Ultraviolet Reactivation in Bacteriophage Lambda

By M. G. R. HART AND J. ELLISON
Sub-Department of Genetics, The Queen's University, Belfast, N. Ireland

(Accepted 7 May 1970)

SUMMARY

Reactivation of damaged phage \( \lambda_v \) by recombination with prophage (prophage reactivation) and by u.v. irradiation of host bacteria (u.v. reactivation) were similar in that u.v.- and nitrous acid-damaged phage was reactivated in both, both were eliminated by three \( \text{rec} \) alleles, neither was much affected by \( \text{her} \) alleles, and both were dependent on the degree of homology between phage and bacterial DNA. These results support a mechanism of u.v. reactivation by recombination between damaged phage DNA and host bacterial DNA. No evidence was obtained to support the hypothesis that u.v. reactivation acts by enhancing host cell reactivation.

Prophage reactivation occurred only in bacteria lysogenic for a prophage genetically related to the superinfecting phage \( \lambda_v \) and integrated at the gal/bio attachment site.

Non-lethal periods of thymine starvation of the host bacteria caused reactivation of u.v.-irradiated phage \( \lambda_v \), comparable with u.v. reactivation.

INTRODUCTION

Garen & Zinder (1955) found that some u.v.-irradiated bacteriophages could be reactivated by the host bacterium. This process, later named host cell reactivation, depended upon the integrity of the bacterium, and it was suggested that damaged phage DNA might be replaced by recombination with homologous bacterial genetic material. The hypothesis was supported by the discovery that u.v. light, which stimulates recombination in bacteriophages (Jacob & Wollman, 1955), increased the survival of u.v.-irradiated phage \( \lambda \) or other dependent-virulent bacteriophages when given in small doses to their host bacteria (Weigle, 1953; Tessman, 1956). The increased survival of u.v.-irradiated phage after u.v. irradiation of host bacteria, called u.v. reactivation, was accompanied by an increase in the frequency of mutants among the surviving phages and this was explained as the result of inaccurate recombination or imperfect homology between phage and bacterium (Tessman, 1956; Stent, 1958).

Sauerbier (1962a, b) challenged the recombination hypothesis of host cell reactivation with results which showed that X-ray and \( \text{HNO}_3 \) damage to phage was not repaired by host cell reactivation. He proposed a chemical repair model involving a host cell reactivation enzyme. The isolation of u.v.-sensitive bacterial mutants which were also unable to perform host cell reactivation (Hill, 1958; Howard-Flanders & Theriot, 1962) supported the chemical repair hypothesis. Subsequently, Harm (1963) suggested a mechanism for u.v. reactivation, dependent on two antagonistic enzyme systems—enzymes which repair lethal lesions and nuclease enzymes which convert non-lethal lesions into lethal ones. Ultraviolet
reactivation might occur because host DNA damaged by u.v. acts as a substrate for the nucleases, competing for their action with bacteriophage DNA and shifting the balance in favour of its repair. Kneser, Metzger & Sauerbier (1965) criticized this view on the grounds that u.v. reactivation of phage can occur when host cell reactivation is inhibited in her mutants, by caffeine or when 5-bromo-uracil is substituted for thymine in the phage DNA. The inhibition may, however, have been incomplete (Harm, 1966; Rupert & Harm, 1966).

Ogawa, Shimada & Tomizawa (1968) showed that in contrast to a uvr D strain, her strains at the uvr A, uvr B and uvr C loci did not show much u.v. reactivation. Mutants at the rec A locus showed no u.v. reactivation, which was thus attributed to an enhancement of repair synthesis necessitating the rec function (Shimada, Ogawa, & Tomizawa 1968).

The different results obtained with her strains and the involvement of the rec function again raise the possibility that u.v. reactivation may not be dependent on excision repair but may involve a recombinational process, perhaps related to that proposed by Rupp & Howard-Flanders (1968) for postreplication repair of bacterial u.v. damage. A mechanism of phage repair which almost certainly depends on recombination between a damaged infecting phage and a homologous region of the bacterial chromosome is that of prophage reactivation: a u.v. damaged virulent mutant of a temperate phage has greater survival when plated on a bacterium lysogenic for the related wild-type phage than when plated on a non-lysogenic strain. This process was described by Devoret & Coquerelle (1966) for the Escherichia coli—phage λ system and has since been investigated by Yamamoto (1967) for the Salmonella—phage P22 system. Rescue of u.v.-irradiated transducing λdg phage was described much earlier by Campbell (1964).

