SPONTANEOUS PRODUCTION OF LAMBDA PARTICLES
WITH TRANSDUCING ACTIVITY¹

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THE transductions by lambda bacteriophage have been described previously
(MORSE, LEDERBERG and LEDERBERG 1956a,b; MORSE 1957; E. LEDERBERG
1960; WEIGLE 1957). The lambda particles accomplishing transduction have
been shown to be defective (ARBER, KELLENBERGER and WEIGLE 1957; ARBER
1958; CAMPBELL 1957; CAMPBELL and BALEBINDER 1959). The origin of trans-
ducing particles in haploid cultures of Escherichia coli K-12 has not been es-

tablished. One possibility is that they are produced from pre-existing cells
(homogenotes) which are merely induced to form phage by the radiation ex-
posure. Alternatively the transducing particles could be the direct consequence
(fortuitous chromosome breaks) of the ultraviolet radiation used to induce phage
growth. A high statistical variance in number of transducing particles would be
observed if there were pre-existing cells because of fluctuations in the time of
formation of these cells. If the UV irradiation were the cause of transducing
particles, lysates produced from a series of independent cultures by a constant
UV dose might be expected to have a low statistical variance with regard to the
number of transducing particles they contain.

We wish to report that some lambda particles produced spontaneously are

capable of transducing Gal genes. This provides, in cultures capable of adsorbing
lambda, a mechanism for forming homogenotic clones spontaneously (Gal⁻ — x
Gal⁻ → Gal⁺/αGal⁻) and leading to high “between culture” variance. How-

ever, analysis of a series of cultures incapable of adsorbing lambda particles also
yielded a high variance, a finding which indicates that homogenotes are not the
sole source of high “between culture” variance.

MATERIALS AND METHODS

The cultures employed were: (only relevant genotypes given)
W945   F⁻ Lp⁺ Mal⁻ Gal⁻
W2580  F⁻ Lp⁺ Gal⁻
M550   F⁻ Lp⁺ Gal⁻/αGal⁻
(F, fertility factor; Lp⁺, lambda lysogenic; Lp⁻, lambda sensitive; Mal, maltose
fermentation; Gal, galactose fermentation.) W945 is the parent culture of

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W2580, and differs from W2580 only in the pleiotropic Mal\(^-\) mutation which prevents adsorption of lambda by \textit{E. coli} K-12 cells and which also results in a failure to ferment maltose (E. Lederberg 1955). Thus W2580 is capable of adsorbing spontaneously produced phage, whereas W945 is not.

To detect transductions unequivocally, it is necessary to eliminate \textit{Gal}\(^+\) reversions in the culture used as an indicator. For this purpose a doubly mutant culture was selected. Such doubly mutant cultures are produced by recombination in a heterogenotic clone. The culture employed, M550, is \textit{Gal}\(^-\)\textit{Gal}\(^-\). The spontaneous probability per bacterium per division for reversion of each of these loci is about 10\(^{-10}\) (Morse, unpublished experiments). The probability of change of phenotype of M550 from \textit{Gal}\(^-\) to \textit{Gal}\(^+\) should be about 10\(^{-20}\) per bacterium per division. \textit{Gal}\(^+\) reversions of M550 have never been encountered in this laboratory.

To ascertain whether spontaneously produced lambda particles possess transducing activity, the supernates of 24-hour cultures in penassay broth were collected. These cultures, started from separate single colonies, were grown at 37\(^\circ\)C on a circular rotating rack. After incubation the cells were sedimented, the supernate removed and preserved with a few drops of chloroform. The supernates were assayed for plaque-forming lambda particles on nutrient agar. Transduction assays were made by mixing 0.1 ml of undiluted lysate with about 10\(^8\) M550 cells on cholate indicator agar (Morse and Alire 1958) containing galactose. The method for assaying for transducing particles, because of the low multiplicities employed may underestimate the real number of transducing particles (Campbell 1957). However, such low multiplicities may yield information on the nature of the particles accomplishing transduction.

RESULTS

Supernates of 39 independent W945 cultures were examined for plaque and transducing particles. The results are shown in Table 1. Nine supernates produced 27 \textit{Gal}\(^+\) clones on application to M550 cells. These are believed to have been transductions of the form:

\[
\text{Gal}_5^- \text{Gal}_1^+ \text{Gal}_3^+ \rightarrow \text{Gal}_3^+ \text{Gal}_1^- \text{Gal}_5^- \quad \text{(gene order not implied)}
\]

The \textit{Gal}\(^+\) clones formed were streaked out serially four times, each time from a single \textit{Gal}\(^+\) colony to test whether they were heterogenetic or not. Of the 27 \textit{Gal}\(^+\) clones, three were heterogenetic, for they segregated \textit{Gal}\(^-\). A number of independently derived \textit{Gal}\(^-\) were collected from each heterogenote and typed for \textit{Gal} genotype (Morse et al. 1956b). The results of the typing are given in Table 2. These results conclusively show that in these cases the transductions had been of the form proposed above and had resulted in heterogenotes of the genotype:

\[
\text{Gal}_5^+ \text{Gal}_1^- \text{Gal}_3^-/\text{ex} \text{Gal}_3^+ \text{Gal}_1^- \text{Gal}_5^+
\]

