Interactions Between the Vegetative States of Phages λ and T1

JOYCE M. GEIMAN, J. R. CHRISTENSEN, AND HENRY DREXLER

Department of Microbiology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642, and Department of Microbiology, The Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina 27103

Received for publication 22 July 1974

Bacteria containing phage lambda in the vegetative state were produced either by induction of λ lysogens or by infection of sensitive cells with λ . These cells were superinfected with T1, and assayed for the production of λ , T1, or both. Although most of the cells produced only λ or T1, approximately 10% of the infectious centers were dual yielders. Examination of the progeny phage produced by the population of mixedly-infected cells showed that there was little, if any, phenotypic mixing, as determined by adsorption phenotype. T1am mutants in a variety of T1 genes were tested for their ability to exclude λ , but none were defective in this ability. One gene of T1, gene 4, can be complemented by λ .

Despite their morphological similarity (4), there is no evidence that phages λ and T1 are related. T1 is a virulent phage with a short latent period, which degrades the host chromosome to provide precursors for its own DNA synthesis (6; D. Figurski and J. R. Christensen, unpublished results). The T1 DNA molecule has an unusually long, and somewhat variable, terminal redundancy (7). In contrast, λ is temperate, has a much longer latent period, and its DNA molecule has short, cohesive ends (14).

If T1 is propagated on λ lysogens, the lysate contains particles that have the adsorption properties and antiserum sensitivity of T1, but which contain complete λ genomes (H. Drexler and J. R. Christensen, to be reported separately). Thus, during its lytic cycle, T1 seems to have the ability to package λ DNA.

One way in which T1 might be able to pick up λ DNA during growth on the lysogens would be to induce the prophage, and to mature replicating λ DNA into T1 coats. We therefore decided to study the interaction between replicating λ and replicating T1, with particular emphasis on whether mutual exclusion or phenotypic mixing or both might occur between these two phages. We report here that mutual exclusion is strong, but not complete, and that phenotypic mixing is rare. We also report the unexpected observation that one of the genes of T1 can be complemented by λ .

MATERIALS AND METHODS

Bacterial and phage strains. All phage strains have been previously described (1, 4). *Escherichia coli* W3350(λ c1₁) was from R. Huskey. *E. coli* C. was from

G. Bertani, and from it C/ λ and C/T1 were isolated for this study. C strains are nonpermissive for amber mutants. For plating T1 in experiments involving T1am mutants, *E. coli* KB3/ λ was used. This was isolated from KB3, the amber-permissive strain used in the original isolation of the T1am mutants (8).

Media. Tryptone: 1% tryptone, 0.5% NaCl. Tryptone + maltose is the same with the addition of 0.2% maltose. Cyanide broth: 0.8% nutrient broth, 0.5% NaCl, and 0.01 M KCN.

Induction of W3350(λ c1₁) and superinfection with T1. Log-phase W3350(λ c1₁) was grown in tryptone without salt, resuspended in an equal volume of the same medium, transferred to an aeration tube, and placed in a 45 C water bath (time = 0). At 15 min, the tube was moved to a 37 C bath while aeration continued. At the desired time, T1 was added; 5 min later the tube was moved to an ice bath, and the suspension was diluted with an equal volume of cyanide broth. The suspension was centrifuged, and the pellet was resuspended in cyanide broth. Infectious centers were assayed on a mixed plating culture consisting of equal volumes of C/ λ and C/T1. In some experiments, a sample of the T1-infected cells was also diluted 10⁴-fold into tryptone at 37 C (growth tube) and incubated until the total incubation time at 37 C was 60 min.

Infection with λ c₈₀ and superinfection with T1. Log-phase C cells were grown in tryptone plus maltose. MgCl₂ was added to a final concentration of 0.01 M and the cells were infected with λ c₈₀ in an aeration tube at 37 C (time = 0). At 15 min, the suspension was chilled and then centrifuged. The pellet was resuspended in tryptone without salt and transferred to a tube at 37 C without aeration. T1 was added, and 7 min was allowed for adsorption, after which the suspension was chilled, diluted, centrifuged, and resuspended as described above. Since T1am mutants were involved, KB3/ λ was used in place of C/ λ in the assay of infectious centers.

