Host Conversion by Prophage Lambda in a Recombination-deficient Mutant of Escherichia coli

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SUMMARY

A recombination-deficient (rec-) strain, AB2463, of Escherichia coli K12 yielded λ lysogens of two types. A rare form, AB2463(λ)(ind), differed from the usual type, AB2463(λ), in showing ultraviolet (u.v.) irradiation induction of the prophage, forming normal numbers of conjugational recombinants and displaying a u.v. response curve similar to that of the parental rec+ strain, AB1157. Superinfection of the inducible form with phage 21b2 led either to loss of the prophage or to the infrequent production of a form which was still lysogenic but no longer inducible. In both cases the bacteria regained their rec- condition as a result of the curing treatment. The phage released from AB2463(λ)(ind) was normal λ. It is concluded that the rec+ phenotype of AB2463(λ)(ind) derives from the presence of the prophage and represents a case of host conversion. It is proposed that AB2463(λ)(ind) is doubly lysogenic for λ and that partial curing yields the rec- single lysogen AB2463(λ).

INTRODUCTION

Production of phage and immunity to the homologous phage are properties common to all lysogenic strains of bacteria. Sometimes, however, the lysogen acquires properties whose relationships to lysogeny are not immediately evident; that is, the bacteriophage itself appears to be playing a part in determining the bacterial phenotype. Examples of this phage-directed conversion include changes in the morphology of cells (Wollman & Wollman, 1938), in the appearance of colonies (Ionesco, 1953) and, more strikingly, the synthesis of surface antigens (Izeki & Sakai, 1953; Uetake & Hagiwara, 1961) or the production of toxin by diphtheria bacilli (Freeman, 1951). In each example there is a perfect correlation between the acquisition of new heredity properties and the establishment of lysogeny. Lieb (1967) reported a change in colonial morphology in Escherichia coli due to the presence of defective λ prophage; this example of conversion differs from those already referred to in that the host can regain its normal phenotype without loss of the prophage.

A description is given here of another case of host conversion. After lysogenization with wild-type λ phage a rec- mutant of Escherichia coli K12 displays rec+ properties in respect of conjugational recombinant formation and response to u.v. irradiation: the bacteria show normal sensitivity and are proficient in the system that leads to liberation of phage. Loss of the prophage coincides with a return to rec- status. That is, prophage λ can, albeit very rarely, complement the recombination deficiency of its host.

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METHODS

Bacteria. The following derivatives of Escherichia coli K12 were used: AB1157F\(^{-}\), thr\(^{-}\), leu\(^{-}\), gal\(^{-}\), str\(^{-}\) kindly supplied by Dr P. Howard-Flanders; AB2463, a rec\(^{-}\) mutant of AB1157 (Howard-Flanders & Theriot, 1966); HfrHthy\(^{-}\), str\(^{-}\)s kindly supplied by Dr W. Hayes.

Bacteriophages. Phage λ, a clear plaque mutant λc and a virulent mutant λv (Jacob & Wollman, 1954); phage 21b2 kindly supplied by Professor R. Pritchard; phage T4 and a T4rII mutant (Benzer, 1955).

Media. Nutrient broth was prepared from Oxoid No. 2 broth powder at a concentration of 25 g./l. and nutrient agar (NA) was made by adding Davis agar, 12.5 g./l. of broth. The composition of minimal medium was that described by Tatum & Lederberg (1947), but lacking asparagine, and minimal agar was obtained by adding Davis agar, 15 g./l. of minimal medium. Amino acid supplements were added to a final concentration of 20 μg./ml and thiamine to a final concentration of 1 μg./ml. Soft agar used in overlays contained Difco agar, 6 g./l. Streptomycin (250 μg./ml) was added to agar.

Phage techniques. The general phage techniques used were those described by Adams (1950).

