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Behavior of λ Bacteriophage in a Recombination Deficient Strain of *Escherichia coli*

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The behavior of λ phage in the Rec⁻ strain JC-1569 is compared with that in the Rec⁺ strain JC-1557. No difference deemed significant was noted in the adsorption rate, latent period, burst size, frequency of lysogenization, and frequency of vegetative phage recombination. The location of the prophage and its mode of insertion in the Rec⁻ lysogen of wild-type λ (λ⁺) were inferred to be normal from the results of conjugational crosses. Spontaneous and ultraviolet (UV) irradiation induction of λ⁺ were markedly reduced in the Rec⁻ lysogen. On the other hand, thermal induction of a mutant lambda (λ*cI857*) lysogen of the Rec⁻ strain was not reduced and was only slightly affected by UV irradiation. Phage subject to inhibition by λ immunity failed to multiply in UV-irradiated cells of the Rec⁻ λ⁺ lysogen, whereas those not inhibited by this immunity did multiply. It was concluded that the failure of UV to induce λ⁺ in the Rec⁻ lysogen was not due to damage to the prophage, but rather to the inability of the irradiated cells to respond by lifting immunity. Preliminary evidence indicates that a single mutation confers recombination deficiency and the inability to lift immunity after UV irradiation. Possible relationships between recombination and the lifting of immunity are enumerated.

Recombination-deficient mutants of an F⁻ strain of *Escherichia coli* K-12 have been isolated (5). These mutants were characterized primarily by their reduced ability to form conjugational recombinants but their normal ability to form conjugational zygotes with certain Hfr donor strains of *E. coli*. A further characterization of these strains revealed that they had gained an increased sensitivity to ultraviolet (UV) irradiation (5) and X irradiation (7). Further study was then carried out to determine the influence of the mutation in one of the strains on the recombination of bacteriophages. No influence was found on the recombination of T4 (W. Eckhart, *personal communication*) and on the recombination of T7 (D. Freifelder, *personal communication*). Some influence of the mutation on the recombination of phage S-13 has been reported (15). The present study was undertaken to investigate the ability of λ, a temperature bacteriophage, to recombine in cells of the recombination-deficient mutant.

MATERIALS AND METHODS

Bacteriophages. The bacteriophages used are given in Table 1a. Lysates of λ⁺ and 434 hy were prepared by UV induction of the respective lysogens. Lysates of λ*hc*, 434hy *hc*, and λ*vir* were prepared by mass infection of broth cultures of C600G and aeration for about 5 hr to allow lysis. Lysates of λ*cI857* and the λ*sus* mutants were obtained from M. Meselson.

Bacteria. The bacterial strains used are all derivatives of *E. coli* K-12 and are listed in Table 1b.

Since the strain JC-411 from which the recombination-deficient mutants were isolated was resistant to λ, spontaneous mutants sensitive to this phage were isolated. The mutation conferring λ resistance on the strains conferred at the same time the inability of the strains to use maltose as the sole carbon and energy source for growth.

Media. For most experiments, bacteria were grown in 1% tryptone broth with 5 g of NaCl and 1 mg of thiamine added per liter. For bacterial assays, tryptone broth was solidified with 20 g of agar per liter, and for phage assays, 10 g of agar per liter. Dilutions for bacterial assays were done in tryptone broth; dilutions for phage assays were in 0.01 M $MgSO_4$. Samples of appropriate dilutions were plated by use of tryptone broth to which 6.5 g of agar had been added per liter.

For mating experiments, bacteria were grown in Luria broth containing 10 g of tryptone (Difco), 5 g of yeast extract, and 10 g of NaCl per liter of distilled water. Viable bacteria were titered on Luria medium solidified with 20 g of agar per liter. Recombinants were selected on half-strength mineral salts medium 56 (10) supplemented with glucose at a final concentration of 0.2% (w/v) and the appropriate growth factors at predetermined optimal concentrations. Composition of phosphate buffer used in irradiation experiments was: KH_2PO_4, 2 g per liter; K_2HPO_4, 7 g per liter; $MgSO_4{:}7H_2O$, 0.25 g per liter. Although this

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TABLE 1a. *Bacteriophage strains*

Strain	Characteristics	Source	Reference
λ^+	The wild type of Kaiser	Induction of AC-169 obtained from A. M. Campbell	Kaiser, 1957 (8)
434hy	A hybrid between λ^+ and 434 which carries most of the λ genome but the immunity specificity of 434	Induction of JC-1171 obtained from M. Meselson	Kaiser and Jacob, 1957 (9)
λ*hc*	Carries immunity λ, a host range mutation (*h*), and a *cI* mutation	R. Thomas	
434hy *hc*	Carries immunity 434, a host range mutation (*h*), and a clear plaque mutation	M. Ptashne	
λ*vir*	Able to plate on λ-lysogenic strains	T. Nagata	
λ*cI857*	Carries a temperature-sensitive *cI* mutation and the *ind*$^-$ mutation	M. Meselson	Sussman and Jacob, 1962 (13)
λ*sus*	The particular *sus* mutants used are described in the text	M. Meselson	Campbell, 1961 (2)

TABLE 1b. *Bacterial strains*

Strain designation	Characteristics[a]	Prophage carried	Source	Reference
JC-1557	Rec^+ UV^R Leu^- Gal^- His^- Str^RND Mal^+ λ^S	None	Spontaneous mutant of JC-411 which is Mal^- λ^R	Genotype of JC-411 is listed by Clark and Margulies, 1965 (5)
JC-1158	Same as JC-1557 except λ^I	λ^+		
JC-1188	Same as JC-1557 except $Mal^-\lambda^R\lambda^I$	λ^+	Spontaneous mutant of JC-1158	
JC-2207	Same as JC-1557 except λ^I	λcI857		
JC-1569	Rec^-UV^S $Leu^-Gal^-His^-Str^R$ND $Mal^+\lambda^S$	None	Spontaneous mutant of Mal^- λ^R JC-1553	Genotype of JC-1553 is listed in reference 5
JC-1163	Same as JC-1569 except λ^I	λ^+		
JC-1189	Same as JC-1569 except $Mal^-\lambda^R\lambda^I$	λ^+	Spontaneous mutant of JC-1163	
JC-2209	Same as JC-1569 except λ^I	λcI857		
JC-1190	Str^S His^+ UV^R Rec^+ D^b	None	UV irradiation of W1485	
JC-182	Str^S Leu^+ D^c	None		Clark, 1963 (4)
C600 G	Permissive host for λ sus mutants	None		
AC-169[d]	Source of λ^+	λ^+	Allan Campbell	
JC-1711[e]	Source of 434 hy	434 hy	Matthew Meselson	
AC-594	Nonpermissive host for λ sus mutants	None	Matthew Meselson	
CR-63	Host for h mutants of λ	None		Appleyard, McGregor, and Baird (1)

