Defective Particle Assembly in Wild Type P2 Bacteriophage and its Correction by the lg Mutation

By G. BERTANI, E. LJUNGQUIST, K. JAGUSZTYN-KRYNICKA* AND S. JUPP†

Microbial Genetics Laboratory, Karolinska Institutet, 104 01 Stockholm, Sweden

(Accepted 15 August 1977)

SUMMARY

The mutation lg of phage P2 has been located on the genetic map of P2 to the right of, and closely linked to, the del2 deletion, probably within tail gene F. The lg mutation causes larger burst sizes, compared with the wild type, especially at high incubation temperatures. The frequency of defective particles is lower in preparations of P2 lg than in those of wild type P2. It seems that the mutation lg improves the efficiency of particle assembly.

INTRODUCTION

The temperate coliphage P2 has an icosahedral head and a contractile tail. We show here that its mutation lg, long known for its effect on plaque size and burst size, affects some property of the phage tail and increases the efficiency of assembly of the virus particles.

METHODS

Bacteriological substrates and other solutions. LB broth and agar, TPG+CAA, low PTPG+CAA, SSC, are described either in Lindqvist (1971) or in Bertani & Bertani (1970). EGTA is aminoethoxyethane-tetra-acetic acid (Hopkin & Williams).

Bacteriophages. All are derivatives of P2 (Bertani & Bertani, 1971). P2 lg (Bertani, Choe & Lindahl, 1969) is presumably a point mutation since it cannot be seen in DNA heteroduplexes with P2 wild type DNA (Chattoraj, Younghusband & Inman, 1975) and does not affect particle density significantly (Bertani, 1975). P2 vir1 (a mutation in gene C: clear plaques, immunity sensitive) is described by Bertani (1960); amB116, amD6, amR3, amH13, amT5 and their combinations with vir1 are described by Lindahl (1971); tsD4 vir1 by Lindahl (1969); del2 lg, lg vir1, tsD4 vir22 lg? cc? by Bertani (1975).

The combination del2 lg tsD4 was isolated as a recombinant from a cross del2 lg amD6 (see Table 1) × tsD4 vir1; the presence of the lg marker was recognized by means of a progeny test (crossed with vir1 amB116 it gave some lg plaques among ts+ am+ recombinants).

The combination amL302 del2 lg was isolated as a recombinant from a cross lg del2 × amL302 lg? tsD4 vir22, through the following steps. Mutation amL302 was isolated from lg after hydroxylamine mutagenesis; it gives no complementation with amL9 of Lindahl (1971). Crossed with tsD4 vir22 lg? it gave an amL302 tsD4 recombinant, unclassified as to the lg

* Permanent address: Institute of Microbiology, University of Warsaw, Poland.
† Present address: Institute of Molecular Biology, University of Paris, France.
marker. The latter was again crossed with tsD4 vir22 lg?, and amL302 lg? tsD4 vir22 was obtained.

The origins of other marker combinations used are given in the footnotes to Table 1.

For preparation of phage stocks and for plating techniques, see Bertani & Bertani (1970). Bacteria. All are derivatives of *Escherichia coli* strain C. C-1757 (Sunshine et al. 1971) was the standard indicator for plating and making stocks of P2: it is auxotrophic, streptomycin resistant, and suppresses amber mutations. Other derivatives used were: C-1a (Bertani, 1968), C-1100 (Sironi, 1969), C-1844 (Sunshine et al. 1971), C-1966 (Bertani, 1975), and the thymine-requiring, host-cell reactivation defective HF4704 of Howard-Flanders (Lindqvist & Sinsheimer, 1967).

Bacteriophage crosses. The techniques employed were exactly as in Bertani (1975, Table 5). C-1757 were used as the host bacteria. All crosses were performed with ultraviolet irradiated phage to promote recombination which is otherwise exceptionally low in P2. This probably accounts for a greater degree of irreproducibility in the recombination data than one might expect for other phage material. The genetic map for ultraviolet irradiated P2 is circular (Bertani, 1975), and this should be taken into account when interpreting recombination data.