Bacterial crosses. Logarithmic phase donor and recipient cultures were mixed in appropriate volume ratios to give final concentrations of about 2 \(\times\) 10\(^7\) Hfr cells and 4 \(\times\) 10\(^8\) F\(^{-}\) cells per ml. The mating mixtures were incubated on a rotor at 37\(^\circ\) for 2 hr. The cells from 5 ml of each mixture were then centrifuged, washed in buffer and resuspended in 1 ml buffer. After suitable dilution, 0.1 ml samples were plated in triplicate for the isolation of recombinants on appropriate selective media. The donor was contraselected by addition of streptomycin to the plating medium.

Ultraviolet-light irradiation. Ultraviolet (u.v.) light irradiation was carried out with a 15 W Hanovia Bactericidal Ultraviolet Unit with the suspension placed at a distance of 29 cm from the centre of the lamp (intensity: 3.9 ergs/mm.\(^2\)/sec. at a distance of 57 cm.). Except where stated otherwise, phages or bacteria in complex media were resuspended in buffer and 3 ml samples were irradiated in uncovered watch-glasses.

Infection with λ and determination of host cell response. A log. phase culture of each strain, grown in broth supplemented with 0.01 M-MgSO\(_4\), was mixed with wild-type λ phage and the mixture incubated at 37\(^\circ\) for 20 min. to allow adsorption of phage. Free phage was subsequently removed by diluting into broth containing λ antiserum (\(k = 10\)) After incubation for 10 min. at 37\(^\circ\), 0.2 ml samples were withdrawn from the mixture, appropriately diluted and plated with λ-sensitive indicator bacteria on NA. Further 0.2 ml samples of the adsorption mixture were withdrawn and appropriate dilutions spread on NA plates. Plates were incubated at 37\(^\circ\) for 24 hr, when the cells giving a lytic response formed plaques. Surviving colonies of the inducible strains were replica-plated (Lederberg & Lederberg, 1952) to NA layered with soft agar containing λ-sensitive indicator bacteria and these plates were u.v.-irradiated for 30 sec. to induce lysogenic bacteria. After incubation the replica plates showed the lysogenic colonies surrounded by a halo due to lysis of the indicator bacteria by the liberated λ, while the non-lysogenic colonies had no halo. Each lysogenic colony was tested for stability by growing it overnight in broth in the presence of antiserum, plating for colonies on NA and replica-plating to indicator bacteria in soft agar on a NA plate, followed by u.v. induction. Only stable lysogenic colonies were taken into account.
Surviving colonies of non-inducible strains were picked into broth and incubated overnight at 37°C before plating samples in soft agar on NA and spotting phages λν, λc, T4 and T4rII on the bacteria to identify lysogenic and resistant colonies; λ lysogens are not lysed by λc and T4rII, while λν and T4 serve as controls for detecting λ-resistant and T4-resistant colonies.

_U.v. induction._ To determine their response to u.v. irradiation, bacterial survivors of infection with wild-type λ were picked from the centres of isolated plaques formed by the phage on each host strain. Colonies obtained in this way were purified by streaking twice on NA plates followed by replica-plating to duplicate plates layered with soft agar containing λ-sensitive indicator. Half of these plates were then u.v.-irradiated for 30 sec. and all plates were incubated at 37°C for 24 hr. For rec+ strains, colonies liberating phage were picked and streaked out twice on agar for purification. In the case of rec- lysogens not liberating phage after u.v. treatment, a random selection of colonies was picked and purified in the same way. For both rec+ and rec- strains a single colony from each purified isolate was then transferred to nutrient broth and, after overnight incubation, each culture was examined for its lysogenic or resistant character in the manner described above.

_Prophage curing._ A young broth culture of each λ lysogen was superinfected with phage 21 b 2 at a multiplicity of 5 and the mixture incubated at 37°C for 20 min. before appropriate dilution and spreading of samples on NA plates. Non-lysogenic survivors were identified by their lack of response to u.v. induction in the case of inducible lysogens, and their sensitivity to phages λν, λc, T4 and T4rII in the case of non-inducible lysogens.