It was the purpose of this investigation to re-examine the effects on u.v. reactivation of mutations which confer u.v. sensitivity on the host bacteria and to look for analogies between u.v. reactivation and prophage reactivation. These two processes appear to have much in common and favour a hypothesis for u.v. reactivation based on a genetic interaction between bacterial and phage DNA rather than on enhancement of the excision repair system.

**METHODS**

**Organisms.** Escherichia coli strains hfrH(λ+), hfrH(λ−) and c600 were kindly supplied by Professor W. Hayes. Strains of c600 carrying prophages 18, 186, 424 and 434 were kindly provided by Dr F. Jacob. Strains AB2433, uvr A and AB2434, uvr B were those described by Howard-Flanders, Boyce & Theriot (1966). Strains hfrB7 met (Broda), KMBL 146, KMBL 239 rec3, KMBL 240 recA, KMBL 241 recB (rec A) and KMBL 243 recC (rec C) (van de Putte, Zwenk & Rorsch, 1966), and strain k125 her (Harm, 1963), all non-lysogenic for phage λ, were kindly provided by Dr A. Rorsch. All of these strains were checked for u.v. sensitivity and for the rec character during the course of the experiments. The virulent mutant of phage λ was given to us by Professor R. C. Clowes. The following strains were a gift of Professor R. Thomas: E. coli 594 (434 hy dg 30), E. coli w3350 (434 hy dg 27), w3350 (434 hy dg 27), w3350 (434 hy dg 21), w3350 (434 hy dg 20), w3350 (434 hy dg 4). These strains were isolated by Campbell (1963). The extent of their deletions is shown in Fig. 4b. Strain hfrC thy− (λ−) was derived from hfr (Cavalli) by aminopterin treatment and curing by u.v. irradiation (Hart, 1966).

Ultraviolet irradiation was from a Hanovia Chromatolite Portable Ultraviolet Lamp, wavelength 2537 Å delivering 6-8 ergs/mm.²/sec. at 10 cm. distance. All operations were performed in dim yellow safelight.

X-irradiation was from a Newton Victor Raymax ‘140’ Industrial X-ray unit. The
Ultraviolet reactivation in bacteriophage lambda

estimated energy per unit mass to the irradiated particles was 3 krad./min. at 7 cm. (Schall, 1961).

Nitrous acid treatment was by the method of Sauerbier (1962a).

Media. Oxoid Nutrient Broth no. 2 (from Oxoid Ltd, Southwark Bridge Road, London, SE1) and Oxoid Blood Agar Base no. 2 were used for cultivation of bacteria and phage.

Phage techniques were those described by Adams (1959).

Ultraviolet reactivation experiments were done as follows.

Overnight broth cultures of host bacteria were diluted 1/10 in 200 ml. of fresh broth and shaken at 37° for 1.5 hr, when the cell density was 2 to 4 x 10^8/ml. The cultures were centrifuged and resuspended in 10 ml. T1 adsorption medium (Benzer, 1952). 1.5 ml. samples were irradiated in glass Petri dishes and 1 ml. volumes were transferred to warmed test-tubes in a water bath at 37°. After temperature equilibration, 1 ml. of phage λv irradiated to a survival of 10^-3 was added. Multiplicity of infection was not greater than 10^-2. Staggered addition times enabled all adsorption mixture to be left for precisely 15 min. before plating. Preliminary experiments had shown that this was sufficient for adsorption of more than 90% of the phage and that no bursts occurred during this period. The mixtures were diluted when necessary and plated by the agar layer method. In experiments with u.v.-irradiated phage an hcr indicator strain was used, on which the survival of the irradiated phage was so low that unadsorbed phage did not form plaques. Elimination of free phage by this method was not possible when nitrous acid damage or hcr bacteria were being tested, but the reduced phage titre using heavily irradiated host bacteria confirmed that efficient adsorption had occurred. In these experiments, the final phage titre was adjusted so that the adsorption mixtures could be plated without dilution.