[a] The following abbreviations are used: Rec for recombination, UV for ultraviolet light, λ for lambda, Mal for maltose, Leu for leucine, His for histidine, Gal for galactose, Str for streptomycin, ND for nondonor, D for donor, superscript R for resistance, superscript S for sensitivity, superscript I for immunity; "+" stands for "independence" when used with the abbreviation of an amino acid, for "proficiency" when used with Rec, and for "ability to utilize" when used with Gal; "−" stands for "dependence" when used with the abbreviation of an amino acid, for "deficiency" when used with Rec, and for "inability to utilize" when used with Gal. F1 stands for the wild-type sex factor F. The gene symbols are those used by Taylor and Thoman (14).

[b] F1 is integrated between *serA* and *lys* so that markers are transferred in the order *lys his trp* . . . etc.

[c] F1 is integrated both between *serA* and *argE* so that markers are transferred *argE str*, *xyl*, etc., and between *thi* and *thr* so that markers are transferred in the order *thr*, *leu*, *lac*, etc.

[d] Alias C600 (λ^+).

[e] Alias C600G(434hy).

buffer is 0.001 M in $MgSO_4$, an additional amount of $MgSO_4$ was added routinely, bringing the final concentration to 0.002 M.

Experimental methods. For most experiments, cultures were prepared by diluting a small inoculum from a nonaerated overnight culture into fresh medium, and aerating at 37 C to a density of 2×10^8 to 4×10^8 cells per milliliter. These will be referred to as log-phase cells.

Determination of UV sensitivity. Log-phase cells were centrifuged and resuspended in an equal volume of phosphate buffer containing 0.002 M $MgSO_4$. Ultraviolet irradiation was accomplished at 46 cm from a General Electric 15-w germicidal lamp G15T8, at which distance the intensity of irradiation was estimated to be approximately 35 ergs per mm^2 per sec. Samples of 5 or 10 ml were transferred to glass petri dishes which were shaken during irradiation. All subsequent operations were carried out in dim light to prevent photoreactivation.

UV induction. Log-phase cells were centrifuged and resuspended in one-half the volume of phosphate buffer containing 0.002 M $MgSO_4$. Samples of no more than 10 ml were transferred to glass petri dishes, shaken during irradiation, and then added to an equal volume of tryptone broth made to 0.001 M $MgSO_4$. The culture was aerated for 2 to 3 hr at 37 C. Turbidity was followed in a Klett-Summerson colorimeter, and, when lysis was complete, chloroform was added and the mixture was centrifuged to remove cells and debris. The supernatant fluid was assayed for free phage.

Superinfection experiments. Cultures were prepared and irradiated as for UV induction. Superinfecting phage were added at the desired multiplicity either immediately after dilution into tryptone broth or after an additional specified time of aeration. After 10 to 20 min of incubation to allow phage adsorption, a sample was added to antiserum for 10 min to inactivate unadsorbed phage. The cells were then diluted $1:10^4$ into prewarmed broth and aerated at 37 C for 2 hr. Chloroform was added, and the culture was diluted and assayed for phage.

Measurement of frequency of lysogenization. Cultures grown at 37 C in tryptone broth for 4 to 5 hr to approximately 2×10^9 cells per milliliter were resuspended in 0.01 M $MgSO_4$ and aerated for an additional 1 hr.

The cells were then diluted to approximately 10^8 cells per milliliter, and phage were added at the desired multiplicity. After 20 min of adsorption at 37 C, antiserum was added; 10 min later, the cells were diluted 1:100 into fresh prewarmed tryptone broth and aerated for an additional 30 min at 37 C to allow establishment of the lysogenic condition; the culture was then plated for survivors. The next day, colonies were transferred on sterile toothpicks and spread in small patches arranged in geometrical array. After overnight incubation, these patch plates were replicated onto fresh tryptone plates on which 10^9 cells of JC-1171, a 434hy lysogen, had been spread. Subsequent incubation of the replica plates leads to the detection of lysogens by the homogeneous zone of lysis within and around the patch. Patches showing no lysis were scored as nonlysogens, as were the small percentage of patches which gave a heterogeneous response.

Phage crosses. The following procedure was suggested to us by Matthew Meselson. Stationary phase cells from an aerated overnight tryptone broth-grown culture were centrifuged, resuspended, and diluted in 0.01 M $MgSO_4$ to give approximately 2×10^8 cells per milliliter. Phage was added at the desired multiplicity, and the mixture was incubated at 37 C for 7 min to permit adsorption. Unadsorbed phage were removed by passing the mixture through a membrane filter (0.45 : pore size; Millipore Filter Corp., Bedford, Mass.) at 4 C and washing the cells which remained on the filter with cold tryptone broth. The washed cells were resuspended in 10 ml of cold tryptone broth by vigorous agitation of the filter disc in a test tube. Approximately 50% of the cells were lost by this procedure, yielding a cell density of 10^7 cells per milliliter. A further dilution of 1:100 into warm tryptone broth was made, and this mixture was aerated for 60 min at 37 C. Finally, the cells in the mixture were lysed by vigorous shaking with a few drops of chloroform. The resulting lysate was assayed and stored.

Bacterial crosses. Log-phase cultures of Hfr and F^- strains, mixed in a ratio of approximately 10 F^- cells to 1 Hfr cell, were incubated at 37 C without agitation for the desired time of mating. The mating mixture was then diluted and plated onto selective agar media plates which were incubated at 37 C for 2 days. Recombinants were tested for their inheritance of unselected markers by making patch plates on fresh selective agar plates and, after overnight incubation, replicating onto the appropriate media. The parent strains were always assayed for viable count and the presence of particular spontaneous mutants just before the mating.