Identification of infectious centers. T1 infectious centers and λ infectious centers could be reliably distinguished by inspection of the plaques formed on the mixed plating cultures. After overnight incubation of the assay plates at 37 C, T1 forms large turbid plaques and λ forms much smaller turbid plaques. In those cases where a cell produced both kinds of phage, the plaques were large and turbid, with a small clear area in the exact center. Random superposition of the two kinds of plaques were also seen; in this case, the small clear area was eccentric. The proportion of this latter kind of plaque was strongly influenced by the total number of plaques on the plate, whereas in some experiments a significant proportion of clear-centered plaques was seen even when the suspension was diluted such that there were only a few plaques per plate.

RESULTS

Incomplete mutual exclusion between λ and T1. The initial experiments on the interaction between these two phages were done by heat-inducing a population of W3350(λ c1₁₁) bacteria for 15 min, returning the population to 37 C, and, after the desired interval, superinfecting the cells with T1. Five minutes after the addition of T1, a sample was withdrawn for assay of the infectious centers on a mixed lawn of C/ λ and C/T1 bacteria. A second sample was also withdrawn, diluted into a growth tube at 37 C, incubated for 45 min, and then assayed for progeny phage. Results of a typical experiment, in which the superinfection was carried out at 25 min (10 min after the end of the induction period) are shown in Table 1. It is interesting that 13% of the infectious centers are

TABLE 1. *Partial mutual exclusion when W3350 (λ c1₁₁) was induced and then superinfected with T1 at 25 min (MOI added = 20)*

Time (min)	Colony count (ml ⁻¹)	Plaque count (ml ⁻¹)		
		λ	T1	Mixed
0	8.6×10^7			
15	3.4×10^8	8.3×10^7		
30 ^a		4.3×10^7	3.4×10^7	1.2×10^7
75 ^b		4.4×10^8	2.2×10^8	None seen

^a This assay of infectious centers was based on the classification of 275 plaques distributed on 30 assay plates using the mixed plating culture: C/ λ plus C/T1. A few large, turbid plaques with small clear areas not exactly in the center, were considered to be random overlaps, and were scored as 1 λ plus 1 T1.

^b This assay of progeny phage was based on the classification of 294 plaques, plated on the same mixed plating culture. Although there were more plaques per assay plate than in the assay of infectious centers, no clear-centered plaques were seen. Twenty-five random overlaps were observed.

cells that yield both λ and T1. Note that these dual yielders were scored on plates that had, on an average, less than 10 plaques per plate. The existence of dual yielders was also confirmed in an experiment using the single burst type of protocol (data not shown).

Table 2 presents the results of a similar kind of experiment which demonstrate the effect of superinfecting induced cells at different times after the induction. As might be expected, early superinfection favors the production of T1 infectious centers, whereas later superinfection favors λ . A fairly common feature of early superinfection, seen here in the 20-min case, is that some of the superinfected cells produce neither T1 nor λ . This is indicated by the fact that the sum of the number of infectious centers of all types (83) is substantially less than the number of induced cells plated (2.0×10^2). The low number of dual yielders after early superinfection, seen here, was not a regular feature of early superinfection, however.

In summary, in cells superinfected with T1 after induction of a λ prophage, exclusion of one of the two phages is the usual rule, but mixed bursts do occur. In a considerable number of experiments of this general type, the proportion of mixed bursts was usually between 5 and 15%, and did not vary in any regular way with the time of superinfection.