The remaining 24 \textit{Gal}\(^+\) clones failed to yield \textit{Gal}\(^-\) segregrants in four consecutive streaks. All 27 transductants were lysogenic for plaque-forming lambda. Pre-
TABLE 1

Transducing activity of spontaneously produced lambda

<table>
<thead>
<tr>
<th>Supernate number</th>
<th>Number transductions</th>
<th>Classification of transductions</th>
<th>Number lambda plaque particles (x 10^8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Heterogenotic</td>
<td>Nonheterogenotic</td>
</tr>
<tr>
<td>1-30*</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>9</td>
<td>13</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>16</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>24</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>29</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>33</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>34</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>37</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>39</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>40</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>27</strong></td>
<td><strong>3</strong></td>
<td><strong>24</strong></td>
</tr>
</tbody>
</table>

* Thirty supernates containing 1.3 x 10^7 plaque particles were assayed and yielded no transductions. All of the transductions listed above were lysogenic for lambda.

** The Gal+ papillae occurring on the transduction assay plates were streaked out serially four times and the streaks examined for Gal-, an indication of segregation from the heterogenotic condition.

TABLE 2

Classification of segregants from heterogenotic transductions

<table>
<thead>
<tr>
<th>Genotypes of segregants</th>
<th>Heterogenote number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gal-&lt;sup&gt;-&lt;/sup&gt; Gal-&lt;sup&gt;-&lt;/sup&gt; (Endogenotic)</td>
</tr>
<tr>
<td>24</td>
<td>39</td>
</tr>
<tr>
<td>37</td>
<td>67</td>
</tr>
<tr>
<td>40</td>
<td>27</td>
</tr>
</tbody>
</table>

It was observed previously (Morse 1955) in transductions to Lp<sup>-</sup> recipients with but a single Gal<sup>-</sup> mutation, about ten percent of Gal<sup>-</sup> papillae (putative transductions) observed after exposure to LFT lysates (low frequency transducing lysates, transduction frequency 1/10<sup>5</sup>λ) were found Lp<sup>-</sup>. The observation above, that all transductions were lysogenic indicates that the Lp<sup>-</sup> observed earlier were probably spontaneous Gal<sup>-</sup> reversions of the recipient culture which were not lysogenized secondarily on the assay plates.

The incidence of lysogenicity (100%) among the transductions reflects the amount of secondary lysogenization on the assay plates, which in a separate reconstruction experiment was ascertained at not less than 95 percent, and not that nondefective lambda particles were accomplishing the transductions. The amount of secondary lysogenization observed is significantly higher than that observed previously (Morse et al. 1956a) and is presumably owing to the difference in media between the two experiments.

It was observed previously (Morse et al. 1956a) that 0.4-0.9 of lambda transductions resulted in clones of cells heterogenotic for Gal. The difference in frequency of heterogenotes between these values and the frequency obtained with
spontaneous lambda (three of 27 segregating, 0.11) is significant (least \( x^2 = 9.8, \) 1 df) and suggests that spontaneous lambda particles are different (with regard to yielding heterogenotes) from transducing particles in LFT lysates.

Since spontaneously produced lambda particles may possess transducing activity and thereby form homogenotic clones, a variance analysis of samples from 20 independent cultures capable of adsorbing phage should show high variance, which is the case (Table 3). In contrast with this, replicate samples from a single culture show the expected variance, which is equivalent to the mean. If the formation of homogenotes by lambda transduction were the sole mechanism involved in the formation of LFT lysates, then a culture incapable of adsorbing lambda particles would not be expected to yield a lysate with transducing activity. This is not the case (Table 3). A perhaps surprising observation is the high variance of samples from the series of cultures incapable of adsorbing phage. This finding may be explained by several possibilities: (1) if lambda transduction is the mechanism for the origin of spontaneous homogenotes in these cultures, then lambda need not reach the stage of free particles, and the whole process may take place intracellularly; (2) there is a separate process (or processes) with the proper statistical characteristics which results in lambda particles with transducing activity.

Previously (Morse, unpublished) attempts were made to detect clones giving HFT (high frequency transducing) phage in haploid cultures and to isolate them. The experiments were as follows: (1) a master plate prewarmed to 37\( ^\circ \)C was inoculated with a film of \( 10^8 \) Gal\( ^{-} \) cells and this plate incubated at 37\( ^\circ \)C for several hours to allow for growth; (2) from the master plate after growth a number of replicates were made by replica plating. These replicas were also incubated for several hours at 37\( ^\circ \)C after which all but one was replicated to indicator plates spread with Gal\( ^{-} \) recipient bacteria, and the indicator plates irradiated with ultraviolet. The indicator plates were incubated for 2–3 days at 37\( ^\circ \)C. The single unused replicate was refrigerated.