RESULTS

Phage recombination. A temperate phage such as λ can detectably engage in recombination either when recombination occurs between the deoxyribonucleic acid (DNA) of two phage strains or when recombination occurs between phage DNA and the host chromosome, leading to the establishment of lysogeny. One of the first questions we wanted to answer was whether or not recombination deficiency in the host cell would impair the ability of infecting bacteriophage to recombine.

The frequency of vegetative recombination was determined by mixed infection of a Rec^- host, JC-1569, and a Rec^+ control, JC-1557, with two strains of λ. The first is a double mutant with a clear plaque mutation *c26* in addition to a suppressor-sensitive mutation *susO29*, and the second carries a suppressor-sensitive mutation *susP3* (2). The position of these markers on the map of λ is shown in Fig. 1. Neither mutant can multiply alone in either the Rec^- or the Rec^+ strain, indicating that the mutations are not suppressed in these hosts. Multiplication in mixed

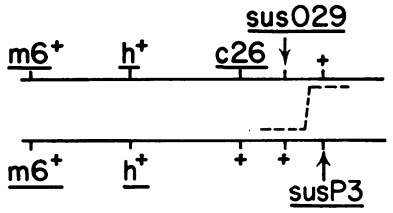

FIG. 1. *Diagrammatic representation of the cross of two λ phages, described in Table 2. The genetic map of both vegetative phage is shown. The dotted line indicates a cross-over event leading to a sus⁺ recombinant.*

infection is possible, however, presumably because of complementation. The yield from such a mixed infection was plated on C600G to determine the total number of phage produced. Wild-type recombinants were scored by plating on AC-594, a strain in which suppression of the original mutations does not occur and therefore on which the parents cannot multiply. From Table 2, it can be seen that the percentages of recombinants formed in the Rec⁻ and Rec⁺ hosts are very similar, 0.8 and 1.1%, respectively. In addition, the fraction of *sus*⁺ recombinants which also inherit the clear plaque marker, *c26*, by a second recombinational event, is approximately the same in both strains. Furthermore, the finding that the efficiency of plating of the recombinants formed in the Rec⁻ host JC-1569 is the same whether the indicator strain is Rec⁻ JC-1569 or Rec⁺ JC-1557 strongly suggests that these recombinants are normal, mature phage particles in no way impaired by their formation in a recombination-deficient host.

The reduction of vegetative phage to prophage, and the resulting conversion of the sensitive host cell into an immune lysogenic cell, occurs by a recombination event between the phage chromosome and the bacterial chromosome (3). The ability of a phage to lysogenize a given bacterial host is, therefore, also a measure of the ability of the phage chromosome to recombine with the host chromosome. Table 3 shows the percentage of Rec⁻ and Rec⁺ cells which become lysogenic when infected with wild-type λ over a range of multiplicities of infection from about 0.4 to 14. There is no obvious correlation of the frequency of lysogenization with multiplicity of infecting phage with either strain, and it is quite clear that the recombination-deficient host is able to be lysogenized to substantially the same extent as the recombination-proficient control at any of the multiplicities used.

Standard parameters of λ biology. We found the adsorption constant for λ⁺ to be 5×10^{-10} ml/min for both the Rec⁻ and Rec⁺ strains, JC-1569 and JC-1557, respectively. The latent period for both was about 40 min, and the average burst size was approximately 100 plaque-forming units (PFU) per cell for the Rec⁻ host and approximately 150 PFU per cell for the Rec⁺ host. Furthermore, the efficiency of plating, a composite

TABLE 2. *Nature of recombinants of phage λ produced in a recombination-deficient strain*

Determination	JC-1557 Rec⁺	JC-1569 Rec⁻
Multiplicity of infection	20	20
Burst size	7.3	4.4
Recombinant frequency (per 100 phage produced)	1.1	0.8
Percentage of recombinants inheriting *c26*	27	19
Titer of recombinants on different tester strains		
JC-1557	2.6×10^3	6.0×10^2
JC-1569	2.5×10^3	6.1×10^2
AC-594	2.6×10^3	7.6×10^2

TABLE 3. *Frequency of lysogenization by λ⁺ of a recombination-deficient strain*

Rec⁺ Host JC-1557[a]		Rec⁻ Host JC-1569[a]	
Multiplicity of infection	Percentage of infected[b] cells which are lysogenic	Multiplicity of infection	Percentage of infected[b] cells which are lysogenic
12	23	14	26
9.5[c]	33	12[c]	42
8.9	48	11	35
3.8[c]	43	5.8[c]	58
1.2	16	1.4	13
0.89	67	1.1	13
0.38	37	0.58	23

[a] For each strain at each multiplicity, 160 survivors were picked and tested for whether or not they now carried the prophage as described in Materials and Methods. In the second experiment shown, 100 survivors were picked and used to inoculate 1 ml of tryptone broth. These cultures were incubated on a shaker overnight at 37 C and were then cross-streaked with λ⁺, λ*vir*, and a T4r$_{II}$ mutant. Nonlysogens show an area of lysis at the cross-streak with all three phages; lysogens show lysis only with λ*vir*.

[b] The number of infected cells was calculated on the basis of a Poisson distribution; $1 - e^{-m}$ is the fraction of infected cells where m is the input multiplicity.

[c] Corrected for unadsorbed phage.

measure of these and other parameters of λ biology, was the same on both strains.

Spontaneous induction. A standard λ lysogen can be defined by two characteristics—the ability to produce phage spontaneously without reinfection (dubbed "spontaneous induction"), and immunity to infection by the same or closely related superinfecting phage. Lysogens of the Rec⁻ strain JC-1569 can be shown to be immune by a cross-streak test (Table 3, footnote), but they do not produce phage by spontaneous induction. The phage titer of the supernatant fluid from an early stationary-phase culture of JC-1188, a λ^R derivative of the Rec⁺ lysogen, is 10^6 to 10^7 PFU/ml, whereas that from JC-1189, the λ^R Rec⁻ lysogen, is 10^2 PFU/ml or less. Furthermore, the titer is the same whether plated on a standard λ-sensitive indicator such as C600G, or on a lysogen carrying the heteroimmune phage 434hy, indicating that large numbers of defective phage particles which might be rescued by recombination or complementation are not produced by the Rec⁻ strain during vegetative growth. Among the few PFU which are produced by the Rec⁻ lysogen, the majority are clear plaque formers, suggesting that phage are produced mainly when mutation in the prophage leads to derepression of vegetative multiplication.

