$ transductants, producing λ, per P1 adsorbed. The above concentration of mitomycin C is also the optimum

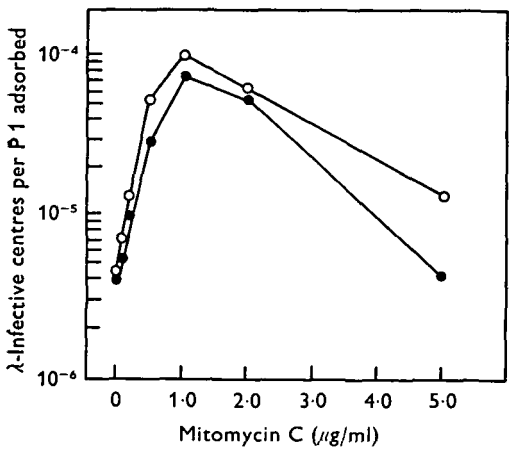


Fig. 6

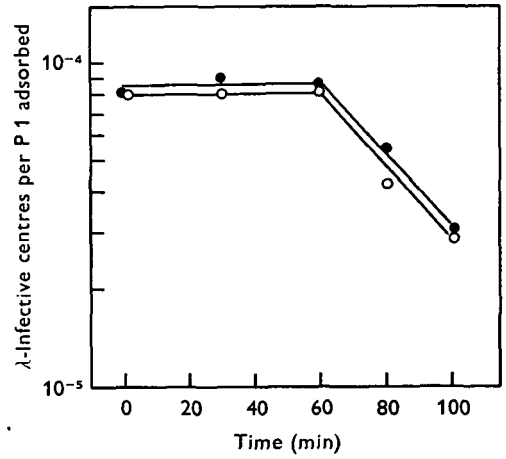


Fig. 7

Fig. 6. Effect of various concentrations of mitomycin C on production of γ-infective centres by *E. coli recA uvrB* (λ) and *E. coli recA* (λ) pretreated with mitomycin C and infected with P1.*recA*$^+$. Young cultures ($OD_{615}$ = 0·3 units) were treated with mitomycin C, harvested, washed, resuspended in mitomycin C-free medium and infected with P1.*recA*$^+$; λ-productivity was determined by count of infective centres. ●—●, *E. coli recA uvrB* infected with P1.*recA*$^+$ at m.o.i. 5·2; ○—○, *E. coli recA* infected with P1.*recA*$^+$ at m.o.i. 2·0.

Fig. 7. Effect of the *recA*$^+$ gene when introduced into *E. coli recA uvrB*(λ) and *E. coli recA* (λ) after treatment with mitomycin C. Young cultures were treated with 2 μg mitomycin C per ml., washed, incubated in mitomycin C-free medium at 37 °C. Sample withdrawn at intervals were infected with P1.*recA*$^+$ and production of λ-infective centres was determined. ●—●, *E. coli recA uvrB*(λ) infected with P1.*recA*$^+$ at m.o.i. 3; ○—○, *E. coli recA* (λ) infected with P1.*recA*$^+$ at m.o.i. 2.

concentration for prophage induction in the *recA*$^+$ strains. The next step was to study the effect of introducing the *recA*$^+$ gene into *recA uvrB* and *recA* lysogens pretreated with mitomycin C and incubated at 37 °C. Young mitomycin C (2 μg/ml) treated cultures were incubated at 37 °C in mitomycin C-free TGAT medium, samples were withdrawn after various incubation intervals, infected with P1.*recA*$^+$ and after 20 min plated for λ-infective centres. The results of such an experiment are shown in Fig. 7. The number of bacteria inducible to phage production by the incoming *recA*$^+$ gene decreased slowly: all transductants produced phage after 1 h of incubation and 30–40 % of the transductants produced phage after 100 min at 37 °C. Very similar results were obtained with *recA uvrB* and *recA* lysogens.