The indicator plates were compared (after incubation) for the location of Gal\( ^{+} \) papillae (transductions plus reversions) on them. If there had been clones of cells on the master plate with an increased potential for giving transduction,

TABLE 3

<table>
<thead>
<tr>
<th>Genotype ( ^{+} )</th>
<th>Number of cultures</th>
<th>Survival</th>
<th>Number of transductions per 0.1 ml sample</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Mal^{+} )</td>
<td>20</td>
<td>3.2 ± 1.7</td>
<td>436</td>
<td>14896.0</td>
</tr>
<tr>
<td>( Mal^{-} )</td>
<td>20</td>
<td>3.2 ± 1.6</td>
<td>142</td>
<td>3347.6</td>
</tr>
<tr>
<td>( Mal^{+} )</td>
<td>1</td>
<td>1.7</td>
<td>438</td>
<td>591.0</td>
</tr>
<tr>
<td>( Mal^{-} )</td>
<td>1</td>
<td>1.6</td>
<td>194</td>
<td>133.8</td>
</tr>
</tbody>
</table>

* \( Mal^{+} \) cultures are capable of absorbing lambda; \( Mal^{-} \) incapable.

† In the case of multiple cultures the cell survival is the mean of cultures induced to yield LFT phage. In the case of single cultures the values given are for the culture assayed. Similarly for numbers of transductions per 0.1 ml sample. Assay of transducing particles was made at a multiplicity of five plaque-forming lambda particles per cell. A plot of numbers of transductions against survival in the two series experiments indicated no correlation between numbers of transductions and survival in the induced cultures. Thus the minor variations in survival cannot be the source of the variances given.
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these would have served as a template and been replicated, and the Gal+ arising from transductions on the indicator plates would show a consistent pattern, corresponding to this template. No pattern of Gal+ was found on the indicator plates suggesting a template.

The Gal+ on the indicator plates were picked and streaked serially in CGal medium to see if they were heterogenotic. Many Gal+ were segregating. The areas corresponding to randomly selected segregating Gal+ on the indicator plates were located on the unused, refrigerated replicate, and cultures were inoculated from these areas. With these cultures the entire experiment was repeated, with the hope that the selected areas might show an increased frequency of Gal+ on the second round indicator plates. No increased yield of Gal+ was observed.

A portion of these experiments have been repeated recently employing an indicator culture which was doubly Gal-, and thereby lowering the background owing to spontaneous reversions of the indicator culture. Again no pattern among the Gal+ was observed on the indicator plates.

DISCUSSION

The results presented show that spontaneously produced lambda particles may have the ability to transduce Gal genes and that these particles are similar, except in yielding more nonheterogenotic clones, to transducing particles in lysates produced by irradiating haploid cultures with UV. The transducing particles are produced rarely, about one per $3 \times 10^5$ plaque-forming lambda particles. This frequency is probably not significantly different from that observed previously in LFT lysates (Morse et al. 1956a), and suggests that the effect of UV is to stimulate phage production generally, and not to produce transducing particles specifically.

Because spontaneously produced particles may transduce Gal genes, clones of homogenotic cells may be formed in cultures of cells capable of adsorbing phage. Attempts to isolate clones of homogenotic cells from haploid cultures have been unsuccessful. The failure could be explained on the basis of the small number of cultures studied (less than ten), since the data of Table 1 show that only nine of 39 cultures produced spontaneous phage with transducing activity. This frequency, 9/39 is an underestimate, but indicates an overall low probability of success in an experiment employing a small number of cultures.

The high variance observed with cultures incapable of adsorbing lambda particles can be explained on the basis of several hypotheses. At the present time there is no basis for choosing among the alternatives. The isolation from a non-adsorbing culture of a homogenotic double lysogen, lysogenic for normal lambda, and for lambda dg, would suggest an intracellular "transduction," but could also be explained on the basis of a transitory lapse in expression of the Mal- gene which allowed the adsorption of a free transducing lambda particle. It has been found previously (Morse, unpublished) that rare cells in E. coli K-12 cultures incapable of adsorbing lambda will serve as recipients for lambda transduction if
exposed to HFT lambda preparation. Approximately one transducing particle in $10^6$ has been found to transduce to these recipients.

**SUMMARY**

Spontaneously produced lambda particles have been found to transduce $Gal$ genes. Production of spontaneous transducing particles in cultures capable of adsorbing lambda should result in homogenotic clones ($Gal^-_{+e}Gal^-_{-}$). Attempts to isolate homogenotic clones from haploid cultures were unsuccessful. Because homogenotes can be formed spontaneously, high statistical variance in numbers of transducing particles should occur between a series of independent cultures. A high variance was found, for both a series of lambda adsorbing and non-adsorbing cultures. The high variance with nonadsorbing cultures indicates that a mechanism, other than the formation of homogenotic cells, exists for the formation of transducing lambda particles.

**LITERATURE CITED**


**CAMPBELL, A., and E. BALBINDER, 1959** Transduction of the galactose region of *Escherichia coli* K-12 by the phages $\lambda$ and $\lambda-434$ hybrid. Genetics 44: 309–319.