One possibility for the failure of spontaneous induction in the Rec⁻ strain is that the mode of insertion or location of the prophage is aberrant. To test this, we performed several crosses between a Rec⁻ lysogen, JC-1163, which is also His⁻ Gal⁻ Leu⁻ Str^R λ^I and a His⁺ Gal⁺ Leu⁺ Str^S λ^S Hfr strain, JC-1190. This strain had been isolated as a donor uniquely capable of giving high yields of recombinants with the Rec⁻ strain, JC-1569, apparently because it transfers the wild-type *rec* allele early in conjugation, thereby permitting recombination to take place. Its point of origin lies between *serA* and *lys* on the map of Taylor and Thoman (14), and the direction of transfer is counter-clockwise. In one cross, His⁺ (Str^R) recombinants were selected, whereas in a second cross, Gal⁺ (Str^R) recombinants were selected. These two types of recombinants were scored for whether or not they had inherited the λ immunity of the lysogenic F⁻ parent, (i.e., the presence of the prophage) or the λ sensitivity (i.e., absence of prophage) of the nonlysogenic Hfr parent. We found that 85% (170 of 200) of the recombinants which inherited Gal⁺ from the nonlysogenic donor also inherited λ sensitivity. As the prophage is responsible for immunity, it is clear that the prophage is not inherited by 85% of the Gal⁺ Str^R recombinants, a result expected only if the prophage is closely linked to the *gal* mutation carried by the lysogenic recipient. A majority of the His⁺ Str^R recombinants would be expected to result from zygotes to which *gal*⁺ was not transferred by the donor (as they are separated by more than 20 min on the genetic map); hence, most of them should retain the prophage of the lysogenic F⁻ parent and be immune. We found, in fact, that 40 of 40 of the His⁺ Str^R recombinants tested were immune. These results led us to the conclusion that the location of the prophage is normal.

To determine whether or not the prophage was also integrated normally, we first tested the His⁺ Leu⁻ Str^R recombinants for their inheritance of the Rec⁺ or Rec⁻ characteristic. This was done by replicating a patch plate made from these colonies onto a selective plate spread with a Leu⁺ Str^S donor, JC-182, which does not transfer the *rec*⁺ allele early in conjugation. We found that 37 of 40 were able to form Leu⁺ (Str^R) recombinants at high frequency, and therefore had inherited the *rec*⁺ gene in the original cross, whereas 3 of 40 were still Rec⁻. The same 37 Rec⁺ isolates were able to undergo spontaneous induction, whereas the 3 Rec⁻ isolates were not. Hence, inheritance of the *rec*⁺ allele by Rec⁻ cells confers upon them both normal recombinant-forming ability and ability to undergo normal spontaneous induction. It therefore seems likely that the mode of integration of the prophage in forming a Rec⁻ lysogen is normal.

UV induction. When we purified one each of the lysogenic Rec⁺ His⁺ Str^R and the lysogenic Rec⁻ His⁺ Str^R recombinants previously described and tested for UV induction, we found that the Rec⁺ recombinant gave normal lysis and phage production, whereas the Rec⁻ recombinant failed to lyse or produce phage. Thus, the inability of a Rec⁻ lysogen to undergo spontaneous induction of wild-type λ is correlated with its inability to undergo induction after exposure to UV light.

UV induction was further studied with JC-1158 and JC-1163, the original Rec⁺ and Rec⁻ lysogens, respectively. At a dose of UV which allows 5% survival of the Rec⁺ lysogen, JC-1158, induction appears to follow the normal course (Fig. 2), and results in a phage yield of 2×10^{10} PFU/ml (i.e., about 100 PFU per input cell). The same dose fails to effect lysis of the Rec⁻ lysogen, JC-1163 (Fig. 2), and produces a phage yield of about 10^3 PFU/ml (Table 4).

The apparent absence of UV induction might be caused by the reduction in the phage-producing capacity of irradiated Rec⁻ cells or by inactivation of the prophage. Figure 3 shows that at the UV dose used for induction of the Rec⁺ lysogen

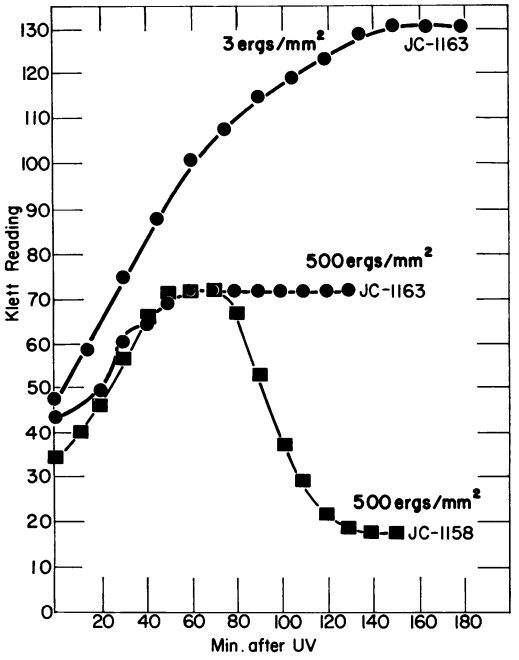

FIG. 2. *Growth of Rec⁺ and Rec⁻ lysogens after UV irradiation. JC-1158 is Rec⁺ and JC-1163 is Rec⁻. Cells of two strains, both lysogenic for λ⁺, were prepared for irradiation as described in Materials and Methods. After irradiation, the cells were incubated at 37 C in a complex growth medium, and the turbidity o, the culture was determined at intervals. Irradiation of approximately 3 ergs per mm² was achieved by attenuating the UV source with a Saran wrap filter and exposing the cells for 10 sec to this source.*

less than 10^{-4}% of either the Rec⁻ nonlysogenic cells or the Rec⁻ lysogenic cells survive. Hence, the viability of these Rec⁻ cells is very sensitive to UV irradiation. To treat the Rec⁻ lysogen with a UV dose equivalent in its lethal effect to the optimal inducing dose applied to the Rec⁺ lysogen, we chose a dose which permitted 5% survival of the Rec⁻ cells. No lysis of the Rec⁻ lysogen was observed (Fig. 2, top curve), and again a very low phage yield was obtained. In fact, the growth pattern of the Rec⁻ lysogen after high or low doses of UV is almost identical with that of the nonlysogen (Fig. 4).

