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On the induction of a recombination-deficient mutant of *Escherichia coli* K-12

By RENANA BEN-GURION

Israel Institute for Biological Research, Ness-Ziona, Israel

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1. INTRODUCTION

It was reported (Clark, personal communication) that a recombination-deficient mutant (*rec*$^-$) of *Escherichia coli* K-12, when lysogenized with bacteriophage λ, is not induced by u.v. light. This observation could be explained in several ways: (*a*) The prophage cannot become released from the bacterial chromosome because of the nature of the *rec*$^-$ mutant; possibly an enzyme that can normally free the prophage from its insertion into the bacterial chromosome, by a recombination event or otherwise, is absent in the *rec*$^-$ mutant. (*b*) A possible product(s) produced after irradiation of *rec*$^+$ cells, and responsible for the loss of prophage immunity, is not produced in the *rec*$^-$ mutant in an active or available form; thus irradiated *rec*$^-$ (λ) cells remain immune after irradiation. This paper describes experiments which support the latter hypothesis.

2. MATERIALS AND METHODS

With the exceptions noted below, the materials and methods have been described previously (Ben-Gurion & Hertman, 1958; Ben-Gurion, 1963*a*, 1963*b*, 1965).

(*i*) *Bacteria*

Strains of *E. coli* K-12: (1) A2463 *rec*$^-$ (Howard-Flanders & Theriot, 1966; kindly supplied by Dr R. C. Clowes), a recombinant-deficient mutant, which is *thr*$^-$ (threonineless), *leu*$^-$ (leucineless), *pro*$^-$ (prolineless), *arg*$^-$ (arginineless), *his*$^-$ (histidineless), *ste-r* (streptomycin resistant) and λ *s* (phage λ-sensitive). (2) Two lysogenic derivatives of this strain: A2463 *rec*$^-$ (λ) carrying wild-type phage λ; A2463 (λ *ind*$^-$ C857) carrying a λ mutant, which yields clear plaques at 40°C. and turbid plaques at 30°C., and is inducible by a temperature shift (Sussman & Jacob, 1962; kindly supplied by Dr W. F. Dove). (3) A derivative of A2463 *rec*$^-$, colicinogenic for Colicins E2 and I (prepared as described previously, Ben-Gurion 1965). (4) *E. coli* K-12 PA417, which is *arg*$^-$ (arginineless), *his*$^-$ (histidineless), *pro*$^-$ (prolineless) and λ*r* (λ-resistant).

(*ii*) *Assay of the capacity of irradiated, sensitive bacteria to produce phage*

The standard experiment used for measuring the capacity of irradiated bacteria to produce phage λ is as follows: the infected sensitive bacteria, after treatment with phage-neutralizing antiserum, are plated at appropriate dilutions (sufficient to eliminate any residual antiserum) on a series of plates with *E. coli* K-12, strain C600, as indicator. The background of plaques produced by the free phage that was not completely eliminated by

the serum was determined by killing the infected bacteria with chloroform and then plating as before. The free phage titre is not reduced by this treatment.

(*iii*) *Assay for zygotic induction*

Crosses of *E. coli* strain of HfrC (λ), and of HfrC (λ *ind*$^-$ C857), with a nonlysogenic F$^-$ strain which is λ resistant, were carried out as follows: log phase bacteria were grown and crossed at 28°C. in order to reduce the rate of induction at 37°C. of the males lysogenized with λ *ind*$^-$ C857. The males were washed several times on a millipore filter before mixing with the females, in order to reduce the number of free phage particles in the mixtures. A culture of males alone, diluted with medium instead of the female-culture, was also tested in order to determine the number of free phage particles produced by spontaneous lysis. Since the females used were resistant to λ, they could yield phage only following transfer of prophage by conjugation. The ratio of females to males in mixtures was 5 to 1. These mixtures, and the males controls, were incubated at 28°C. without shaking in 100 ml Ehrlenmeyer flasks for various periods of time, following which samples were withdrawn and tested for infectious centres on an indicator strain resistant to streptomycin, on agar-streptomycin plates. Thus only the free phage, and the streptomycin-resistant females into which the prophage entered and that were zygotically induced, could produce plaques. The number of free phage produced by the males, prior to plating on streptomycin-agar, was determined from the controls, and was substracted from the total number of infectious centres present in the mixture of males and females; thus the frequency of zygotic induction could be calculated.