The decay of a thermosensitive prophage in MC-treated cells during post-treat-

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ment incubation was next studied. *E. coli recA uvrB* and *E. coli recA* bacteria were lysogenized with λc*Itl*, a mutant of phage λ which produces a repressor sensitive to heat (45 °C) and ultraviolet light or mitomycin C (Lieb, 1966). Young cultures of such lysogens were treated with 2 μg of mitomycin C per ml (30 min, 37 °C). The treated bacteria were incubated in mitomycin C-free TGAT medium at 37 °C. Portions of the cultures were taken between 0 and 100 min of incubation, diluted tenfold into medium kept at 45 °C and after 15 min plated for λ-infective centres. The total number of colony formers at 32 °C and the number of infective centres

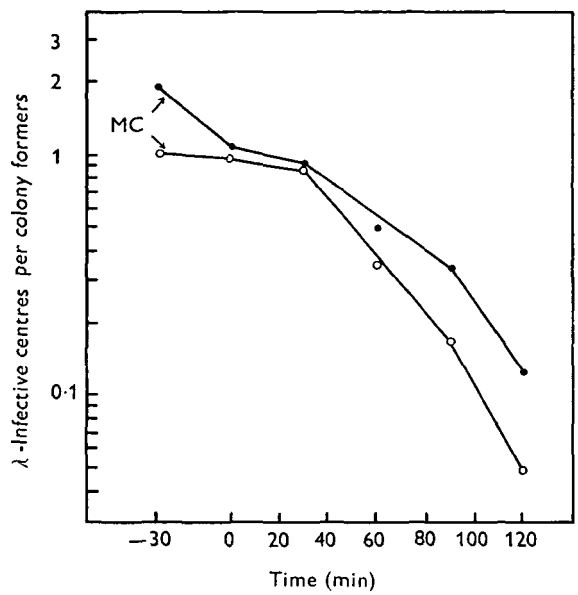


Fig. 8. Heat induction of *E. coli recA uvrB* (λc*It*1) and *E. coli recA* (λc*It*1) pretreated with mitomycin C and incubated at 37 °C. Young cultures were treated with 2 μg mitomycin C per ml, harvested, washed and incubated at 37 °C in mitomycin C-free medium. Samples withdrawn at intervals were diluted 100-fold into medium kept at 45 °C and after 15 min plated for λ-infective centres. The number of colony formers (37 °C) and the number of heat-induced infective centres before mitomycin C treatment were determined. The ratio of heat-induced infective centres to the number of colony formers in the untreated culture is plotted as a function of post-treatment incubation time. ●—●, *E. coli recA uvrB* (λc*It*1); ○—○, *E. coli recA* (λc*It*1).

produced after heating untreated cultures was also determined. The ratio of heat-induced infective centres to the number of colony formers in the untreated culture (32 °C) as a function of post-treatment incubation time is shown in Fig. 8. For *E. coli recA uvrB* (λc*It*1), this ratio was 2 in untreated cultures, it decreased to 1·1 immediately after exposure, and decreased to 0·3 after 90 min of post-treatment incubation. *E. coli recA* (λc*It*1) behaved in a similar way, except that the number of infective centres in untreated cultures was equal to the number of colony formers.

These findings eliminate the possibility that the failure of mitomycin C-treated *recA uvrB* (λ) bacteria to produce phage is due to general cell damage. They suggest, rather, that they fail to produce phage because of persisting immunity. They

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also show that in these bacteria mitomycin-C treatment creates conditions which render the λ prophage inducible by a later arrival of the *recA*$^+$ gene. These conditions persisted for at least 90 min at 37 °C.

*Effect of introducing the* recA *gene into* recA uvrB *cells pretreated with ultraviolet light.* Prophage induction in *E. coli* K 12 (λ) by exposure to ultraviolet light occurs at a dose smaller by a factor of about 10 in a *uvr* mutant than in *uvr*$^+$ strains