Table 1. Response to infection with wild-type λ phage

<table>
<thead>
<tr>
<th>Strain</th>
<th>Multiplicity of input</th>
<th>Multiplicity of infection*</th>
<th>Response (%) to λ infection†</th>
<th>No. of survivors tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB 1157</td>
<td>10</td>
<td>8.8</td>
<td>Lytic 8, Resistant 7, Lysogenic 85</td>
<td>100</td>
</tr>
<tr>
<td>AB 2463</td>
<td>10</td>
<td>8.7</td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

* Calculated from the phage remaining unadsorbed at the end of the adsorption period.
† Expressed as number of lysogenic/lytic/resistant colonies/total number of cells.

**RESULTS**

The frequency of λ lysogenization in a rec− strain of *Escherichia coli* K12, AB 2463, was comparable with that in the rec+ parental strain, AB 1157 (Table 1). While AB 1157(λ) releases its prophage after u.v. irradiation, reports from other workers and earlier experiments in this laboratory indicated that the rec− lysogen AB 2463(λ) was not u.v.-inducible. However, in the course of examining the response to u.v. irradiation of 300 lysogenic isolates of AB 2463, each one derived from an independent lysogenization event, two of the isolates were found to release λ phage after an inducing dose of 30 sec. irradiation. In subsequent tests both behaved identically and one only is referred to below as AB 2463(λ)(ind). It seemed likely that such an inducible variant would be simply a lysogenic rec+ revertant of the rec− AB 2463 strain.

AB 2463(λ)(ind) was compared with the rec− non-inducible form AB 2463(λ) and the parental rec+ lysogen AB 1157(λ) in respect of u.v. sensitivity and showed a u.v.
response curve close to that of the parental rec+ type (Fig. 1). In a test of recombining ability the three lysogens were compared in crosses with the donor HfrH. AB 2463(λ)(ind) could be classed as phenotypically rec+ (Table 2).

To determine whether AB 2463(λ)(ind) was merely a rec+ revertant or whether it represented a unique association between the prophage and its host, an attempt was made to cure the lysogen. Clearly, if after losing its prophage the strain retained its rec+ characteristics then it was a rec+ revertant. However, if prophage curing led to a loss of its rec+ properties then the rec+ phenotype could be equated with the presence of the prophage and the phenomenon would represent a case of host conversion. Superinfection of the λ lysogens with phage 21b2 was found to be the only reliable means of effecting curing.

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**Fig. 1. U.v. survival curves for λ lysogens.** Logarithmic phase broth cultures were harvested and washed twice in buffer before irradiation. Immediately after irradiation, samples were withdrawn and appropriate dilutions plated in soft agar on NA before incubating for 24 hr at 37°. ▲—▲, AB 1157(λ); ◦—◦, AB 2463(λ); ■—■, AB 2463(λ)(ind).

**Table 2. Characteristics of λ lysogens**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Spontaneous induction</th>
<th>U.v. induction</th>
<th>U.v. sensitivity</th>
<th>thr+leu+ recombination frequency* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB 1157(λ)</td>
<td>+</td>
<td>+</td>
<td>R</td>
<td>11</td>
</tr>
<tr>
<td>AB 2463(λ)</td>
<td>−</td>
<td>−</td>
<td>S</td>
<td>0.02</td>
</tr>
<tr>
<td>AB 2463(λ)(ind)</td>
<td>+</td>
<td>+</td>
<td>R</td>
<td>9.5</td>
</tr>
</tbody>
</table>

+, positive response; −, negative response; R, resistant; S, sensitive.

* Frequency recombinants per 100 input HfrH cells.
Phage 21 is a temperate coliphage related to λ serologically but having a different attachment site; bacteria lysogenic for λ are still sensitive to this phage. 21b2 is a deletion mutant which cannot be integrated as prophage since it lacks the region of its chromosome whose function is required for integration at its specific site on the host chromosome; it gives instead an abortive lysogenic response. In the abortively lysogenized cell the phage genome does not form part of the bacterial chromosome but remains as a singular, non-multiplying structure in the cytoplasm of the host. At each

Fig. 2. U.v. survival curves for non-lysogens and cured λ lysogens. ■—■, AB 1157; ●—●, AB 2463; ○—○, AB 2463(λ)(ind) cured; △—△, AB 2463(λ) cured.