Search for phenotypically mixed particles. In the experiment of Table 1, the mixed yielders represent 22% of the total λ yielders and 26% of the total T1 yielders. It was logical, then, to inquire whether phenotypically mixed particles might be produced. If phenotypic mixing were random in the mixed yielders, as it is in the classical cases of phenotypic mixing (for instance, see ref. 11), we might expect to find approximately 10% of the T1 genomes in particles with λ adsorption phenotype and similarly about 10% of the λ genomes in particles with T1 adsorption phenotype. The proportion of mixed particles could be even higher if, say, T1 could make a phenotypic contribution even in those

TABLE 2. *Effect of time of superinfection on the type of infectious centers obtained*

Time of superinfection (min)	T1 MOI	Classification of plaques from a sample representing 2.0×10^2 superinfected cells		
		λ	T1	mixed
20	8.4	21	60	2
30	5.7	120	20	21

cells in which no genotypic T1 particles are made.

A particle with T1 adsorption phenotype should be able to infect a λ -resistant, T1-sensitive host, and the infectious center so produced should make a typical λ plaque on a λ -sensitive, T1-resistant lawn. To search for such particles, we grew a culture of C/ λ cells, and resuspended them in 5×10^{-4} M MgCl_2 (optimal for T1 adsorption) (9). To one portion of this suspension, a sample from the growth tube of the experiment in Table 1 was added, and to another a suspension of λc_{60} phage containing approximately the same number of λ particles as the sample from the growth tube. After allowing 15 min at 37 C for phage adsorption, the suspensions were chilled, and the unadsorbed phage removed by five cycles of centrifugation and resuspension in cold cyanide broth. Samples of each washed suspension were plated on lawns of C/T1 cells. The results are shown in Table 3.

At first glance, it appears that about 1% of the λ genomes in the growth tube have T1 phenotype, but this conclusion must be tempered by the result in the control tube, where about 0.2% of the "ordinary" λc_{60} , which had not replicated in the presence of T1, also appear to have T1 adsorption phenotype. We were surprised that such a high proportion of presumably unadsorbed phage should be associated with C/ λ bacteria after the extensive washing employed here. A further investigation of this phenomenon has shown that these plaques are not due to a simple carry-over of unadsorbed or reversibly adsorbed λ , nor to the presence of λh mutants, but that λ preparations regularly contain a small proportion of phenotypically abnormal particles. The particles have the following properties (J. R. Christensen, unpublished observations): they are poorly neutralized by anti- λ

serum; they adsorb poorly to *E. coli* C600; they can infect a variety of strains selected for resistance to λ *vir*; they are subject to the same host restriction as λ in such strains if their host of origin differed in host specificity from the resistant strain being infected; the progeny of the abnormal particles are normal λ phage.

Whatever the nature of the abnormal λ particles, their existence means that the finding in the experiment of Table 3 that about 1% of the progeny from the growth tube of Table 1 seemed to be phenotypically mixed must be taken only as an upper limit, to be compared with the expectation of 10% phenotypically mixed particles if phenotypic mixing were to occur randomly in the mixed yielders of Table 1. It is possible that all the λ plaques seen in this experiment to be associated with the washed C/ λ suspension are phenotypic variants of the type discussed above, rather than true phenotypically mixed particles.

A similar experiment, *mutatis mutandis*, was done to look for T1 particles with λ adsorption phenotype in the growth tube from the experiment of Table 1. These experiments (data not shown) indicated that approximately 1 T1 in 10^3 attached to C/T1 and produced a plaque when plated on C/ λ . The T1 control for this experiment indicated a negligible level of abnormal T1 particles or of carry-over of normal T1 particles through the washing procedure. This may indicate that a very low level of phenotypic mixing can actually occur between T1 and λ , but it seems certain that such a process occurs rather rarely under the conditions employed here.

Exclusion of λ by T1 amber mutants. Since T1 infection of W3350($\lambda c1_{11}$), soon after thermal induction of the prophage, efficiently prevents the replication of the λ phage in a large majority of the cells, we were interested in testing the ability of various T1 am mutants to carry out this exclusion function. We were particularly interested in mutants in gene 1 and gene 2, since these mutants fail to make T1 DNA in nonpermissive hosts, and are defective in producing cell lysis (4).

We first tried to test exclusion in heat-induced W3350($\lambda c1_{11}$), but we found that several of the T1 am mutants showed such high transmission in this bacterial strain, with or without heat induction, that this approach was abandoned. (The high transmission of T1 am mutants is not related to the λ prophage, since W3350 also gives high transmission.)