Comparing the viabilities of the Rec⁻ lysogen and nonlysogen over a wide range of UV doses (Fig. 3) shows, however, that the presence of the prophage may have a small effect on the survival. This could be interpreted as indicating that a small amount of induction does occur. This interpretation is further bolstered by the results in Table 4, showing that 30 times as many phage are produced from an irradiated Rec⁻ lysogen as from the unirradiated control. Furthermore, all the phage which are detected from the irradiated culture form turbid plaques, whereas about 80% of the phage produced spontaneously are clear plaque mutants.

Lifting of immunity. Formally, the first event in prophage induction may be thought of as the lifting of the repression of vegetative phage multiplication. This repression also extends to closely related phages introduced by infection and is designated as immunity. Lifting of immunity can be detected operationally by exposure of lysogenic cells to UV or other inducing agents and subsequent superinfection with a genetically marked phage. The resulting lysate should contain representatives of both prophage and superinfecting phage. Table 5 shows the results of such an experiment with the Rec⁺ and Rec⁻ lysogens. The prophage was wild-type λ which carries the h^+ and the c^+ markers, and the superinfecting phage carried the *h* and the *c* markers. The lysogens were exposed to a dose of UV normally sufficient for induction. The yield from the Rec⁺ lysogen contains prophage and superinfecting phage types as well as recombinants; the Rec⁻ lysogen produces undetectable amounts of phage of any type. It thus appears that ultraviolet irradiation of the Rec⁺ lysogen is followed by the lifting of immunity, so that both the prophage and the superinfecting phage

TABLE 4. *Phage yield from an irradiated Rec⁻ λ⁺ lysogen*

Phage prepn	Titer	Fraction of clear plaque formers in yield
Free phage in culture prior to treatment[a]	1.8×10^2	61/72
Free phage in culture of unirradiated cells[b]	3×10^1	9/12
Free phage in culture of irradiated cells[c]	1.1×10^3	0/225

[a] A culture of JC-1163 grown to 2.5×10^8 cells per milliliter was centrifuged and the supernatant fluid was assayed for free phage.

[b] The pellet from the centrifugation indicated in footnote *a* was resuspended in phosphate buffer (see Materials and Methods), and was divided into two portions. One portion was added to an equal volume of tryptone broth to bring cell concentration to approximately 2×10^8 cells per milliliter, and was aerated at 37 C for 3 hr. The culture was then centrifuged and the supernatant fluid was assayed for free phage.

[c] The second portion mentioned in footnote *b* was first irradiated for 20 sec before dilution with tryptone broth. Otherwise, its treatment was identical to that described in footnote *b*.

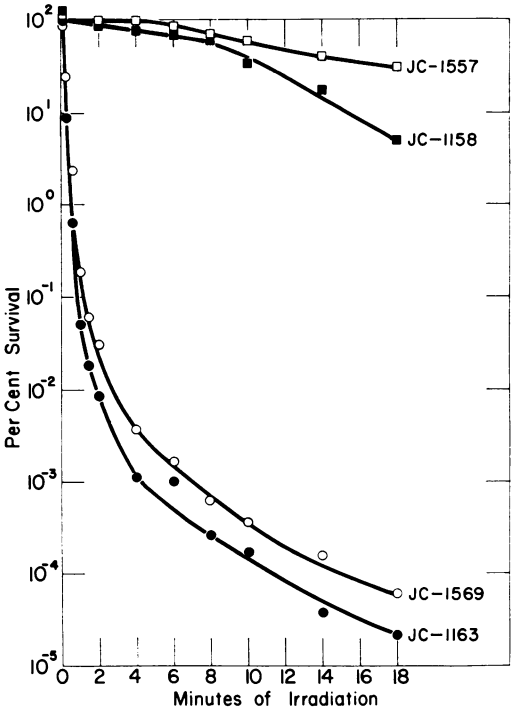

FIG. 3. *Survival curves. Nonlysogens were the Rec⁺ strain JC-1557 and the Rec⁻ strain JC-1569. Lysogens for λ⁺ were the Rec⁺ strain JC-1158 and the Rec⁻ strain JC-1163. Rec⁺ and Rec⁻ strains were grown to approximately 2 × 10⁸ cells per milliliter. The cells were harvested and resuspended in phosphate buffer to which addition of $MgSO_4$ had been made. Cumulative doses of UV irradiation were applied; each minute of exposure corresponds to a dose of approximately 20 ergs per mm².*

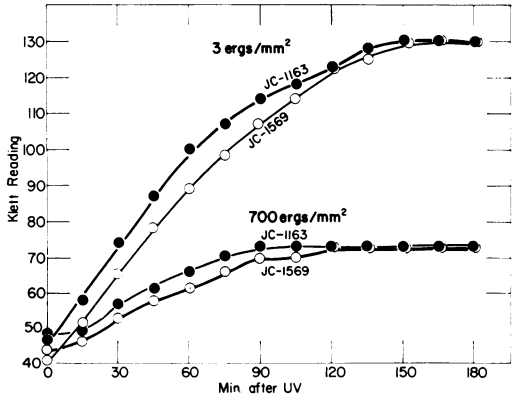

FIG. 4. *Growth of a lysogenic and a nonlysogenic Rec⁻ strain after UV irradiation. Data obtained after irradiation of JC-1163, a Rec⁻ lysogen of λ⁺, similar to that illustrated in Fig. 2, are included for purposes of comparison with data obtained after similar treatment of the Rec⁻ nonlysogenic strain, JC-1569.*

can multiply. The fact that in the irradiated Rec⁻ lysogen neither phage can multiply may be due to the failure of immunity to be lifted or to the inability of the irradiated cells to support the growth of any phage.