Kill-reduction factors (KRF) were calculated as

\[ \log \frac{N}{N_0} (\lambda^- \text{ strain}) \]

\[ \log \frac{N}{N_0} (\lambda^+ \text{ strain}) \]

using log. N/N₀ values at doses where the relationship between log. N/N₀ and dose was linear. (N₀ = initial phage titre, N = phage titre after treatment.) Five replicate plates were examined for each determination and the results analysed statistically.

RESULTS

Characteristics of ultraviolet reactivation

Effects of different inactivating agents

Fig. 1 shows the effects of small doses of u.v. light given to HfrH(λ^-) host bacteria on the survival of phage λv damaged by u.v. light or HNO₂. Ultraviolet reactivation occurred in each case. There was no reactivation of X-ray damaged-phage.

Phage λv (titre 10^8/ml.) in T1 adsorption medium was u.v.-irradiated to 10^-3 survival, then mixed in equal volume with HfrH(λ^-) bacteria (10^9/ml., u.v.-irradiated for various times in 1 mm. layers) and held at 37° for 15 min. Appropriate dilutions were then plated out in 0.7% agar layers using the hcr strain K12s hcr as plating bacterium (Fig. 1a).

Phage λv (titre 10^8/ml.) was warmed to 37° in acetate buffer pH 4.5. NaNO₂ was then added to 0.5M conc. and the mixture incubated for a period allowing 10^-3 survival (about 7 min.) HNO₂ action was terminated by diluting 10^-2 in buffer to which an equivalent concentration of NaOH had been added. The final pH was 7.1. Treated phage was adsorbed and plated as in (a) (Fig. 1b).
Ultraviolet reactivation by different u.v.-sensitive bacterial mutants

Fig. 2a shows the results of experiments on u.v. reactivation of u.v.-irradiated phage λ, using four different rec derivatives of the rec+ strain KMBL 146. Phage plated on strains KMBL 239, 240 and 241 was not reactivated, but strain KMBL 243 gave normal u.v. reactivation. The four rec strains behaved consistently in repeated experiments. Their reduced ability to form recombinants with hfrH(λ−) and hfrB7 donors, and increased sensitivity to direct

Fig. 1. Ultraviolet reactivation by irradiation of the host. Graphs showing survival of phage λ, treated with (a) u.v. light or (b) HNO2 and adsorbed to Escherichia coli strain hfrH(λ−) bacteria u.v.-irradiated for different periods.

Fig. 2. Ultraviolet reactivation by irradiation of the host. Graphs showing the survival of u.v.-irradiated phage λ, pre-adsorbed to (a) strains KMBL 146 (rec−) X, KMBL 239 (rec) ▲, KMBL 240 (rec) ○, KMBL 241 (rec) ■, and KMBL 243 (rec) ◇; (b) strains K128 her O, AB2434 □, and AB2433 ●; (c) strains hfrH (λ−), circles and K128 her squares. Filled symbols, plating on minimal agar; open symbols, plating on nutrient agar. General methods as in Fig. 1.
u.v. irradiation were as found by van de Putte et al. (1966), who also noted the exceptional behaviour of strain KMBL 243.

Fig. 2b shows the results with the three her strains. No reduction in u.v. reactivation was found; in fact, in this experiment, all gave greater reactivation than wild-type strains. However, strain K12s her was usually less efficient than the wild-type (cf. Fig. 2c; Table 1). The u.v. dose for maximum reactivation was always lower for her strains than for wild-type strains.

Effects of postirradiation treatments

If u.v. reactivation depends on increased excision repair it might be affected by holding the infected bacteria in liquid or plating on minimal medium. These conditions are believed to improve the survival of fil+ bacteria after u.v. irradiation by allowing additional time for excision repair before damage becomes irreversible (Witkin, 1968). The results of experiments in which Escherichia coli strains HfrH (λ−) and K12s her were plated on minimal medium or held in buffer before or after phage adsorption are given in Fig. 2c and Table 1. In all cases u.v. reactivation was within the range normally found with direct plating on complex medium.