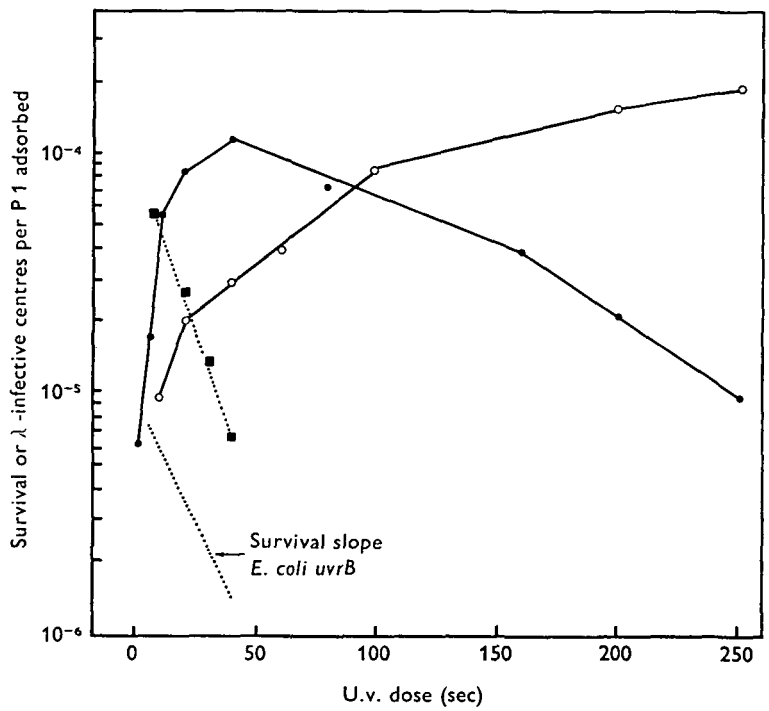


Fig. 9. Effect of various doses of ultraviolet light on survival of *E. coli recA uvrB*λ and on production of λ-infective centres by *E. coli recA uvrB* (λ) and *E.c oli recA* (λ) irradiated and then infected with P 1.*recA*$^+$. Young cultures at $OD_{615}$ = 0·3 units were irradiated and infected with P 1.*recA*$^+$. Bacterial survival was determined by colony count, λ-productivity by count of λ infective centres. ■—■, *E. coli recA uvrB*λ$^s$ infected with P 1.*recA*$^+$ at m.o.i. 4; ●—●, *E. coli recA uvrB* (λ) infected with P 1.*recA*$^+$ at m.o.i. 5; ○—○, *E. coli recA* (λ) infected with P 1.*recA*$^+$ at m.o.i. 3.

(Howard-Flanders & Boyce, 1966; Rörsch *et al.* 1966). It was of interest to test this effect of the *uvr* mutation on induction in the *recA uvrB* lysogens, exposed to ultraviolet and subsequently transduced to *recA*$^+$. The results of such an experiment are shown in Fig. 9. The optimum dose for *E. coli recA uvrB* (λ) was 40 sec, compared to 240 sec for *E. coli recA* (λ). These were also the optimum doses for induction of λ in the respective *recA*$^+$ counterparts of these strains.

The effect of various doses of ultraviolet on the survival of *E. coli recA uvrB* λ$^s$ first irradiated and then transduced to *recA*$^+$ is shown in Fig. 9. The slope of the survival curve of the transductants is very similar to the slope of the survival curve

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of *E. coli uvrB*. This implies that the newly introduced *recA* function is active in repairing some ultraviolet lesions otherwise lethal in the double mutant.

## 4. DISCUSSION

The recombination-deficient and excision-defective (*recA13uvrB*) double mutant, studied in the present report, was constructed by crossing Hfr *recA13* with F⁻ *uvrB5*. Isogenic single mutant and 'wild type' derivatives of the double mutant strain were obtained by P1-mediated transduction of the relevant characters.

As expected, the double mutant is extremely sensitive to ultraviolet light. As with the *recA uvrA6* double mutant constructed by Howard-Flanders & Boyce (1966), the sensitivity of the *recA13uvrB5* double mutant to ultraviolet light is enhanced by a factor of 40, compared to the *uvrB5* single mutant; and by a factor of 10, compared to the *recA13* single mutant. The enhancement in the sensitivity to mitomycin C is not as dramatic. The double mutant is 4 times more mitomycin C-sensitive than the *recA13* single mutant.

More interesting is the combined effect of the *recA13* and *uvrB5* mutations on growth and cell survival under normal conditions of culture. This effect is demonstrated in *recA13uvrB5* cultures by the longer doubling time and greater ratio of optical density to the number of colony formers, as compared to cultures of the *recA13* and *uvrB* single mutants. Moreover, evidence is presented for the existence of a substantial fraction of cells in *E. coli recA uvrB* (λc*I857*) cultures, which are unable to form colonies at 32 °C, but produce phage at 42 °C. No such cells can be detected in cultures of the single mutants or 'wild type' bacteria.

The following interpretation is suggested for these findings. The genetic survival of multiplying bacteria depends to some extent on properly functioning repair mechanisms; in *rec*⁺ bacteria the *uvrB* function is not essential; in the absence of a working *recA* function, however, the *uvrB* function contributes to cell survival; the fraction of cells which cannot form colonies, but produces phage after heating, carries lesions repairable by each one of the two repair mechanism and is, therefore, found exclusively in cultures of the *recA uvrB* double mutant.

