Transfection of *Escherichia coli* Spheroplasts: Infectious Lambda Prophage DNA

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**SUMMARY**

High mol. wt. DNA was extracted from *Escherichia coli* lambda lysogens and was shown to be infectious. Its infectivity was due to prophage DNA integrated into the host chromosome rather than to DNA released from mature phage particles, as established by the following criteria: the titre of infectious DNA exceeded by 100-fold the titre of infectious units present before DNA extraction; mild shear selectively reduced prophage DNA infectivity to 2% of the un-sheared DNA while lambda phage DNA infectivity retained 50% of its infectivity; DNA extracted from an *E. coli* (lambda c857 tsxlsaz) lysogen yielded 200 times as many plaques on sup+ than on sup- spheroplasts. Thus lambda prophage DNA infectivity depends on expression of the excision gene while the infectivity of non-integrated forms of lambda does not. About 10^4 genome equivalents of *E. coli* DNA yielded one infectious centre unit in this assay system; this high infectivity should make prophage DNA a useful marker in genetic transformation experiments.

DNA extracted from lysogens of *Haemophilus influenzae* (Harm & Rupert, 1963; Boling et al. 1972) and *Bacillus subtilis* (Romig, 1968; Rutberg, 1969) was infectious and several properties of this infectivity showed that it was caused by infectious prophage DNA and not some form of replicating phage DNA (Peterson & Rutberg, 1969; Rutberg et al. 1969; Rutberg, 1971; Boling et al. 1972). The infectivity of prophage DNA has been a useful property for examining prophage excision and the replication of φ105 DNA (Armentrout & Rutberg, 1971; Rutberg, 1973), for studying the sensitivity of various forms of DNA to host nucleases (Rutberg et al. 1969; Rutberg, 1971; Boling et al. 1972) and for investigating the requirement of host recombination functions for excision and replication (Boling et al. 1972; Barnhart & Cox, 1973). In coliphage systems, a special lambda phage carrying both the phage and host attachment sites in a larger than normal chromosome was constructed (Nash, 1974); the sensitivity of this phage to the presence of chelating agents in the plating agar was used to distinguish it from the wild type product of intramolecular recombination which is insensitive to chelating agents (Nash, 1974). With this assay, recombination of lambda DNA, both in vivo (Gottesman & Gottesman, 1975a; Nash, 1975a) and in vitro (Gottesman & Gottesman, 1975b; Nash, 1975b) has been studied in detail.

In this paper we report the results of a study of the infectivity of linear DNA extracted from *E. coli* cells lysogenic for phage lambda.

Lysogenic cultures were grown in modified Fraser & Jerrel medium (Henner et al. 1973) to a titre of 5 × 10^8 cells/ml, centrifuged at 8000g for 10 min and resuspended in one-twentieth volume of 0.05 M-tris buffer, pH 8.5, containing 0.02 M-EDTA. The number of infectious units/ml was measured and the cells were treated with lysozyme, followed
by pronase and sodium dodecyl sulphate as described by Schekman et al. (1971). The viscous suspension was extracted three times in a slowly rotating tube with distilled phenol and the aqueous phase was dialysed against 0.01 M-tris buffer, pH 8.1, containing 1 mM-EDTA, and stored in a sterile tube at 4 °C.

The size distribution of DNA extracted from a lambda lysogen was examined by sedimentation of 3H-labelled DNA on neutral 4 to 30 % sucrose gradients; 90 % of the label and 90 % of the infectivity sedimented ahead of an infectious lambda phage DNA marker and 10 % sedimented ahead of a 14C-labelled T4 phage DNA marker. Therefore, it will be assumed that our DNA extraction procedure did not break a significant fraction of the lambda prophage DNA molecule and the number of prophage DNA molecules will be equated with the number of cells present before DNA extraction.