Table 1. Effect of liquid holding on u.v. reactivation on phage λ

<table>
<thead>
<tr>
<th>Strain</th>
<th>0 min</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
<th>40 min</th>
<th>0 hr</th>
<th>6 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>hfrH(λ−)</td>
<td>×11·3</td>
<td>×9·0</td>
<td>×10·2</td>
<td>×9·8</td>
<td>×8·0</td>
<td>×17·3</td>
<td>×11·1</td>
</tr>
<tr>
<td>K12s her</td>
<td>×5·0</td>
<td>×6·0</td>
<td>×5·1</td>
<td>×5·4</td>
<td>×7·3</td>
<td>×3·6</td>
<td>×4·4</td>
</tr>
</tbody>
</table>

N.B. Phage adsorption 30 min.

In Expt. 1 phage λv (titre 10⁸/ml.) in T1 adsorption medium was u.v.-irradiated to 10⁻² survival, mixed in equal volume with irradiated or unirradiated bacteria (10⁶/ml.) in T1 adsorption medium, held at 37° for adsorption, then diluted in buffer and the infected irradiated bacteria held for the times shown before plating.

In Expt. 2 the host bacteria were irradiated at their usual concentration in T1 adsorption medium, then either used to adsorb irradiated phage λv for 30 min. before plating or held at 10⁻² dilution, in T1 adsorption medium at 23° in the dark for 6 hr before centrifuging and resuspending at the original concentration, adsorbing and plating as before.

Characteristics of prophage reactivation

Survival of phage λv treated with u.v. light or HNO₂ and plated on Escherichia coli strains HfrH(λ−) and HfrH(λ+).

Fig. 3 shows the survival of phage λv treated with the two agents and plated on lysogenic and non-lysogenic strains of Escherichia coli. Prophage reactivation occurred with both u.v.- and HNO₂-treated phage, but not with X-ray damaged phage. It was always greater with u.v.-damaged phage, as would be consistent with the known stimulating effect of u.v. light on genetic recombination.

Effects of different prophages on reactivation

Fig. 4a shows results obtained when u.v.-irradiated phage λv was plated on E. coli strains carrying the lambda-related prophages 424 and 434, both of which are inducible and
Fig. 3. Reactivation by prophage. Graphs showing survival of phage \( \lambda_v \), treated with (a) u.v. light and (b) \( \text{HNO}_2 \) and plated on \textit{Escherichia coli} strains \( \text{Hfr} \)\( \lambda^+ \). Phage \( \lambda_v \) was u.v.-irradiated or treated with \( \text{HNO}_2 \), as described for Fig. 1, then plated out in 0.7% agar layers containing \textit{E. coli} strain \( \text{Hfr} \)\( \lambda^+ \) or \( \text{Hfr} \)\( \lambda^+ \) as plating bacteria.

Fig. 4. Reactivation by prophage. (a) Graphs showing the survival of u.v.-irradiated phage \( \lambda_v \), plated on \textit{Escherichia coli} strains carrying various prophages. Individual points are omitted for clarity but lines are regression lines calculated from at least five individual mean values from duplicate plate counts. (b) Graph showing the kill-reduction-factors obtained by comparing the survival of u.v.-irradiated phage \( \lambda_v \) on \textit{E. coli} strain \( c600(\lambda^+) \) and the five \textit{E. coli} strains carrying 434 by \( dg \) prophages. The deletions run from gene J towards gene A and the data are plotted so as to show the extent of the deletion, all genes to the left of a point being deleted in that strain. The deletions are given by Campbell (1963). Methods as for Fig. 3.
Ultraviolet reactivation in bacteriophage lambda

Prophage reactivation in u.v.-sensitive strains of Escherichia coli

Table 2 shows the effect of lambda prophage in various u.v.-sensitive strains. The her strains all reactivated u.v.- and HNO₂-damaged phage to the same extent as wild-type bacteria. The behaviour of the rec strains was not uniform. Strain KMBl 243 was not significantly different from the wild type. Prophage reactivation was virtually eliminated in strains KMBl 240 and 241. In strain KMBl 239 it was strongly reduced with u.v.-damaged phage and completely eliminated with HNO₂-damaged phage. In all the strains in which reactivation occurred, u.v.-damaged phage was reactivated more than HNO₂-damaged phage. This
suggests that u.v. lesions in the superinfecting phage DNA contribute to reactivation and this may be unaffected by the KMBL 239 rec mutation.