3. RESULTS

(*i*) *Irradiation of rec*$^-$ (λ) *and superinfection with* λ

If *rec*$^-$ (λ) bacteria lose their immunity after u.v. irradiation, but are still not induced because the prophage cannot get 'unattached' from the bacterial chromosome, or for some

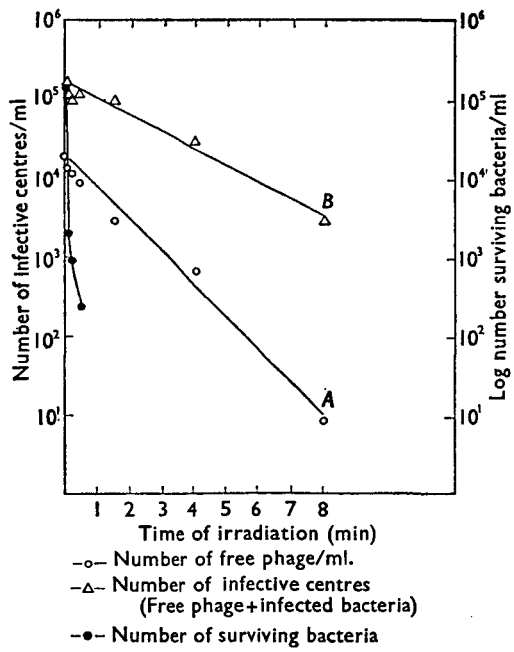

Fig. 1. The capacity of non-lysogenic *rec*$^-$ cells to produce phage λ after various doses of u.v. irradiation.

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other reason inherent in the *rec*⁻ cell, then infection with phage λ from the outside should be productive. If, on the other hand, the reason for the noninducibility of the *rec*⁻ (λ) cells is due to the retention of immunity after irradiation, then superinfection of irradiated lysogenic bacteria with λ should be unproductive. Superinfection of irradiated *rec*⁻ (λ) bacteria was carried out in order to distinguish between these possibilities. The cells were irradiated with various doses corresponding to exposures of 0, 3, 6, 15, 60, 90, and 120 sec. Superinfection of the cells treated with these doses was not productive. To discover the extent to which these results might be ascribed to a lowered capacity to propagate phage λ, due to the high u.v. sensitivity of the *rec*⁻ bacteria (Clark & Margulies, 1965; Howard-Flanders & Theriot, 1966) the capacity of non-lysogenic *rec*⁻ bacteria to produce phage λ was determined. It was found, as may be seen from the results in Fig. 1, that at the doses used for the superinfection experiments of irradiated lysogenic bacteria, the capacity of the *rec*⁻ cells to produce phage λ after infection was hardly impaired. Therefore, the reason for the inability of the irradiated bacteria to produce phage λ upon superinfection is not due to the loss of the capacity to produce phage, but because immunity is probably still unimpaired in these cells.

(*ii*) *Induction of colicinogenic* rec⁻ *cells by u.v.*

Since lysogenic and bacteriocinogenic bacteria that are u.v.-inducible are also induced by other common agents, it seems probable that the primary product(s) that triggers the

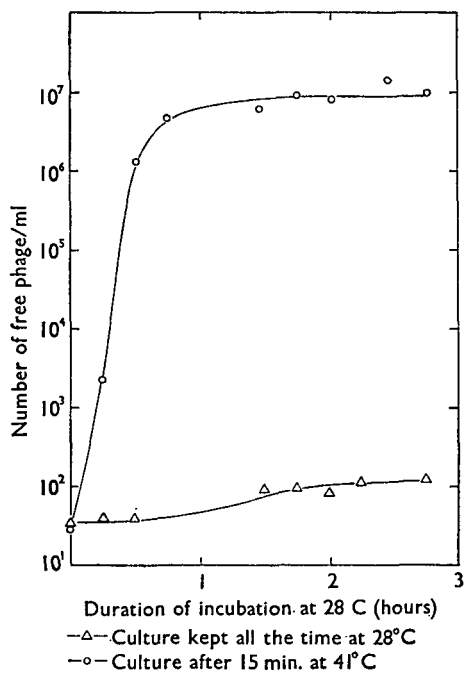

Fig. 2. Induction of *rec*⁻ (λ *ind*⁻ C857) by a temperature shift. Log phase bacteria (*rec*⁻ λ *ind*⁻ C857) grown in minimal medium were washed then resuspended in fresh minimal medium. One sample of these bacteria was kept all the time at 28°C., a second sample was immersed in a water bath at 41°C. for 15 min. then transferred to 28°C. and incubated there for 3 hours. To samples withdrawn from the two cultures at various time intervals, chloroform was added and the number of free phages determined.

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induction in these two systems might be identical. If this product(s) is responsible for the loss of immunity in the lysogenic system and, in the light of the above evidence, is also not produced in *rec*$^-$ (λ) cells, then colicinogenic bacteria of the *rec*$^-$ type should not be inducible by u.v. although, presumably, the production of colicin in no way depends on recombination. We therefore infected *rec*$^-$ cells with the colicinogenic factor E2 which is inducible in *rec*$^+$ cells (the infection was carried out with *col* I and *col* E2 in the manner described by Ben-Gurion, 1965) and these bacteria were irradiated and tested at u.v. doses of 0, 3, 6, 15, 30, 60, 120 sec. Unlike the *rec*$^+$ controls, there was no induction of *col* E2 at these doses.