We have found, however, that λ can multiply in nonlysogenic Rec⁻ cells irradiated to as low as 10^{-5}% survival. To test the ability of the irradiated Rec⁻ lysogen to support phage multiplication, we repeated the superinfection experiments, using as superinfecting phage two different phages insensitive to λ immunity. The first phage was λ*vir*, which forms clear plaques on lysogenic or nonlysogenic hosts. From Table 6, it can be seen that λ*vir* multiplies as well in the irradiated Rec⁻ lysogen as in the Rec⁺ lysogen. The second phage used was 434hy, which can multiply in λ lysogens. Conversely,

TABLE 5. *Superinfection of an irradiated Rec⁻ lysogen with λhc*[a]

Markers scored	Origin	Time of superinfection after UV	Yield (phage/input cell) from Rec⁺ lysogen		Yield (phage/input cell) from Rec⁻ lysogen	
			Expt 42	Expt 46	Expt 42	Expt 46
		min				
c⁺	Prophage	0	17	14	<0.4	<0.0005
		30	27	12	<0.4	<0.0005
c	Superinfecting phage	0	26	30	<0.4	<0.0005
		30	13	23	<0.4	<0.0005
h⁺	Prophage	0	12	13	<0.4	<0.0005
		30	17	9	<0.4	<0.0005
hc	Superinfecting phage	0	23	23	<0.4	<0.0005
		30	13	23	<0.4	<0.0005
hc⁺	Recombinant	0	0.3	1.6	<0.4	<0.0005
		30	0.6	1.8	<0.4	<0.0005

[a] The UV dose was 525 ergs/mm². The multiplicity of superinfection of the Rec⁺ lysogen in experiment 42 was 1; in experiment 46 it was 7. The multiplicity of superinfection of the Rec⁻ lysogen in experiment 42 was 5; in experiment 46 it was 18. The prophage, λ*h*⁺*c*⁺, makes turbid plaques on the standard indicator C600G. The superinfecting phage, λ*hc*, makes clear plaques on C600G and also on CR63 because of the host range marker. Because the prophage is *h*⁺, it will not make plaques on CR63 but will make speckled plaques on a mixture of C600G and CR63. Turbid plaques on C600G were inferred to be *c*⁺; clear plaques, to be *c*. Speckled plaques on the mixed indicator were inferred to be *h*⁺; clear plaques, to be *hc*; and turbid plaques, to be *hc*⁺. It was not possible to distinguish between speckled clear and speckled turbid plaques, so the reciprocal recombinant class *h*⁺*c* could not be scored.

λ can multiply in lysogens carrying 434hy. Neither phage, of course, can plate on lysogens carrying the same type because of the immunity restriction. Table 7 shows that UV irradiation does not prevent the multiplication of 434hy in the Rec⁻ lysogen, although it appears that it decreases the number of prophage type particles recovered after lysis.

TABLE 6. *Superinfection of an irradiated Rec⁻ lysogen with λvir*[a]

Phage type	Time of superinfection after UV	Yield (phage/input cell) from	
		Rec⁺ lysogen	Rec⁻ lysogen
	min		
Prophage	0	3.6	≦0.01
	40	11	≦0.01
Superinfecting phage	0	32	89
	40	51	112

[a] The UV dose was 525 ergs/mm². The multiplicity of superinfection of the Rec⁺ lysogen was 2; the multiplicity of superinfection of the Rec⁻ lysogen was 8. The yield of free phage was determined by plating on C600G. Turbid plaques were inferred to be prophage type, and clear plaques, superinfecting phage. In other experiments, the yield of free phage was also determined by plating on a λ⁺ lysogen, AC-169; titer of clear plaque formers was the same. No turbid plaques were ever found on the lysogenic indicator.

TABLE 7. *Superinfection of irradiated and unirradiated Rec⁻ lysogens by 434 hy*[a]

Phage type	Exposure to UV	Yield (phage input cell) from	
		Rec⁺ lysogen	Rec⁻ lysogen
	sec		
Prophage λ⁺	0	0.4	0.17
	20	1.5	0.002
Superinfecting phage 434hy	0	32	17
	20	39	23

[a] The multiplicity of superinfection of the Rec⁺ lysogen was 5; the multiplicity of superinfection of the Rec⁻ lysogen was 7. The prophage carries the immunity of λ and will not plate on λ lysogens but will plate on 434hy lysogens. Superinfecting phage is 434hy and will only plate on λ lysogens. The yield was plated on JC-1171 which is a 434hy lysogen and on AC-169 which is a λ lysogen. All plaques appearing on the λ lysogenic indicator were inferred to be progeny of the superinfecting phage; all plaques appearing on the 434hy lysogenic indicator were inferred to be progeny of the prophage.

Having shown that an irradiated Rec⁻ lysogen can support the multiplication of superinfecting phage insensitive to λ immunity, we turn to the other possibility mentioned above, that UV does not result in the lifting of λ immunity, and, hence, no UV-induced multiplication of phage is observed. This interpretation implies that the host must participate in the UV-induced lifting of immunity, because the mutation affecting UV inducibility occurred in the host chromosome prior to lysogenization. One prediction of this hypothesis is that induction of λ in a Rec⁻

