Expression of T4 Early Genes 62, 44, 45 and 46 in the λ–T4 Recombinant Phage λ806-17

By CHACK-YUNG YU AND KAI-KEUNG MARK*
Department of Biology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong

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SUMMARY

A λ–T4 recombinant phage, λ806-17, which carries the T4 early genes 62, 44, 45 and 46, was studied inside a homoimmune lysogen. Under such conditions, gene expression from the λ promoters is represented. Results showed extensive expression of gene 46, and significant expression of genes 62 and 45. The expression of these early T4 genes is presumed to depend on T4 promoters included in the cloned fragment. A new promoter proximal to gene 46 is implicated. The results also indicate that the extent of gene expression, in terms of complementation, increases with the time allowed for expression.

INTRODUCTION

T4 is one of the most intensively studied bacteriophages, but analysis of the regulatory mechanisms of T4 gene expression has been a difficult task. Recent advances in recombinant DNA technology have now opened up a new range of opportunities to investigate these problems.

In this study, a λ–T4 recombinant phage, λ806-17, or Q (Wilson et al., 1977), was used as a simplified system for the study of early T4 gene expression. This λ–T4 recombinant phage carries a 5.6 kb EcoRI restriction fragment of T4 DNA, corresponding to genes 62, 44, 45 and 46 (Wilson et al., 1977). We asked whether these T4 genes, cloned into the λ vector, can be expressed and, if this is the case, whether their transcription is initiated from T4 promoters present in the cloned segment.

METHODS

Bacterial strains. Escherichia coli strain B, Sup°, λ-resistant, was used as host for bacteriophage T4. Strain CR63, Sup1+, λ-resistant, was used as host for T4 amber mutants (Appleyard et al., 1956). Strain W3350, Sup°, λ-sensitive, was used as host for λ and λ–T4 recombinants and some complementation tests (Campbell, 1961). Strain W3350 (λ120) was used for other complementation tests.

Bacteriophages. T4D+ was our laboratory standard strain, originally obtained from Dr G. Streisinger. Amber mutants of phage T4 in gene 46 (ambB14), in gene 45 (ambE10), in gene 44 (ambE2058) and in gene 62 (ambNG485) were obtained from Dr E. P. Geiduschek. λc147 was from Dr A. D. Kaiser, and λ121 from Dr K. C. Luk. λ–T4 recombinant phages λ806-17 (Q) and λ806-16 (J), which carry an imm21 region, were from Dr N. E. Murray (Wilson et al., 1977). λ806-17 (Q) (see Fig. 1) contains a 5.6 kb T4 DNA fragment corresponding to the T4 early genes 62, 44, 45 and 46 (Wilson et al., 1977), and possibly the gene regA, as well as parts of genes 43 and 47 (Spicer et al., 1982). λ806-16 (J) is a similar recombinant which harbours a T4 DNA fragment corresponding to the late genes 13 to 20 (Wilson et al., 1977).

Media. Tryptone broth and tryptone agar plates were used (Mark, 1973).

Preparation and concentration of phages. The methods for preparation and concentration of phages were similar to those described previously (Mark, 1973).

Complementation in vivo. Because T4 interferes with the development of phage λ (Pearson & Snyder, 1980), a modification of the usual in vivo complementation test (Revel, 1981; Vorozheikinia et al., 1980) was used to check whether λ–T4 can synthesize functional T4 gene products (with their own promoter). Suppressor-free E. coli W3350 (λ121) cells were pre-infected with λ–T4 recombinants at 37 °C to allow some expression prior to infection with T4 mutant phage.
E. coli W3350 (λ121) was grown in tryptone broth to a concentration of 3 × 10⁸ to 3.5 × 10⁹ cells/ml (OD₆₅₀ 0.6 to 0.7). The bacterial culture was then separated into several vials, each was supplemented with 2 mM-MgSO₄ and infected with λ806-17 (Q), λ806-16 (J) or λ21, at a multiplicity of 5.0. After 10 min on ice, to allow for adsorption, the mixture was then supplemented with L-tryptophan to a final concentration of 20 μg/ml and incubated at 37 °C in a shaking water-bath. After a further 5 to 10 min, the mixture was diluted (by a factor of 10⁻², 10⁻³ or 10⁻⁴) into prewarmed tryptone broth. The unadsorbed phage were assayed after chloroform treatment.

At intervals, T4 phage progeny were assayed on strains CR63 (Su⁺) and B (Su°). The phage yields were calculated by dividing the number of T4 progeny phage by the parental phage input. Recombination frequencies were calculated from the titres obtained on B.

RESULTS

Table 1 shows the complementation of a T4 gene 46 amber mutant, amB14, with λ806-17 (Q) on E. coli W3350 (λ121). Complementation tests were carried out in parallel with two other superinfecting phages, λ806-16 (J) and λ21. J is genetically identical to Q except that it carries a cloned DNA fragment corresponding to T4 late genes 13 to 20. At 90 min after infection with the phage T4 mutant, the burst size for Q plus T4 46amB14 (46⁻) was 174, and the recombination frequency about 4%. When Q was replaced by λ21 or by J, the burst sizes were 2-7 and 0-9, respectively. The background for the T4 46amB14 alone was 7-6 (this is a rather leaky mutant; Hercules & Sauerbier, 1973). These results clearly indicated that Q produced functional gene 46 gene product (gp46) from a T4 promoter, since the promoters of the lambda vector were repressed in the homoimmune lysogen.