Temperature shift-up (-down) experiments. Bacteria of strain C-1a exponentially growing with aeration in LB broth with 2.5 mM-CaCl₂ at 30 °C (37 °C for the shift-down experiment) were concentrated by centrifugation and suspended in similar medium at 4 x 10⁸/ml (8 x 10⁸/ml in Fig. 3b). They were infected with a small volume of phage at 30 °C (42 °C for the shift-down experiment) at multiplicities well below 1. After 5 or 10 min the mixture was appropriately diluted into growth tubes, at the same temperature, to be assayed at intervals for phage. A set of growth tubes was also prepared at the higher temperature (lower for the shift-down experiment), and samples of infected bacteria were diluted at intervals into such tubes from one of the former growth tubes. After 90 min (80, for the shift-down experiment) this second set of tubes was chilled, and each tube was assayed for phage. Adsorption was always very good so that unadsorbed phage could be neglected. Although calcium was also present in the growth tubes, dilutions in the growth tubes were such that losses of progeny phage through readsoption were negligible.

Measurement of ³H-thymidine incorporation. An overnight stationary culture of HF4704 in TPG+CAA medium with 10 μg thymine/ml was diluted 1:100 in the same medium and incubated with aeration at 37 °C until a cell density of about 10⁸/ml was reached. Mitomycin C was added to a final concentration of 44 μg/ml, and incubation continued for 10 min in darkness. The bacteria were then centrifuged in the cold, resuspended in 1/10 volume of TPG+CAA with 2 μg thymine/ml and infected while on ice with a small volume of phage. After 5 min the mixture was shifted to 42 °C and diluted 10-fold with warm TPG+CAA containing 2 μg unlabelled thymine/ml and (methyl-³H)-thymidine (Radiochemical Centre, Amersham, U.K.: about 20 Ci/mmol) to obtain a final activity corresponding to 1 μCi/ml. At various times, 0.5 ml samples were added to 0.5 ml aliquots of 20% TCA, left on ice for 30 min, and centrifuged. The pellets were resuspended in 0.5 ml 1 N-NaOH, left overnight at 37 °C, neutralized with 0.5 ml 1 N-HCl, and again precipitated by addition of 1 ml 20% TCA and 1 drop of fish sperm DNA solution (1 mg/ml). After 30 min at 0 °C, the samples were filtered on Whatman GF/C filters, washed, dried and counted in a scintillation counter with POPOP + PPO (Packard) in toluene. As part of the same experiment, at 10 min after infection, appropriate dilutions were made in TPG+CAA with thymine (10 μg/ml), and further incubated at 42 °C for burst size measurements.

³H-labelled phage preparation. A culture of HF4704 was grown with aeration in TPG+
Defective phage assembly

CAA medium with thymine (10 μg/ml) to a density of 1 to 2 × 10^6/ml. It was then centrifuged and resuspended to volume in TPG + CAA with 2.5 μg thymine/ml. 3H-thymidine (50 μCi/ml) and phage (to obtain an input phage to bacteria ratio of 5) were added, and the culture shifted to 40 °C, with aeration. After 20 min, EGTA was added to 0.01 M. Lysis occurred 5 to 10 min later. The lysates were clarified by centrifugation at low speed, then spun for 3 h at 25,000 g. The pellets were resuspended in SSC, treated with DNase (140 μg/ml) for 30 min at 37 °C in the presence of 14 mM-MgSO4, spun again for 3 h at high speed, resuspended in SSC, and clarified at low speed.

32P-labelled phage preparation. Cultures of C-1757 in low P TPG + CAA supplemented with arginine and tryptophan were grown at 37 °C with aeration to a cell density of 4 × 10^8/ml. Carrier-free 32P-phosphate was added (about 100 μCi/ml) and the cells were infected with phage (to obtain an input ratio of 5), and shifted to 30 °C. After 90 min EGTA (0.01 M final concentration) was added. The lysates were clarified by low speed centrifugation, then spun for 3 h at 25,000 g, and the pellets were resuspended in SSC. They were then diluted 10-fold in 0.15 M-NaCl, and incubated for 30 min at 37 °C after addition of MgSO4 (to 14 mM) and DNase (to 140 μg/ml).

Fractionation of phage preparations. Phage samples about 2 ml in volume were layered on top of a CsCl step gradient in SSC (0.5 ml of density 1.7 g/ml, and 2.5 ml of density 1.4 g/ml) and spun at 20,000 rev/min in an SW50 Spinco rotor for 90 min. Fractions of about 0.2 ml were collected dropwise from the bottom of the centrifuge tubes. A small sample from each fraction was precipitated with TCA and counted for radioactivity (see section on thymine incorporation).