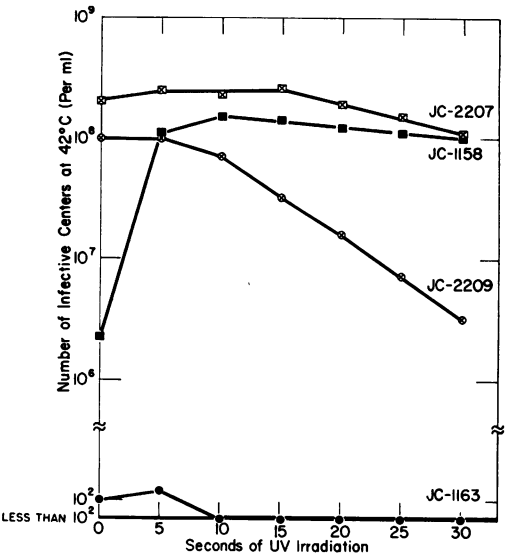

FIG. 5. *Influence of UV irradiation on the transition from prophage to vegetative phage induced by incubation at 42 C. Lysogens of wild-type λ (grown in L medium at 37 C) and lysogens of a temperature-sensitive λ (grown in L medium at 30 C) were harvested during exponential growth and prepared for irradiation as described in Materials and Methods. Doses of UV were given cumulatively, with time for dilution and plating before each additional exposure. All operations were carried on in dim light. The titer of infective centers was determined by adding a sample of irradiated cells to C600G, plating immediately with soft agar, and incubating at 42 C as soon as the agar overlayer had solidified. Plaques were counted after 10 to 15 hr of incubation except in the experiment with JC-1158 when 5 hr of incubation was used. The number of viable cells per milliliter in each suspension, assayed just prior to irradiation, was as follows: JC-1158, the Rec⁺ (λ⁺) lysogen, 2.2 × 10⁸; JC-1163, the Rec⁻ (λ⁺) lysogen, 1.7 × 10⁸; JC-2207, the Rec⁺ (λcI857) lysogen, 2.3 × 10⁸; JC-2209, the Rec⁻ (λcI857) lysogen, 1.3 × 10⁸. After 5 sec of irradiation, the number of viable cells per milliliter of JC-1163 and JC-2209 had been reduced to 3 × 10² and 2 × 10², respectively, whereas the number of viable cells per milliliter of JC-2207 was 2.5 × 10⁸. After 10 sec of irradiation, the number of viable cells per milliliter of JC-1158 was 7.0 × 10⁷.*

lysogen will occur if immunity is lifted without participation of the host. There are two methods for accomplishing this: zygotic induction and temperature induction. In the first case, as a result of conjugation, the prophage escapes the repressive immunity substance in the cytoplasm of the donor, and hence may enter the vegetative phase in the recipient. In the second case, if a phage mutant which produces a thermosensitive immunity substance is present as prophage, induction can occur at a temperature high enough to inactivate the repressor.

It is already known that zygotic induction will occur when a Rec$^-$ recipient is crossed with a λ lysogenic donor (5). Hence, it is clear that all the events subsequent to the lifting of immunity which occur in the transition of prophage to mature phage particles can occur successfully in the Rec$^-$ strain.

To determine whether temperature induction would occur, JC-1557 and JC-1569 were lysogenized with λcI857 (13), which lysogenizes at 30 C and can be induced at 40 C. The two lysogenic strains, JC-2207 and JC-2209, respectively, were found to give normal yields of phage (i.e., about 100 per input cell) incubated at 40 C. We have therefore demonstrated that, under conditions where the repressor can be destroyed by heat, the Rec$^-$ lysogen is induced as efficiently as the Rec$^+$ lysogen.

This discovery permitted us to test the hypothesis that UV induction of λ in the Rec$^-$ lysogen fails because the prophage is inactivated by the irradiation. Rec$^+$ and Rec$^-$ lysogens were exposed to various doses of UV light and were then plated together with C600G at 42 C. Those cells capable of permitting phage multiplication at 42 C to the extent of producing at least one mature virus particle will form plaques and be counted as infective centers. As can be seen from Fig. 5, most of the cells of JC-1158, the Rec$^+$ lysogen of λ$^+$, can be induced to become infective centers by as little as 5 sec of exposure to UV light. By comparison, less than 10^{-4}% of the cells of JC-1163, the Rec$^-$ lysogen of λ$^+$, can be induced by the same exposure. Cells of JC-2209, the Rec$^-$ lysogen of λ*cI857*, exposed to the same dose of UV light produce as many infective centers as they do if unirradiated. Hence, irradiation has not destroyed the ability of the prophage to produce an infective center. To support this conclusion, cells of JC-2209 exposed to 5 and 20 sec of UV irradiation, were incubated in Luria broth at 30 C. At the end of 2 hr of incubation, 65% of the cells initially capable of giving rise to infective centers at 42 C were still capable of this response. Hence, it is clear under these conditions that repression may be lifted at least 2 hr after irradiation with only a slight decrease in the ability of the prophage to become vegetative.

Enzyme activity associated with induction. The production of certain enzymes and phage-specific proteins is correlated with the transition of λ prophage to vegetative phage and is thought to occur as a result of the lifting of immunity after various inducing treatments. The synthesis of one of these enzymes, an exonuclease, is present even after induction of certain suppressor-sensitive mutants in which DNA replication does not occur (11); consequently, its synthesis proceeds independently of DNA replication and is therefore a sensitive measure of the derepression of a prophage function. Table 8 shows the results obtained by Charles Radding, who assayed this protein in mitomycin-treated Rec$^+$ and Rec$^-$ λ lysogens. The table also shows the results of the assay of another protein, β-protein, formed after the induction of λ lysogens (12). Neither protein was detectable in the mitomycin-treated Rec$^-$ lysogen, indicating that repression of phage-specific protein synthesis is not lifted after this inducing treatment.

TABLE 8. *Activity of λ-exonuclease and β-protein in a mitomycin-treated Rec$^-$ lysogen*[a]

Strains and properties	Mitomycin C	Surviving cell[a]	Infective centers[b]	λ-Exonuclease		β-Protein precipitin
				Units /mg	Precipitin	
	μg/ml	%	%			
JC-1158 Rec$^+$ (λ$^+$)	6	28	120	15	+	+
JC-1163 Rec$^-$ (λ$^+$)	1	3	2.5×10^{-4}	0[c]	0[d]	0[d]
JC-1163 Rec$^-$ (λ$^+$)	6	6×10^{-4}	2×10^{-4}	0[c]	NT[e]	NT

[a] We are greatly indebted to Charles Radding of The University of Michigan, who performed the experiments and obtained the data shown in this table.
[b] Measured 20 min after mitomycin treatment.
[c] Measured 40 min after mitomycin treatment.
[d] No detectable activity. Would have detected ~1% of Rec$^+$ (λ) activity under conditions of assay.
[e] No trace of activity. Tail of adjacent precipitin band not deflected at all.
[f] Not tested.

DISCUSSION

Bacteriophage λ can multiply in cells of Rec⁻ strain, JC-1569, with a normal latent period and burst size; it recombines normally in mixed infection of Rec⁻ cells, and lysogenizes the Rec⁻ strain with normal frequency. Hence, the recombination-deficiency mutation carried by JC-1569 affects neither the vegetative multiplication of λ nor the events leading to the reduction of vegetative phage to prophage. Insofar as recombination is involved in these phases of λ biology, either the phage provides the function missing in the Rec⁻ cells, induces the cells to provide that function, or recombines in such a way that the missing function is unnecessary.