The infectivity of nucleic acid extracts from a lambda lysogen was due to DNA because the infectivity was resistant to pronase and RNase but sensitive to DNase (data not shown). Infectivity of lambda lysogen DNA was stable for one week at 4 °C. E. coli host DNA inhibited transfection assays at concentrations above $3 \times 10^8$ genome equivalents/ml; thus all transfection assays with prophage DNAs were carried out at lower DNA concentration. Even at the lower DNA concentrations used in our experiments, transfection was still inhibited; when lambda phage DNA marked with a c26 mutation was mixed with c+ prophage DNA, the infectivity of c26 phage DNA was 10 % of the value obtained with c26 phage DNA alone. To avoid shear, all prophage DNAs were diluted with 1 ml plastic pipettes. All DNA dilutions were performed with 0.01 M-tris buffer, pH 8.1.

The following evidence is presented to show that the infectivity of the DNA from lambda lysogens is due to integrated prophage DNA and not some form of replicating lambda phage DNA. The titre of DNA infective centres from lambda prophage DNA was $4 \times 10^5$/ml while the titre of infectious units before DNA extraction was only $2 \times 10^5$/ml. Thus most of the DNA infectivity must be derived from DNA other than mature phage DNA synthesized in spontaneously induced cells. The experimental results presented in Table 1 make it even less likely that the prophage DNA is significantly contaminated with replicating phage DNA. DNA from c857ts prophage was mixed with lambda phage DNA marked with a c mutation and assayed for infectivity at 32 °C; a nearly equal number of turbid (prophage DNA) and clear (phage DNA) plaques was obtained (Table 1). However, mild shear selectively reduced the infectivity of the prophage DNA to 2 % of the initial value while the infectivity of the phage DNA remained at 50 % of the unsheared value. This result indicated that the infective prophage DNA is contained in large, shear-sensitive segments of DNA. Table 2 presents the results obtained when prophage DNA from a (lambda c857ts xisam6) lysogen was used to transect sup+ and sup− spheroplasts. The corrected titre on sup+ spheroplasts is 200-fold higher than on sup− spheroplasts. Thus the xis+ gene function was required for the infectivity of 99 % of this prophage DNA; in contrast xisam6 phage do not require xis function to form plaques. The sum of these data shows that the infectivity of our prophage DNAs resides with integrated lambda genomes and not with replicating forms of lambda phage DNA; this is true even for lysogens where spontaneous induction is not blocked by the recA mutation, such as the K 300 sup+ (lambda c857ts xisam6) lysogen.

Previous experiments with linearly integrated, infective prophage DNA from Bacillus subtilis and Haemophilus influenzae lysogens distinguished this infectivity from that of phage DNAs by its faster sedimentation (Boling et al. 1972), by its different behaviour in marker rescue experiments (Armentrout & Rutberg, 1970) or by the presence of bacterial markers which could be transferred along with phage markers (Peterson & Rutberg, 1969).
Table 1. Shear sensitivity of infectious lambda prophage DNA*

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Infective centres on three plates</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, c857ts prophage DNA mixed with c8a phage lambda DNA</td>
<td>441 turbid</td>
<td>100</td>
</tr>
<tr>
<td>c857ts prophage DNA mixed with c8a phage lambda DNA and sheared for 10 s on a Vortex mixer</td>
<td>212 clear</td>
<td>48</td>
</tr>
</tbody>
</table>

* DNA was extracted from E. coli JC 2946 (lambda c857ts) recA12 F- leu- thi- lac Y+ galK- ara-14 xyl-5 mtl- proA74 hisG7 arg E+ str-3 toxA-3 supA44. Five x 10^9 genome equivalents/ml of DNA were mixed with 5 x 10^8 lambda c8a DNA molecules/ml and assayed for infectivity with E. coli W3350 spheroplasts; the remaining DNA was sheared for 10 s on a Vortex mixer at a setting of 6 (1 ml of DNA in a 2 x 150 mm test-tube) and assayed with the same spheroplasts. Each assay tube contained 1 x 10^8 genome equivalents of prophage DNA and 1 x 10^8 lambda c8a phage DNA molecules.