**Survival of phage λ<sub>0</sub> plated on rec bacteria**

The foregoing results suggested that damaged phage DNA was repaired by a recombination process. Ultraviolet irradiation of the host bacterium could either initiate such recombination or raise its frequency, as in other systems (Jacob & Wollman, 1955). To test for reactivation in unirradiated host bacteria, non-lysogenic rec bacteria were compared with the related rec<sup>+</sup> strain (Table 3) as hosts for irradiated phage λ<sub>0</sub>. After correction for the different plating efficiencies on the strains, small but significant decreases in survival of u.v.-damaged phage were found in all rec strains tested, but the decreases with HNO<sub>2</sub>-damaged phage were not significant. The repair process by which u.v. reactivation operates was therefore either absent or undetectable in unirradiated bacteria.

**Reactivation of u.v.-damaged phage λ<sub>0</sub> by thymine starvation of the host bacterium**

The stimulation by u.v. light of recombination between bacteriophages is caused by unexcised pyrimidine dimers in their DNA (Baker & Haynes, 1967). Dimers are evidently not formed during thymine starvation in *E. coli*, since her mutants are not more sensitive to
thymine starvation than wild-type bacteria (Cummings & Taylor, 1966). Ten ml. log. phase cultures of *E. coli* strain hifr(λ⁻)thv in m9 medium with methionine and thymine were membrane filtered, washed three times and resuspended in 30 ml. of the same medium lacking thymine. After thymine starvation for various times the cultures were centrifuged, resuspended in 0.5 ml. T1 adsorption medium and mixed with an equal volume of phage λ (titre 10⁶/ml.) previously u.v.-irradiated to 10⁻³ survival. After 15 min. adsorption, dilutions were plated using the *her* bacterium K12s *her* as plating bacterium (Fig. 5). Thymine starvation of host bacteria caused reactivation of u.v.-damaged phage. Ultraviolet reactivation was thus not dependent on the presence of dimers in the bacterial DNA. The fact that maximal reactivation occurred before the survival of the bacteria was much decreased suggested that damage to bacterial DNA was not essential to reactivation.

**Relationship between u.v. reactivation of different coliphages and their DNA homology with *Escherichia coli* **

The u.v. reactivation of phages λ, T3 and T1 were compared with the % homology between phage DNA and *E. coli* DNA from data obtained from the literature (Table 4). The greater the homology between phage and bacterium the greater the fraction of u.v.-damaged phage which can be rescued by u.v. reactivation.

**Table 4. Ultraviolet reactivation for some *Escherichia coli* phages and their homology with their host bacteria**

<table>
<thead>
<tr>
<th>Phage</th>
<th>Percentage homology DNA/DNA hybridization</th>
<th>Ultraviolet reactivation (phage survival of 10⁻³ before reactivation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ</td>
<td>32 (Cowie &amp; Szafranski, 1966)</td>
<td>× 25 (Harm, 1963)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>× 17 Present experiments</td>
</tr>
<tr>
<td></td>
<td></td>
<td>× 11 (Kneser <em>et al.</em> 1965)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>× 12.5 (Weigle, 1953)</td>
</tr>
<tr>
<td>T3</td>
<td>6 (Cowie &amp; Szafranski, 1966)</td>
<td>× 5.6 (Tessman, 1956)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>× 3.5 (Harm, 1963)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>× 2.8 (Weigle &amp; Dulbecco, 1953)</td>
</tr>
<tr>
<td>T1</td>
<td>0 (Schildkraut <em>et al.</em>; quoted by Cowie &amp; Szafranski, 1966)</td>
<td>× 1.9 (Tessman, 1955)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>× 3.5 (Harm, 1963)</td>
</tr>
</tbody>
</table>

*Note:* values for u.v. reactivation of phage λ of up to ×17 have been obtained on our experiments. Using 10⁻³ survival and the same method it was not possible to detect u.v. reactivation of phages T1 and T3.

**DISCUSSION**

There are striking similarities between u.v. reactivation and prophage reactivation which suggest that both occur by related mechanisms. Nitrous acid-damaged phage is reactivated by both processes; both processes are reduced by the same three out of four *rec* alleles in the host bacterium; neither process is much affected by *hcr* alleles; both processes are dependent on the degree of homology between the phage and bacterial DNA.