Table 3. Prophage curing and characteristics of cured strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>21b2 superinfection λ curing frequency* (%)</th>
<th>Characteristics of cured strains</th>
<th>gal+str-r recombination frequency† (%)</th>
<th>No. of colonies tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB 1157(λ)</td>
<td>33</td>
<td>R</td>
<td>12.5</td>
<td>40</td>
</tr>
<tr>
<td>AB 2463(λ)</td>
<td>41</td>
<td>S</td>
<td>0.01</td>
<td>40</td>
</tr>
<tr>
<td>AB 2463(λ)(ind)</td>
<td>2</td>
<td>S</td>
<td>0.04</td>
<td>40</td>
</tr>
</tbody>
</table>

* Expressed as number of cured colonies/total number of lysogenic colonies tested.
† Frequency recombinants per 100 input HfrH cells.
R, resistant; S, sensitive.
cell division the abortive lysogen gives rise to a non-lysogenic, \( \lambda \)-sensitive daughter cell that does not harbour either phage or prophage.

The precise mechanism for the curing effect of phage 21b2 is not known. For \( \text{AB}2463(\lambda)(\text{ind}) \) the value obtained for efficiency of curing, using this method, was about one-twentieth of that given by \( \text{AB}2463(\lambda) \) (Table 3). All apparently cured colonies, as indicated by non-inducibility, of the lysogen \( \text{AB}2463(\lambda)(\text{ind}) \) were picked into broth and incubated overnight at 37\(^\circ\). These cultures were then checked for the presence or absence of prophage \( \lambda \) (sensitivity of plated culture to phages \( \lambda y, \lambda c, T4 \) and \( T4 r1I \)) and tested for u.v. sensitivity and recombining ability (Fig. 2, Table 3). It is seen that cured derivatives of \( \text{AB}2463(\lambda)(\text{ind}) \) displayed a \( \text{rec}^- \) phenotype; it can be concluded, therefore, that the \( \text{rec}^+ \) characteristics of \( \text{AB}2463(\lambda)(\text{ind}) \) derive from the presence of the prophage.

Among the non-inducible colonies of 21b2-treated \( \text{AB}2463(\lambda)(\text{ind}) \) were found a small proportion (4 \%) of colonies that still contained prophage \( \lambda \) although they possessed the \( \text{rec}^- \) phenotype of demonstrably cured colonies. This anomaly can be explained if it is supposed that \( \text{AB}2463(\lambda)(\text{ind}) \) is doubly lysogenic for \( \lambda \). The observed phenomenon would then represent a case of partial curing involving only one prophage of the doubly lysogenic cells; the partially cured strain might then be identical to \( \text{AB}2463(\lambda) \).

In view of its possible double prophage complement, the u.v. inducibility of
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AB2463(λ)(ind) was examined in more detail and compared with that of the parental rec+ lysogen AB1157(λ). A determination was made of infective centres in dilutions of u.v.-irradiated cultures on λ-sensitive indicator bacteria (Fig. 3). For AB2463(λ)(ind) the number of induced cells increased more rapidly with increasing dose, the maximum of induction was achieved at a lower dose and thereafter the number of plaque-forming centres decreased more rapidly than for AB1157(λ).

Samples, appropriately diluted, of a u.v.-induced lysate of each of the lysogens were plated with a λ-sensitive indicator strain and after overnight incubation at 37° an examination was made for any difference between the two lysates in respect of phage phenotype. No difference was observed. In order to detect any specific genotypic characteristics, the λ lysate obtained from AB2463(λ)(ind) was used to infect AB2463. A lysogenization frequency value of 76 % was obtained and this compares with a value of 78 % obtained using wild-type λ (Table I). One hundred lysogenic colonies derived from independent isolations in this infection experiment were characterized and all conformed to the rec– AB2463(λ) type. It may be concluded that AB2463(λ)(ind) contained normal λ and not a mutant prophage endowed with the specific capacity to convert its host from the rec– state to a phenotypically rec+ condition.