Electron microscopy. Phage samples from CsCl step gradients were dialysed against 0.05 M-tris, 0.01 M-MgSO4, pH 7.5, mounted on copper grids coated with collodion, stained with 2 % uranyl acetate, and observed in a Philips 301 electron microscope.

RESULTS

The spontaneous mutation lg (Bertani, Choe & Lindahl, 1969) was detected originally because it made P2 plaques slightly larger compared with the wild type at 37 °C. We have noted that the differential effect on plaque size is temperature dependent. Wild type and lg plaques are in fact indistinguishable at 30 °C; at higher incubation temperatures, the wild type plaques become rapidly smaller, while the lg plaques are only marginally affected. This result is reflected in the burst size which is different for the two phages (Fig. 1). There is no difference, however, in latent period. In summary, phage P2 is naturally sensitive to high temperatures and the lg mutation makes it less so.

The effect of lg is noticeable (Fig. 1) also in the presence of the virr mutation, although, in this case, the effect is smaller. The burst size of P2 virr already tends to be larger than that of P2 wild type (see Discussion).

Since P2 and P2 lg particles (whether virr or not) have identical heat stabilities, we tried to see if the two phages differed in some step of the reproductive process.

First, we measured the rate of incorporation of a DNA precursor in mitomycin C-treated bacteria infected with either P2 or P2 lg at high temperature. The drug blocks host cell DNA synthesis, so that the incorporation observed reflects phage DNA synthesis (Lindqvist & Sinsheimer, 1967). The results (Fig. 2) show that phage DNA synthesis begins at about 8 min from infection and proceeds at a linear rate. No convincing difference is observed between the two phages (especially when the different multiplicities of infection are taken into account). Under the conditions of the experiments, the usual difference in burst size between the two phages was noted (90 for P2 lg virr against 30 for P2 virr).
Next, we investigated the effect on the final burst size of shifting the incubation temperature at different times in the course of one-step growth experiments. The results (Fig. 3) show that P2 infected bacteria differ from bacteria infected with P2 \( l g \) in the latter part of the latent period. One aspect of the curves of Fig. 3 is unexplained: in shift-up experiments (Fig. 3\( a \) to \( d \)), one notes a period, beginning at about 35 min and terminating (see Fig. 3\( d \)) shortly before the end of the latent period, during which the shift itself has a strong yield-depressing effect. It would seem that during this period the infected bacteria are particularly sensitive to a temperature increase. Fig. 3\( (d) \) clearly shows that the effect is much stronger.
Defective phage assembly

Fig. 2. Incorporation of radioactive thymidine in mitomycin C-treated bacteria following infection with P2 vir (m.o.i. 2.4; solid circles) or P2 lg vir (m.o.i. 3.5; open circles) at 42 °C (technical details in Methods). The data as plotted were corrected by subtracting the incorporation data obtained in parallel with control, uninfected bacteria (dashed line). The two continuous lines are fitted by the least squares method.

than the one obtained if the infected bacteria spend the whole latter half of the latency period at the higher temperature (for example with shift-up at 25 or 32 min). [In the only shift-down experiment (Fig. 3f), there is a suggestion of the opposite effect: an increase of the final burst size when the shift occurs after 27 min.] This effect interferes to some extent with the evaluation of these experiments. It is, however, evident with both lg and lg + genotypes. A temperature-sensitive period attributable to the difference in genotype, lg v. wild type, clearly follows the stage discussed above. The difference is noted both in a lysogenizing system (Fig. 3c, d) and when the immunity repressor is inactive due to the vir mutation (Fig. 3a, b), both in shift-up (Fig. 3a, b, c, d) and shift-down (Fig. 3f) experiments. According to Lindahl (1974) the temperature-sensitive period for several P2 ts mutants affected in late functions begins about 12 min before lysis. We obtained a similar result for a mutation in the tail gene D (Fig. 3e). It can be seen that the period of temperature sensitivity of wild type P2 as compared with P2 lg may well coincide with the temperature-sensitive period for late functions.

These facts indicate that P2 lg + phage is somewhat defective for one of the functions required to make or to assemble the protein structures of the phage particles. This view is supported by two other sets of observations.