Table 2. Dependence of transfection efficiency on prophage excision within the spheroplast

<table>
<thead>
<tr>
<th>DNA</th>
<th>Infective centres on</th>
<th>Relative infectivity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sup+ spheroplasts</td>
<td>sup- spheroplasts</td>
</tr>
<tr>
<td>Phage λ</td>
<td>420</td>
<td>1287</td>
</tr>
<tr>
<td>KB-3 su+ (λ c857xisam6)</td>
<td>60</td>
<td>1</td>
</tr>
</tbody>
</table>

* To correct for differences in the competence of sup+ (KB-3) and sup- (W3110) spheroplasts the relative plating efficiency of the prophage DNA was calculated as follows:

\[
\frac{\lambda \text{ prophage DNA infective centres on } sup^- \text{ spheroplasts}}{\lambda \text{ prophage DNA infective centres on } sup^+ \text{ spheroplasts}} \times \frac{\lambda \text{ phage DNA infective centres on } sup^+ \text{ spheroplasts}}{\lambda \text{ phage DNA infective centres on } sup^- \text{ spheroplasts}}
\]

This correction is necessary because of strain and day-to-day variations in spheroplast competence. Lambda c857ts xisam6 phage released from the lysogen plated with equal efficiency on sup+ and sup- bacteria.

We used different criteria to reach the same conclusion, that the infectivity from lambda lysogens is due to integrated prophage DNA: the titre of infectious DNA exceeds the infectious units present before DNA extraction by 100-fold; mild shear selectively reduced the infectivity of prophage DNA while the infectivity of lambda phage DNA was hardly affected (Table 1) – this result indicates that the lambda genome is integrated into large shear-sensitive segments of DNA; most importantly, lambda prophage DNA with a suppressible defect in the excision gene xis plated with greater efficiency on sup+ than on sup- spheroplasts (Table 2). This shows that almost all of the infective DNA required an intact xis function which is required for infectivity of prophage DNA but not of free forms of phage DNA (as shown by the fact that xisam6 phage plate equally well on sup+ and sup- bacteria – Table 2). Since the lysogen from which the excision-defective prophage DNA was isolated carried an efficient suppressor, spontaneous excision of the prophage was not suppressed; thus prophage DNA even from wild type lysogens is probably contaminated with less than 1 % of infectious phage DNA or infectious replicative intermediate (Table 2).

About 1 in 10^4 genome equivalents of lambda prophage DNA will form an infective...
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centre on E. coli W3350 spheroplasts (Table I); this efficiency is very close to that obtained
for lambda phage DNA since the presence of host nucleic acids reduces the phage DNA efficiency
from the usual $10^{-3}$ to $10^{-4}$. The nearly equal infectivities of lambda prophage and lambda phage DNAs also indicate that the prophage excision, which is required to permit a successful infective cycle, takes place efficiently with lambda prophage DNA.

The relatively high efficiency of transfection of lambda prophage DNA and its ease of isolation (purification of the DNA is not necessary) make it a useful marker for genetic transformation studies, now that genetic transformation of E. coli has been demonstrated (Cosloy & Oishi, 1973). Since techniques have been devised to move lambda prophage to other attachment sites besides that between the host gal and bio region (Shimada et al. 1972), it can be used as a marker in many different locations on the host chromosome. Furthermore, φ80, 21 and 434 phage are all close relatives of lambda. It is to be expected that their prophage DNA will have the same properties as infective lambda prophage DNA. Thus it should be possible to construct multiple lysogens with different prophages of different immunities inserted at different sites on the chromosome. Using density- and radioactive-labelling techniques with synchronized cells, the direction and type of DNA replication of E. coli could be analysed in a manner similar to experiments performed with B. subtilis (Quinn & Sueoka, 1970).

We have examined the infectivity of DNA extracted from P1 lysogens; $10^8$ genome DNA equivalents yielded only 1 infective centre. DNA from mutator phage lysogens ($>10^9$ genome equivalents) yielded no infective centres. Thus both of these prophage DNAs possess an infectivity too low to conduct useful experiments.

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