(*iii*) *Induction of* rec$^-$ *bacteria lysogenic for a lambda mutant which is induced by a temperature shift*

The induction of a mutant of phage λ (λ *ind*$^-$ C857) by a temperature shift of the lysogenic bacteria, is probably due to different products of the treated cell than in the case of wild phage λ. Therefore, if the noninducibility of wild λ in *rec*$^-$ bacteria is due to the deficiency of some factor that is produced after irradiation in *rec*$^+$ cells, then prophage λ *ind*$^-$ C857 might be induced in *rec*$^-$ cells. On the other hand, if in *rec*$^-$ cells the prophage is not induced because the prophage cannot escape the chromosome, then a temperature shift should not induce λ *ind*$^-$ C857, assuming that this prophage is also inserted into the bacterial chromosome. Results summarized in Fig. 2 show that *rec*$^-$ (λ *ind*$^-$ C857) cells are indeed induced by a temperature shift.

To test whether λ *ind*$^-$ C857 is transferred during conjugation, a zygotic induction experiment was carried out. As may be seen from Fig. 3, zygotic induction occurs when males lysogenic for λ *ind*$^-$ C857 are crossed with females which are not lysogenic but resistant to λ, so that the prophage enters the female with the male chromosome.

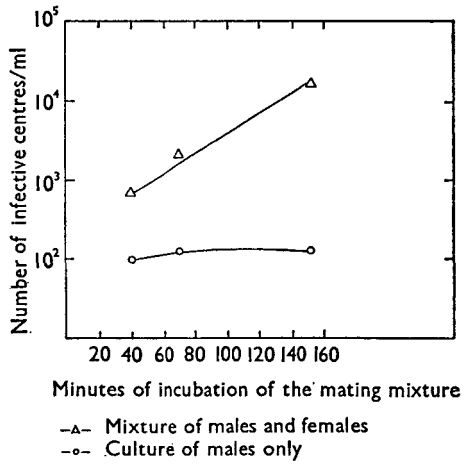

Fig. 3. Zygotic induction in a cross between HfrC (λ *ind*$^-$ C857) and strain PA417 which is F$^-$, nonlysogenic and lambda-resistant. Cells of strain HfrC (λ *ind*$^-$ C857) that had grown in minimal medium at 28°C., were harvested in log phase by washing on a millipore filter. 0·4 c.c. of the washed cells resuspended in minimal medium were added to 1·6 c.c. of log culture of F$^-$ cells (also grown in minimal medium at 28°C.). The final cell concentration was approximately 3×10^7 HfrC cells per ml. and 2×10^7 F$^-$ cells per ml. The mixture was incubated at 28°C. without shaking in 100 ml. Erlenmeyer flasks. At various periods of time 0·5 ml. was withdrawn, shaken and tested for infective centres. A parallel culture in which 0·4 ml. of washed resuspended HfrC cells were added to 1·6 ml. of fresh minimal medium was treated in the same fashion in order to ascertain the titre of phage produced by spontaneous lysis.

4. DISCUSSION

The results summarized in this paper suggest that a product or products produced in *rec*$^+$ strains after irradiation and responsible for the loss of immunity of the lysogenic cells are not produced, at least not in an active form, in the *rec*$^-$ mutant. If, however, such a product was produced in *rec*$^-$ cells, but the reason for the noninducibility of these bacteria was the inability of the prophage to become detached from the bacterial chromosome, then superinfecting phage would be able to grow in the non-immune irradiated cells. Since superinfecting phage does not grow in the irradiated cells, it follows that they have retained their immunity. That is, immunity is not broken in these cells by irradiation at doses that do not reduce their capacity to produce λ.

Since *rec*$^-$ bacteria carrying an inducible, colicinogenic factor are also not induced by u.v. it seems probable that the same product(s) induces both systems in *rec*$^+$ cells, but is lacking in irradiated *rec*$^-$ cells and is unconnected with release of prophage from the chromosome. Moreover, the fact that a prophage which is inducible by elevating the temperature can be so induced in *rec*$^-$ bacteria, supports this contention. It has been shown (Hertman, personal communication) that u.v. irradiation prior to a temperature shift does not affect the induction of *rec*$^-$ (λ *ind*$^-$ C857). This would eliminate the possibility that u.v. interferes with the process of prophage induction in *rec*$^-$ cells.

SUMMARY

A lysogenic, recombination-deficient bacterial mutant (*rec*$^-$) can be induced to produce λ *ind*$^-$ C587 by a temperature shift, although it does not produce phage after u.v. irradiation when lysogenic for wild λ. When this mutant (*rec*$^-$) was made colicinogenic for colicins E2 and I, colicin production was found not to be inducible.

Superinfection of irradiated lysogenic *rec*$^-$ (λ) bacteria, at doses of irradiation that did not reduce their capacity to produce phage λ, was not productive. It seems therefore that a certain product(s) that usually arises following u.v. irradiation in *rec*$^+$ cells, and triggers the induction of lysogenic and colicinogenic cells by causing loss of immunity or repression, is not produced in the *rec*$^-$ mutant. However, immunity is lost and the prophage is induced after a temperature shift in *rec*$^-$ cells which are lysogenic for λ *ind*$^-$ C857.

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