Disturbance of the normal biology of λ is evident only when lysogenic Rec⁻ cells are examined to detect the amount of phage produced, either by the spontaneous or the UV-induced transition of prophage to vegetative phage. The small amount of UV induction supported by the Rec⁻ lysogen inferred from the results in Table 4 may result from residual activity permitted despite the mutational defect. It seems possible that immunity may also not be lifted spontaneously during multiplication of lysogenic Rec⁻ cells. The appearance of a majority of clear plaque mutants among the few phage which are produced spontaneously makes it likely that the phage itself must mutate to escape from the repressive effect of the immunity substance.

We have found, as a result of the superinfection experiments, that the lack of UV induction is not due to the destruction of the lysogenic cells' ability to host phage multiplication. Furthermore, as a result of experiments in which thermal induction follows UV irradiation, we have inferred that the lack of UV induction is not due to the destruction of the prophage's ability to multiply. Consequently, we have concluded that UV induction fails because immunity is not lifted in the irradiated lysogenic cells. This conclusion is further supported by the failure of superinfecting phage, sensitive to the repressor produced by the prophage, to multiply in the irradiated lysogenic cells.

Other workers have reached these same conclusions after studying the behavior of λ in recombination-deficient strains of independent origin. T. Ogawa and J. Tomizawa (*personal communication*) have found that "lambda-immunity of non-lysogenic segregants of λb2 infected Rec⁻ cells is not abolished by UV irradiation." I. Hertman and S. Luria (*personal communication*), in the course of their work on AB-2463 (7), have found that "in the irradiated Rec⁻ bacteria the lambda phage is still subject to repression." R. Ben-Gurion (*personal communication*), in her independent work on AB-2463, has found that "immunity is not broken in these cells by irradiation, at doses that do not reduce the capacity of these cells to produce lambda." Van de Putte, Zwenk, and Rörsch reported, however, that this behavior is not characteristic of all recombination deficient strains (16). We have also found that strain AB-2470, isolated by Howard-Flanders and Theriot, when lysogenized with λ⁺, not only responds to UV induction just like the lysogenic derivative of its recombination proficient ancestor, AB-1157, but also produces just as many free phage by spontaneous induction.

There are two major pieces of evidence which suggest that some function of the bacterial cell is responsible for the destruction of immunity following UV irradiation of a λ lysogen. The first was the discovery of a bacterial mutant in which a lysogen of wild-type λ made at 30 C is induced to produce vegetative phage at 40 C (6). In this mutant, a host-determined thermosensitive process is involved in maintaining or destroying the lysogenic state. Secondly, J. Tomizawa and T. Ogawa (J. Mol. Biol., *in press*) have found, not only that the addition of chloramphenicol immediately after irradiation prevents the loss of immunity from a cell possessing repressor, but also that the phage itself does not participate in the destruction of repressor. Our hypothesis that the mutation which confers recombination deficiency on JC-1569 has also affected the ability of JC-1569 to destroy immunity is consistent with this evidence.

A scheme for the participation of the host cell in the destruction of immunity has been provided by Goldthwait and Jacob (6). In this scheme, a small-molecular-weight precursor of DNA is an antagonist of the λ repressor. When DNA is being synthesized normally, this compound would be present in a concentration insufficient to inactivate the repressor. If DNA synthesis ceases, however (because, for example, replication has reached the end of its round or UV irradiation interrupts synthesis), then the increased concentration of this compound would inactivate the repressor. Since neither spontaneous nor UV induction takes place in the Rec⁻ lysogen, JC-1163, it is tempting to suppose that the inhibition of DNA synthesis is unable to cause inactivation of the repressor in this strain. Thus, one might predict that thymine deprivation would be unable to induce λ in a Rec⁻ Thy⁻ lysogen, and, in fact, this has been confirmed by A. L. Taylor and D. J. Cummings (*personal communication*).

Another prediction would be that methods of induction which act directly on the repressor or which physically remove the repressor will be successful in the Rec⁻ strain, and this is what we have found. When the Rec⁻ strain is lysogenic for a mutant of λ which determines a thermo-

sensitive repressor, induction occurs successfully at 42 C, a temperature which presumably destroys the repressor. Zygotic induction also occurs successfully when the Rec⁻ strain is the conjugational recipient, the repressor presumably being left behind in the lysogenic donor cell. Thus, all the steps necessary for the transition from prophage to vegetative phage subsequent to removal of the repressor can be performed in the Rec⁻ strain.

It therefore appears that certain inducing conditions are unsuccessful in the Rec⁻ strain because they are dependent on cellular functions which are defective in these cells, presumably as a result of the same mutation which makes the cells recombination-deficient. To test this hypothesis, it is necessary to see whether the two characteristics can be separated genetically. A preliminary attempt has been described in this paper. Of 40 His^+ Str^R recombinants tested from a cross of JC-1190 with the Rec⁻ lysogen, JC-1163, 37 were Rec^+. All 37 produced phage by spontaneous induction, indicating that, if two mutations are responsible for the recombination-deficient and λ noninducibility phenotypes, they must be closely linked. Further studies are necessary to determine the point conclusively, and these are in progress. At present, however, we consider the existence of two mutations as unlikely.

It should be pointed out that at present there is no evidence that the *rec* mutation affects the primary structure of a protein. If it does, one can assume that the protein is directly involved in recombination. Under this assumption, one can hypothesize that there might be a step in the lifting of immunity which is a recombination event or is biochemically similar to one of the steps in recombination.

Alternatively, one can assume that the protein is only indirectly involved in recombination. Under this assumption, one can hypothesize that there might be a step involving the enzymatic conversion of Goldthwait and Jacob's small-molecular-weight precursor of DNA into an inactivator of λ repressor, and this step would be defective in the Rec⁻ strain. If the same inactivator were necessary to derepress one or more genes determining enzymes involved in recombination, then conditions which might normally inhibit DNA synthesis and consequently induce the formation of recombination enzymes would not do so in this strain.

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