DISCUSSION

The evidence presented here indicates that λ lysogenization proceeds normally in the rec– strain AB2463. Other workers have arrived at the same conclusion for this and other rec– mutants (Brooks & Clark, 1967) but reports have not hitherto appeared of the unusual host-converting effect of λ discovered in AB2463(λ)(ind), a rare u.v.-inducible form of AB2463. If Campbell’s model for phage integration is correct (Campbell, 1962) and taking into account other observations on this phenomenon (Rothman, 1965; Franklin, Dove & Yanofsky, 1965; Signer, 1966), then for lysogenization to occur in rec– bacteria the necessary recombination event must be mediated by the infecting phage. The demonstration that the rec+ properties of AB2463(λ)(ind) can be attributed to the presence of prophage λ points to another situation in which the recombination function of the phage operates in its host, either complementing the deficient bacterial recombination mechanism or functioning as a mediator of bacterial recombination in toto.

As a consequence of chromosomal integration of infecting phage, the usual result in both rec+ and rec– bacteria is that the cl region of the λ prophage functions to control the expression of other prophage genes, preventing vegetative multiplication and phage release (Campbell, 1962; Jacob & Monod, 1961). Ptashne (1967) found that λ repressor, the protein made by the cl gene, binds specifically and with high affinity to λ DNA and suggested that the simplest model for the action of the repressor is correct, namely that the repressor blocks transcription from DNA to RNA by this direct binding to DNA. The unique phage + host relationship referred to by me would seem, on the basis of a number of observations, to be the result of a rare lysogenization event leading to the formation of a double lysogen in which the λ repressor does not block the recombination function of the phage genome. This lysogen class would not be recognized in wild-type rec+ bacteria if, as it seems, the only indication of its occurrence in rec– bacteria is their conversion to a phenotypically rec+ condition.

Takano (1966) examined recombination between two different R factors, two
mutants of phage \( \lambda \) and two mutants of phage T\(_4\) in a \( \text{rec}^- \) mutant of \textit{Escherichia coli} and found that it occurred normally, without conferring \( \text{rec}^+ \) properties on the host bacteria. Consequently he suggested that non-integrated phage and such episomes as F' factors and R factors produce specific recombination enzymes different from those of the host. Clearly, when \( \lambda \) is integrated in the host chromosome, as it seems to be in AB\(2463(\lambda)(\text{ind})\), a different situation exists in which the phage recombination enzyme is no longer specific but can supplement or substitute for the bacterial recombination enzyme.

In general, when \( \text{rec}^- \) mutants carry the \( \lambda \) prophage they are unable to release \( \lambda \) phage either spontaneously or in response to u.v. irradiation (Brooks & Clarke, 1967; Fuerst & Siminovitch, 1965; Hertman & Luria, 1967). In \( \text{rec}^+ \) cells, induction by u.v. irradiation is probably caused by inactivation of \( \lambda \) repressor (Tomizawa & Ogawa, 1967) by a substance produced after irradiation (Goldthwaite & Jacob, 1964; Hertman & Luria, 1967; Witkin, 1967). If a product of the \( \text{rec}^+ \) gene is required for conversion of a DNA precursor into the substance responsible for inactivation of the \( \lambda \) repressor (Brooks & Clark, 1967; Ben-Gurion, 1967) then in \( AB2463(\lambda)(\text{ind}) \) the prophage complement can supply the recombination function. Additionally, if it is supposed that in the double lysogen \( AB2463(\lambda)(\text{ind}) \) one of the prophages is less effectively repressed, hence the expression of its recombining function, then a smaller amount of the substance will be required to inactivate any repressor activity. A smaller dose of u.v. will then be sufficient to elicit maximum induction. We have seen that this is so. The steeper nature of the latter part of the induction curve for \( AB2463(\lambda)(\text{ind}) \), after maximum induction, suggests that this strain represents a \( \text{rec}^- \) bacterium with \( \text{rec}^+ \) properties contributed by the prophage complement. The structural organization of \( AB2463(\lambda)(\text{ind}) \) is considered elsewhere (Erskine, 1969).

The observation that a prophage can occasionally take over such an important host cell function as recombination may be relevant in studies with latent viruses in other virus+host systems.

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