It is clear from the experiments with *Adg* prophage that prophage reactivation depends on the rescue of the damaged superinfecting phage by genetic recombination with the related prophage. Prophage reactivation is eliminated by *rec* mutations, whereas lytic recombination and multiplicity reactivation are not (van de Putte *et al.* 1966; Brooks & Clark, 1967; Baker & Haynes, 1967). Signer & Weil (1968) found that *rec* host bacteria allowed normal recombination to occur between superinfecting phage λ and cryptic prophage...
and were thus able to use rec bacteria to isolate phage mutants deficient in the red function. In the experiments of Signer & Weil (1968), the superinfecting phage was undamaged and probably capable of excising the prophage by int activity (Zissler, 1967; Gingery & Echols, 1967; Gottesman & Yarmolinsky, 1968; Dove, 1968). Recombination would then be mediated by red genes and be independent of rec functions. In our own experiments, the superinfecting phage was damaged and perhaps unable to excise the prophage until repair was complete. Under these conditions the rec mutation appears to be essential, though there is currently much concern about the possibility that apparent pleiotropic effects of rec mutations are due to associated mutations in other genes (e.g. Clark, 1968). However, the elimination of prophage recactivation and u.v. reactivation by bacterial mutations in our experiments establishes that bacterial systems are involved, whether the effects are due to the rec genes or not.

Bacteria lysogenic for prophage 424 which is genetically related to phage λ did not re-activate u.v.-irradiated phage λ. This suggests either that reactivation is site-specific and occurs only when phage and prophage have affinity with the same attachment site, or that excision of the prophage by int activity is necessary (λ prophage is excised by phages λ and 434 but not by phage 424 (Dove, 1968)). The second alternative is unlikely since the rec function is known to contribute in only a minor way to exchanges between red+ autonomous phages. Furthermore, the involvement of only half of the prophage genome in prophage reactivation (shown in the dg experiments) may reflect the site specificity of the process. If recognition of the attachment regions of lysogen and superinfecting phage led to close pairing between the left arm of the superinfecting phage and the corresponding region of the prophage DNA, permutation of gene order would prevent exchanges between right arms. However, it will be necessary to investigate in more detail the role of phage genes in the two types of reactivation.

The necessity for the rec function may thus be an indication that one component of the exchange is integrated with the bacterial chromosome. If this is correct, u.v. reactivation also depends on genetic interaction between the damaged phage DNA and part of the bacterial DNA. The survival of phage-damaged nitrous acid is not significantly lower on rec strains than on the corresponding rec+ strain. Thus the repair process by which u.v. reactivation operates is absent or undetectable in unirradiated bacteria. Ultraviolet irradiation of the host bacterium therefore initiates repair and the small reduction in survival of u.v.-irradiated phage plated on rec bacteria suggests that u.v. irradiation of the phage also does this to a small degree, a result which is paralleled in prophage reactivation.

Reactivation may be brought about by direct damage to host DNA or by u.v. photoproducts indirectly affecting the activity of appropriate enzyme systems. The efficacy of thymine starvation of the host bacterium in inducing reactivation suggests that direct damage to host DNA is not the mechanism. Significant genetic damage cannot be demonstrated in thymine-starved bacteria, and thymine starvation differs from u.v. irradiation in that excisionless (uvr) and rec A bacteria are not especially sensitive to it (Cummings & Taylor, 1966). Prolonged thymine starvation of the Hfr strain used in our experiments was shown previously (Hart, 1966) to have no effect on recombination of DNA transferred to recipients, where u.v. treatment promotes recombination under similar circumstances (Jacob & Wollman, 1961). The period of thymine starvation which gave maximum reactivation of phage λ, in the present experiments did not reduce the viability of the host bacteria. Gallant & Spottswood (1965) obtained similar results in a study of recombination in merodiploid bacteria. The amount of recombination obtained after thymine starvation was greater than expected from the lethality of the treatment. They suggested that thymine starvation might
have acted by increasing effective pairing necessary for recombination. Agents initiating reactivation may function in this way, allowing interaction between regions of phage and bacterial DNA which are perhaps insufficiently homologous for normal recognition. However, it is premature to attempt a detailed explanation of u.v. reactivation while the mechanism of recombination is obscure. Nevertheless prophage reactivation and u.v. reactivation may help recombination studies, as they require bacterial recombination mechanisms but not bacterial survival.

We thank Mr A. J. Jones and Miss A. Hill for their valuable technical assistance.

REFERENCES


*(Received 10 June 1969)*