Several years ago, D. H. Walker (personal communication), studying P2 lysates, fractionated by means of equilibrium density centrifugation in CsCl, in the electron microscope, noticed that non-infectious, DNA-containing, tailless phage heads were present in some preparations and could be easily separated by their higher density than the normal, tail-bearing
Fig. 3. One-step growth curves at 30 or 42 °C (•—•) with the results of corresponding temperature shift experiments (○—○). The latter indicate the burst sizes eventually obtained after shifting the temperature at various times during phage multiplication.

The abscissa shows the time of sampling (in min) for phage titration (one-step growth curves) or of dilution into broth at the higher or lower temperature (temperature shift experiments). (a, b, c, d, e) One-step growth curves at 30 °C with shift-up to 42 °C; (f) growth curve at 42 °C with shift-down to 30 °C. (a) P2 vir1; (b) P2 lg vir1; (c) P2; (d) P2 lg; (e) P2 tsD4; (f) P2. Note that in (a) and (b) the base-line (number of phage-yielding bacteria) seems to increase at about two-thirds of the latent period. This is probably significant but remains unexplained (residual division of infected cells? early lysis of a small fraction of the population?). It may be connected with the peculiar effect of the temperature shift discussed in the text.
Defective phage assembly

Fig. 4. Fractionation of radioactively labelled phage preparations by sedimentation in a CsCl step gradient (see Methods). (a) P2, and (b), P2 lg, labelled with $^{32}$P at 30 °C. Phage titre/ml in $10^9$ units given by solid bars; $^{32}$P in 20 µl samples, ct/min in $10^3$ units shown by the histogram. (c) P2 virr, and (d), P2 lg virr, labelled with $^3$H-thymidine at 40 °C. Total active phage in original lysates was $6 \times 10^{10}$ and $10^{11}$, respectively.

particles. Similarly, in the course of purification of $^{32}$P or $^3$H-thymidine-labelled P2 lysates by centrifugation in CsCl (equilibrium or band sedimentation) a radioactive, but biologically inactive band, of heavier density, or faster sedimenting than normal, has also been noted (B. Lindqvist, personal communication, and our own observations). Considering what is known for other bacteriophages, it is likely that a variety of factors (the strain of host bacteria
used in the preparation of lysates, medium, temperature, etc.) affect the frequency with which such defective particles are formed. Nevertheless, one such factor is clearly the gene affected by the \( lg \) mutation (Fig. 4). We confirmed by electron microscopy that the biologically inactive peak (as seen for example in Fig. 4a at the left) contains exclusively particles which are easily recognizable as tailless, DNA-filled, P2 heads, whereas the main, biologically active peak contains typical, tailed P2 particles. In fractions to the right of the main peak, a high frequency of empty heads was noted.

A number of phage crosses were made to localize the \( lg \) mutation on the map of P2. The markers used were (in map order): \( amL3O_{2} amR_{3} amH_{13} del2 amT_{5} (tsD_{4}, amD_{6}) \) \( virI \). The \( del2 \) deletion produces the \( fun \) phenotype (Bertani, 1975), and the previously studied point mutation \( fun \) has been shown to be located between the essential genes G (to the left) and F (to the right), themselves located between genes H and T (Lindahl, 1971). The relevant map segment is represented therefore by the sequence:

\[
L \ R \ H \ G \ del2 \ F \ E \ T \ D \ virI,
\]

which covers about half of the P2 chromosome.

It was soon observed that \( lg \) is closely linked to \( del2 \). This is obvious from all crosses in Table 1 (except for crosses 6 and 7, which are controls showing the segregation of \( del2 \) when both parents are \( lg \) or \( lg^{+} \)). Separation of \( lg \) from \( del2 \) does, however, occur (rare recombinant types in crosses 2, 5, 8, 9, 10, 11, Table 1). As expected, crosses 8 and 12 are consistent with a location of \( del2 \) to the right of gene R, and to the left of \( T \). We used in our crosses selective markers in genes \( L \), \( R \), \( H \), \( T \) and \( D \): in all cases \( lg \) remained closely linked to \( del2 \). Since \( del2 \) lies between \( R \) and \( T \), so must \( lg \). If all cases of uncoupling of \( del2 \) from \( lg \) are pooled, the occurrence of the two complementary recombinant types favours the sequence \( del2 \) \( lg \) from left to right on the chromosome, with a total score of 23 favourable cases against 5. According to cross 10 (Table 1) \( lg \) is much closer to \( del2 \) than to \( amT_{5} \). The only known genes between \( T \) and \( del2 \) are tail genes \( E \) and \( F \) (Lindahl, 1971; Sunshine \textit{et al.} 1971). Considering the high degree of saturation of the P2 map, it seems very likely that the \( lg \) mutation is located in either \( E \) or \( F \) and affects the phage tail.