Ninety min after superinfection of the Q-infected lysogen with T4 45amE10, the burst size was 63, and the recombination frequency 2-2%. In the controls, the burst sizes were 0-002, 0-03 and 0-03 for bacteria preinfected by λ21, J, or not preinfected, respectively. These results indicate that λ806-17 can produce functional gp45 also from a T4 promoter.

To our surprise, λ806-17 (Q) was unable to complement T4 gene 44 and gene 62 mutants as efficiently as it did the gene 46 mutant. In the case of T4 46amNG485, complementation with Q at 90 min gave a burst size of only 9-3, about 3% that of T4⁺ under the same conditions. Nevertheless, complementation had occurred, because the corresponding burst was about 20-fold higher than that in the control experiment (no preinfection with the T4 recombinant).

In the case of T4 44amE2058, complementation with Q at 90 min gave a burst size of 1-8, which was only about 0-5% of the corresponding value for T4. This was significantly lower than the leaky T4 44amE2058 level (burst size 15-9). Therefore, no complementation can be inferred in this case.

Since T4 will shut off the expression of a cytosine-containing λ-T4 genome (Pearson & Snyder, 1980; Kutter et al., 1981), the time allowed for expression of the cloned T4 genes is approximately equal to the time interval between infection with λ806-17 and superinfection with a T4 amber mutant. In the experiments described so far, this time interval was 25 min. In further experiments the times allowed for complementation were varied from 0 to 90 min in order to monitor the efficiency of complementation as a function of time. It was thus clearly shown (Table 2) that burst sizes were higher when the period of preinfection with the λ-T4 recombinant increased. Therefore, it is expected that λ806-17 produces gp46 continuously during this period.

DISCUSSION

The results of complementation in vivo strongly suggest that the cloned early T4 genes 46, 45 and 62 of the recombinant phage λ806-17 (Wilson et al., 1977; Spicer et al., 1982) can be efficiently expressed, giving functional products because of T4 promoters present on the cloned fragment. This was shown by the high burst sizes of T4 amber mutants of these genes, observed when λ806-17 preinfected the homoimmune lysogen, E. coli W3350 (λ121). Under these conditions, the major promoters of the λ vector are repressed. Positive results of complementation experiments gave strong support to the notion that the cloned genes are transcribed from T4 promoter(s) included in the cloned fragment (Fig. 1) (Velten & Abelson,
**T4 early gene expression**

(a) λ806-17 (Q)

![Diagram of the T4 region carried by phage λ806-17 (Q) (after Spicer et al., 1982). Arrows indicate the extent of transcriptional units, promoters are at the right. The thick arrow of gene 46 (g46) indicates strong expression of this gene, as observed in this work.](image)

(b) T4 genes 43 to 55

![Segment of the genetic map of T4 relevant to this work (after Hercules & Sauerbier, 1974).](image)

**Fig. 1.** (a) Diagram of the T4 region carried by phage λ806-17 (Q) (after Spicer et al., 1982). Arrows indicate the extent of transcriptional units, promoters are at the right. The thick arrow of gene 46 (g46) indicates strong expression of this gene, as observed in this work. (b) Segment of the genetic map of T4 relevant to this work (after Hercules & Sauerbier, 1974).

**Table 1. Complementation of T4 amber mutants with λ806-17 (Q) in E. coli W3350 (λ21)**

<table>
<thead>
<tr>
<th>Amber mutant and phage</th>
<th>Mean burst size at time</th>
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<tbody>
<tr>
<td></td>
<td>10 min</td>
</tr>
<tr>
<td>Q + T4 am+</td>
<td>0.5</td>
</tr>
<tr>
<td>Q only</td>
<td>No plaques detected on CR63 or B</td>
</tr>
<tr>
<td>46- + Q</td>
<td>0.24</td>
</tr>
<tr>
<td>46- + J</td>
<td>0.08</td>
</tr>
<tr>
<td>46- only</td>
<td>0.23</td>
</tr>
<tr>
<td>46- + λ21</td>
<td>0.19</td>
</tr>
<tr>
<td>45- + Q</td>
<td>0.03</td>
</tr>
<tr>
<td>45- + J</td>
<td>0.05</td>
</tr>
<tr>
<td>45- only</td>
<td>0.01</td>
</tr>
<tr>
<td>45- + λ21</td>
<td>0.48</td>
</tr>
<tr>
<td>44- + Q</td>
<td>0.14</td>
</tr>
<tr>
<td>44- + J</td>
<td>0.11</td>
</tr>
<tr>
<td>44- only</td>
<td>0.07</td>
</tr>
<tr>
<td>62- + Q</td>
<td>0.34</td>
</tr>
<tr>
<td>62- + J</td>
<td>0.43</td>
</tr>
<tr>
<td>62- only</td>
<td>0.40</td>
</tr>
</tbody>
</table>