The existence of a genetic survival mechanism which depends on genetic recombination and operates in ultraviolet-irradiated bacteria in the absence of pyrimidine dimer excision was suggested by Howard-Flanders (1968). This interpretation was offered to explain sensitivity to ultraviolet light of the *recA13uvrA6* double mutant (Howard-Flanders & Boyce, 1966). This assumption is supported by our present finding that the *recA*⁺ gene, introduced after exposure to ultraviolet light, protects *recA uvrB* bacteria as efficiently as the chromosomal *recA*⁺ gene. This finding suggests an active function for the *recA*⁺ product in repairing ultraviolet-light-induced lesions, otherwise lethal in excision defective mutants.

Exposure of *E. coli recA uvrB* (λ) to ultraviolet light or mitomycin C does not induce phage production. Like the exposure to ultraviolet light (Hertman & Luria, 1967), mitomycin C treatment creates conditions which render the λ prophage inducible by a later arrival of the *recA*⁺ gene. According to a repression control model

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suggested in a preceding publication (Hertman & Luria, 1967), exposure to inducing agents causes the production of a substance $X$, possibly a derivative of adenine (Goldthwait & Jacob, 1964), which inactivates the represser in *recA*$^+$ cells; in *recA* mutants, the extensive degradation of DNA, induced by ultraviolet light, causes the accumulation of a substance(s) $Y$ which counteracts the action of $X$ (or possibly prevents its production). This interpretation is not sustained by the present finding that the induction of the prophage is prevented in *E. coli recA uvrB* ($\lambda$) exposed to mitomycin C, whereas the breakdown of DNA in the treated cells does not exceed the level of untreated controls. The suggested repression control model may be, however, still maintained by assuming that a certain amount of spontaneous DNA breakdown occurs, under normal conditions of growth, in all *recA uvrB* cells and causes the accumulation of substance $Y$, which prevents spontaneous, ultraviolet or mitomycin C-induction. DNA-breakdown was observed indeed, in both *E. coli recA* and *recA uvrB* cultures, but there is no evidence, at present, that this spontaneous breakdown occurs in the entire cell population and not in a fraction of the growing cells. A possible alternative model for the role of the *recA* function in the induction of prophage $\lambda$ by ultraviolet light was suggested by Ogawa & Tomizawa (1967). This model is based on the assumption that the *recA* function is involved in the production or activation of the inducer substance. At present, any elaborate interpretation for the role of the bacterial *recA* function in prophage induction by ultraviolet light or other inducing agents, depends on further progress in the understanding of both the *recA* mechanism and the effect of inducing agents on the repression control of prophages.

## SUMMARY

Cellular functions of a double mutant of *Escherichia coli* K 12 deficient in recombination (*recA*) and defective in excision of pyrimidine dimers (*uvrB*) have been compared to those of isogenic *recA* or *uvrB* single mutants and 'wild type' bacteria. A combined effect of the two mutations on cell survival both under normal conditions of growth and after exposure to ultraviolet light or mitomycin C was demonstrated. The ratio of optical density to the number of colony formers in growing cultures of the double mutant is three times greater than in similar cultures of the *recA* single mutant and 9 times greater than in either *uvrB* or in 'wild type' cultures. The doubling time in growing *recA uvrB* cultures is 90 min, compared to 60 min, for the *recA* single mutant and 40 min for the *uvrB* single mutant and 'wild type' bacteria. Growing cultures of *recA uvrB* ($\lambda c$I*857*) bacteria contain a substantial fraction of cells which are unable to form colonies at 32 °C, but produce phage when heated to 42 °C. No such cells were found in cultures of the single mutants or the 'wild type' bacteria lysogenic for $\lambda c$I*857*. The double mutant is 10 times more sensitive to ultraviolet light and twice more sensitive to mitomycin C than the *recA* single mutant. In contrast to *recA* bacteria, exposure of the double mutant to mitomycin C induces little additional breakdown of cellular DNA. Induction of the prophage by mitomycin C is, however, prevented in

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both *recA uvrB* ($\lambda$) and *recA* ($\lambda$) bacteria. Exposure to mitomycin C creates conditions which render the prophage inducible by a newly transduced *recA* gene. This effect of mitomycin C persists and can be revealed in complete medium at 37 °C after 100 min of incubation. The decay of the prophage, in cells exposed to mitomycin C, proceeds at a similar rate in both the double mutant and the *recA* single mutant. The inability of *recA* lysogens to be induced to phage production is discussed in the light of the present findings.

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