Since the identification of the \( lg \) genotype in recombinant plaques is fairly laborious, the genetic analysis was not pursued further.

**DISCUSSION**

We have shown that the \( lg \) mutation of phage P2 is responsible for larger burst sizes as compared with the wild type phage, especially at higher incubation temperatures. The kinetics of phage DNA synthesis is apparently unaffected by the mutation.

In phage crosses, the \( lg \) mutation is located at the right of the \( del2 \) deletion, and closely linked to it. The first known gene to the right of \( del2 \) is the essential gene F, as revealed by the polar mutation \( amF_{4} \), which abolishes the two proteins \( F_{1} \) and \( F_{2} \), totalling a mol, wt. of 66000 (Lengyel \textit{et al.} 1974). Coding for such a polypeptide would require at least a quarter of the DNA length between \( virI \) (in gene C) and the right end of \( del2 \), a segment which from physical measurements (Chattoraj, Younghusband & Inman, 1975) is known to correspond to at most 8000 base pairs. It is then very probable that \( lg \) is a mutation of \( F \) (\( F_{1} \) or \( F_{2} \)). Proteins \( F_{1} \) and \( F_{2} \) are the major components of the P2 tail and are believed to make up, respectively, the tail sheath and the tail tube (Lengyel \textit{et al.} 1974).

Consistent with the involvement of a late gene, we have shown that the differential effect of higher incubation temperatures on the burst size occurs very late during the latent period.
### Table 1. Bacteriophage crosses