1980). However, it is possible that the cloned T4 genes are transcribed from certain weak λ promoters not under the control of the λ repressor (Burt & Brammar, 1982). This is unlikely because in the λ–T4 hybrid we used, a segment of λ DNA [ordinates 22056 to 32578 (Daniels et al., 1982), 44.5 to 65.6% on the physical map (Szybalski & Szybalski, 1979)] in which many weak λ promoters are found (Burt & Brammar, 1982) had been replaced by the T4 DNA fragment (Wilson et al., 1977; Spicer et al., 1982).
Table 2. Production of gp46 from λ806-17 at various times of expression*

<table>
<thead>
<tr>
<th>Time allowed between preinfection and superinfection (min)</th>
<th>Percentage of infected cells that produced infective centres (%)</th>
<th>Average burst size per infected cell</th>
<th>Phage yield based on infective centres formed</th>
<th>Frequency (%) of am+ recombinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7</td>
<td>4</td>
<td>52</td>
<td>5-4</td>
</tr>
<tr>
<td>15</td>
<td>18</td>
<td>17</td>
<td>95</td>
<td>4-1</td>
</tr>
<tr>
<td>25</td>
<td>24</td>
<td>50</td>
<td>212</td>
<td>4-1</td>
</tr>
<tr>
<td>50</td>
<td>36</td>
<td>180</td>
<td>494</td>
<td>3-5</td>
</tr>
<tr>
<td>90</td>
<td>74</td>
<td>257</td>
<td>348</td>
<td>4-0</td>
</tr>
</tbody>
</table>

* E. coli (λ21) was preinfected with λ806-17 (m.o.i. of 5) and, at the times indicated, superinfected with T4 46amB14 (m.o.i. of 1.6 × 10^-3). T4 phage adsorption was efficient (about 2% unadsorbed phage). When λ806-17 infection was omitted, T4 46amB14 produced a phage yield (based on input phage) of 2.1. For details of procedures, see Methods.

By means of genetic complementation experiments, expression of the cloned gene group 46, 45, 44 and 62 has also been observed by two other groups (Vorozheikinia et al., 1980; Velten & Abelson, 1980). Since the complementation efficiencies of these genes are unaffected by the orientations of the T4 DNA fragment within the vector, Vorozheikinia et al. (1980) further suggested that transcription of these genes may be initiated from the included T4 promoters. Unfortunately, their experiments were conducted with non-lysogenic bacteria (for gene 45) and thus their experimental conditions do not completely rule out the possibility of expression of the cloned T4 genes from λ promoters (Wilson & Murray, 1979; Murray et al., 1979).

According to Spicer et al. (1982), the T4 restriction fragment of λ806-17 carries T4 genes 46, 45, 44, 62, regA and part of genes 47 and 43. Based on the sensitivity of gene expression to u.v. radiation (Hercules & Sauerbier, 1973, 1974), it was suggested that gene 46 may be transcribed from a distal promoter near gene 55. Since our complementation tests showed that cloned gene 46, gene 45 and gene 62 can be transcribed from T4 promoters in our cloned segment, such a promoter should exist proximal to gene 46. By means of translation in vitro of pre-replicative T4 mRNA size-fractionated in vivo, Young & Menard (1981) found that gp46 is coded by two species of mRNA. One of these might be monocistronic because its coding capacity was estimated to correspond to only about 300 amino acid residues. These mono- and polycistronic RNAs could be generated from multiple promoters (one of which could be proximal to gene 46) and/or by post-transcriptional processing and degradation.

Under our conditions, in vivo complementation of a gene 46-deficient mutant was very effective since the burst size was 40 to 45% that of the wild-type. In contrast, complementation of mutations in genes 45, 44 and 62 occurred at rather low levels. For example, the complementation yields for amber mutants in gene 62 or gene 44 were only 3% and 0.5% of the wild-type level, respectively. This difference may be due to the fact that genes 45, 44 and 62, but not gene 46, are susceptible to negative translational control by the regA protein (Karam et al., 1981; Gold et al., 1981). It is reasonable to assume that the product of regA is produced from the λ806-17 recombinant phage because regA is an early gene and is included in the cloned 5.6 kb T4 DNA fragment (Spicer et al., 1982). Thus, the production of gp45, gp62 and gp44 may be regulated to a certain level by the translational repressor, the regA gene product, resulting in lower complementation efficiencies of the corresponding mutants. It is known that an amber mutation in gene 45 exhibits a dominant effect on the biological function of gene 44 (Karam et al., 1979; Stahl et al., 1970). A gene 44 mutation will also exert a polarity effect on the expression of gene 62 (Karam et al., 1979; Bowles & Karam, 1979). These complications may contribute in some degree to reduce complementation yields. If the regA protein is expressed from λ806-16, this recombinant could be used as a source for the production of this translational repressor. Consequently, effects of the regA product on the expression of many other T4 early genes could be studied conveniently.
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REFERENCES


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