<table>
<thead>
<tr>
<th>Cross</th>
<th>Parent phages&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Phage yield&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Phage type selected&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Percentage of yield&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Plaques scored&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>del2 + + + +&lt;sup&gt;e&lt;/sup&gt;</td>
<td>128</td>
<td>am&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4.6</td>
<td>72 vir&lt;sup&gt;i&lt;/sup&gt; tested: 71 del2 +, 1 + lg</td>
</tr>
<tr>
<td></td>
<td>+ lg amD6 vir&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>159 vir&lt;sup&gt;i&lt;/sup&gt; tested: 136 del2 lg, 19 + +, 4 + lg</td>
</tr>
<tr>
<td>2</td>
<td>del2 lg + +</td>
<td>(0.5)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>am&lt;sup&gt;+&lt;/sup&gt;</td>
<td>(0.2)</td>
<td>48 vir&lt;sup&gt;i&lt;/sup&gt; tested: 4 del2 lg, 44 + +</td>
</tr>
<tr>
<td></td>
<td>+ + amD6 vir&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>75 vir&lt;sup&gt;i&lt;/sup&gt; tested: 20 del2 +, 55 + lg</td>
</tr>
<tr>
<td>3</td>
<td>del2 lg amD6 +&lt;sup&gt;h&lt;/sup&gt;</td>
<td>170</td>
<td>am&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1.4</td>
<td>75 vir&lt;sup&gt;i&lt;/sup&gt; tested: 13 del2 lg, 61 + +, 1 + lg</td>
</tr>
<tr>
<td></td>
<td>+ + + vir&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>56 vir&lt;sup&gt;i&lt;/sup&gt; tested: 39 del2, 17 +</td>
</tr>
<tr>
<td>4</td>
<td>+ lg + +</td>
<td>47</td>
<td>am&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1.4</td>
<td>195 vir&lt;sup&gt;i&lt;/sup&gt; tested: 10 del2 amD6, 4 del2 +, 181 + amD6</td>
</tr>
<tr>
<td></td>
<td>del2 + amD6 vir&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>No selection</td>
</tr>
<tr>
<td>5</td>
<td>+ + + del2</td>
<td>8</td>
<td>am&lt;sup&gt;+&lt;/sup&gt;</td>
<td>2.4</td>
<td>52 vir&lt;sup&gt;i&lt;/sup&gt; tested: 6 del2 lg, 5 del2 +, 1 + lg, 26 + +</td>
</tr>
<tr>
<td></td>
<td>+ amD6 + vir&lt;sup&gt;i&lt;/sup&gt;</td>
<td>58</td>
<td>am&lt;sup&gt;+&lt;/sup&gt;</td>
<td>7.8</td>
<td>19 vir&lt;sup&gt;i&lt;/sup&gt; tested: 19 del2, 80 +</td>
</tr>
<tr>
<td>6</td>
<td>del2 lg amD6 +</td>
<td>65</td>
<td>am&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1.0</td>
<td>100 am&lt;sup&gt;+&lt;/sup&gt; tested: 90 del2 lg, 22 + +</td>
</tr>
<tr>
<td></td>
<td>+ lg + vir&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>32 vir&lt;sup&gt;i&lt;/sup&gt; tested: 10 del2 lg, 2 del2 +, 20 + +</td>
</tr>
<tr>
<td>7</td>
<td>del2 lg amD6 +</td>
<td>117</td>
<td>am&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.4</td>
<td>About equal numbers of vir&lt;sup&gt;i&lt;/sup&gt; and vir&lt;sup&gt;+&lt;/sup&gt; recombinants were observed.</td>
</tr>
<tr>
<td></td>
<td>+ + amR&lt;sub&gt;3&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td>23 vir&lt;sup&gt;i&lt;/sup&gt; tested: all del2 lg</td>
</tr>
<tr>
<td>8</td>
<td>amL302 + del2 + amR&lt;sub&gt;3&lt;/sub&gt; + +</td>
<td>98</td>
<td>am&lt;sup&gt;+&lt;/sup&gt; +&lt;sup&gt;t&lt;/sup&gt; tsD&lt;sub&gt;4&lt;/sub&gt; +</td>
<td>3.6</td>
<td>About 12% of recombinants were vir&lt;sup&gt;i&lt;/sup&gt; +, 17 vir&lt;sup&gt;+&lt;/sup&gt; tested: 6 del2 lg, 1 + lg, 10 + +</td>
</tr>
<tr>
<td></td>
<td>+ + + vir&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>52 vir&lt;sup&gt;i&lt;/sup&gt; tested: 20 del2 lg, 5 del2 +, 1 + lg, 26 + +</td>
</tr>
<tr>
<td></td>
<td>amR&lt;sub&gt;3&lt;/sub&gt; + amT&lt;sub&gt;5&lt;/sub&gt; +</td>
<td></td>
<td></td>
<td></td>
<td>61 vir&lt;sup&gt;i&lt;/sup&gt; tested: 4 del2 lg, 3 del2 +, 25 + +</td>
</tr>
<tr>
<td>9</td>
<td>+ del2 lg tsD&lt;sub&gt;4&lt;/sub&gt;</td>
<td>22</td>
<td>am&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.8</td>
<td>71 vir&lt;sup&gt;i&lt;/sup&gt; tested: 43 del2 lg, 3 del2 +, 25 + +</td>
</tr>
<tr>
<td></td>
<td>amT&lt;sub&gt;5&lt;/sub&gt; + + + vir&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>79 vir&lt;sup&gt;i&lt;/sup&gt; tested: 55 del2 lg, 9 del2 +, 15 + +</td>
</tr>
<tr>
<td></td>
<td>+ del2 lg amD6 vir&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>100 vir&lt;sup&gt;i&lt;/sup&gt; tested: 90 del2 lg, 10 + +</td>
</tr>
<tr>
<td>10</td>
<td>amH13 + + +</td>
<td>156</td>
<td>am&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1.1</td>
<td>195 vir&lt;sup&gt;i&lt;/sup&gt; tested: 10 del2 amD6, 4 del2 +, 181 + amD6</td>
</tr>
<tr>
<td></td>
<td>+ del2 lg amT&lt;sub&gt;5&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td>No selection</td>
</tr>
<tr>
<td>11</td>
<td>amH13 + + +</td>
<td>210</td>
<td>am&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4.8</td>
<td>100 vir&lt;sup&gt;i&lt;/sup&gt; tested: 90 del2 lg, 10 + +</td>
</tr>
<tr>
<td></td>
<td>+ del2 lg amD6 vir&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>72 vir&lt;sup&gt;i&lt;/sup&gt; tested: 71 del2 +, 1 + lg</td>
</tr>
<tr>
<td>12</td>
<td>del2 lg + +</td>
<td>21</td>
<td>am&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4.8</td>
<td>100 vir&lt;sup&gt;i&lt;/sup&gt; tested: 90 del2 lg, 10 + +</td>
</tr>
</tbody>
</table>

<sup>a</sup> All phages were irradiated with u.v. light before infection, with a dose producing between 2 and 3 lethal hits. The m.o.i. for each phage (based on phage titre before irradiation) varied between 4.4 and 10.0 (average 6.4).

<sup>b</sup> Phage output divided by bacterial input: may not be equal to burst size as a result of u.v. irradiation reducing effective m.o.i.

<sup>c</sup> Percentage of yield is the observed frequency of the relevant recombinant type in percentage of total phage yield. When the class selected also included one of the parents, and a particular recombinant was recognized by plaque type (clear plaque for vir<sup>i</sup>, turbid for vir<sup>+</sup>), the percentage given refers to this particular recombinant. Analysis of recombinants was done by picking individual plaques, streaking out for single plaque isolation at 30 °C, and picking one daughter plaque into SSC for testing. The lg character was recognized by streaking out the daughter plaque on C<sub>3</sub>757 and incubating at 43 °C. The del2 character was recognized by heat stability (survival of phage in daughter plaques after 3 h exposure at 53 °C). For details see Bertani (1975).

<sup>d</sup> Isolated as a recombinant from a cross del2 lg amD6 x tsD4 vir<sup>i</sup>.

<sup*e</sup> A recombinant from cross 7.

<sup>g</sup> The reason for this low yield is unknown. A technical mistake in plating is not excluded.

<sup>h</sup> A recombinant from cross 6.

<sup>k</sup> A recombinant from cross 12.
Finally we have noted that the frequency of defective, tailless particles is much higher in wild type P2 lysates than in P2 lg preparations.

We propose that in wild type P2, particularly at the higher temperatures, particle assembly is inefficient due to the properties of one of the F proteins, with the result that a fraction of the particles remains tailless. The lg mutation would correct this natural shortcoming of P2.

The above interpretation says nothing about the amount of capsid protein synthesized, which may well be in excess of the needs for packaging of all phage DNA. Another alternative, not yet excluded, would be that some of the tail proteins (presumably those of the FETUD operon, see Lindahl, 1971) are not synthesized in sufficient amounts in P2 wild type relative to the phage DNA and other capsid proteins available. The lg mutation would then affect transcription or translation of the FETUD operon.

Six (1975) has shown that phage P2 supplies all its capsid proteins to the satellite phage P4, even under conditions where the multiplication of P2 itself is minimal. He also noted that the burst size of the satellite phage is much larger when the helper P2 is lg rather than wild type. This otherwise puzzling observation is fully explained by our present conclusions concerning the nature of the lg mutation.

Less easily explained is the larger burst size typical of P2 vir+ as compared with the wild type, especially since this difference seems to be relatively greater in the absence than in the presence of the lg mutation (Fig. 1). While the immunity repressor is inactive or absent as a result of the vir+ mutation, the lg mutation seems not to affect, even quantitatively, the potential for lysogenization of the phage. With vir+ phage, it is reasonable to expect that even during the lytic development that takes place in a majority of the infected bacteria a certain amount of repressor is present, although insufficient to give the lysogenic response in those cells. Its possible effects on phage DNA and protein synthesis are not known. Our results could be explained, however, if the repressor indirectly depressed the amount of capsid protein synthesized, disproportionately to its effect on the amount of phage DNA. This would introduce a new limitation on the number of phage particles that could be assembled, superimposed on the limitation resulting from the lg+ condition. In the presence of the lg mutation, capsid protein, normally present in excess, could be efficiently used, and a decrease of its amount due to the repressor would have little or no effect on the phage yield.

We thank Mrs Lisa Mördal for assistance. This work was supported by grant 72 from the Swedish Medical Research Council. K. J.-K. was supported by a traineeship from the Swedish Academy of Engineering Sciences, and S. J. by an EMBO research fellowship. We thank the Department of Histology (Professor J.-E. Edström) for use of the electron microscope.

REFERENCES


Defective phage assembly


(Received 25 April 1977)