We therefore chose to test the effect of the T1 mutants by infecting *E. coli* strain C with λc_{60} , and superinfecting 15 min later with the desired

TABLE 3. Experiment to detect phenotypically mixed λ particles

λ PFU added to C/ λ cells ^a	λ Plaques found from plating $5 \times$ washed cell suspension on C/T1 lawn	Apparent proportion of phenotypically mixed particles ^b
Expt: 2.8×10^3 from growth tube of the experiment of Table 1	32	1.2×10^{-2}
Control: 3.6×10^4 λc_{60} phage	82	2.3×10^{-3}

^a Calculated on the basis of the total amount of sample plated on the C/T1 lawn.

^b See text for a discussion.

T1am. The T1 mutants mostly have a low transmission in C, so the expected result if the mutant is able to exclude is that the infected cells will not yield either kind of phage. Table 4 shows that this was the case in mixed infections of λc_{60} and T1am16 (gene 1) and with λc_{60} and T1am5 (gene 2). The result with T1am16 requires some comment. This mutant, unlike most, has a high transmission in C, with nearly half of the cells yielding phage in the T1am16-infected control. In the cells infected with λc_{60} and T1am16, however, the number of λ yielders is only about 2% of what is seen in the λ control. Therefore, T1am16 can exclude λc_{60} in a much larger proportion of C cells than it can successfully infect. Furthermore, in the dually infected cells, a much smaller proportion is making T1 than in the T1 control, although this limitation of the growth of T1 by λc_{60} does not necessarily involve a limitation in the amount of T1 gene 1 product being produced.

Although T1 genes 1 and 2 were our chief candidates for a possible role in the exclusion of λc_{60} , we tested a considerable variety of mutants in other genes. In most of the cases, the results resembled those seen with T1am5, i.e., low transmission of the T1am and efficient exclusion of λc_{60} . These mutants included: T2am3 (gene 2); T1am41 (gene 3); T1am15 (gene 5); T1am1 (gene 8); T1am13 (gene 9); T1am20 (gene 10); T1am29 (gene 11); T1am10 (gene 13); T1am45 (gene 14); T1am11 (gene 15); T1am4 (gene 16); T1am7 (gene 17). The results with T1am46 (gene 7) resembled those with T1am16 in Table 4.

TABLE 4. Exclusion of λc_{60} by T1am16 (gene 1) and by T1am5 (gene 2)

Phage added ^a	Infectious centers per cell	
	λ	T1
λc_{60}	0.74	
λc_{60} and T1am16	0.015	Nil ^b
T1am16		0.46
λc_{60} and T1am5	0.01	Nil ^b
T1am5		Nil ^b

^a The cells were first infected (or mock infected) with λc_{60} , incubated for 15 min with aeration at 37°C, and then infected (or mock infected) with T1am.

^b "Nil" means that the T1 plaques obtained by plating a dilution of the resuspended cells was approximately the same whether or not the suspension was treated with CHCl₃. The measurement after CHCl₃ measures the amount of unattached T1 carried over, and was usually about 10% of the number of cells. Therefore, if 0.01 of the cells yielded T1, this would have escaped detection, but a T1 yield of 0.1 of the cells would have been seen.

A case of exclusion of λ accompanied by complementation of the T1 mutant. When we tried the exclusion experiment with T1am23 (gene 4), we observed a surprising phenomenon: λ was efficiently excluded, but at the same time the T1am23 was efficiently complemented (Table 5). Several experiments of this type have been done with T1am23. The transmission with this mutant in strain C is typically around 5 to 10%, but with complementation, from about 50 to 100% of the cells yield T1am23. The complementation shows itself not only as an increase in the proportion of cells yielding T1, but also as an increase in the burst size. When C is infected with T1am23, the burst size is typically about three to five progeny phage per yielding cell; with complementation by λ , the T1am23 burst size is typically around 30 to 60 (data not shown).

DISCUSSION

The observation that λ and T1 show considerable mutual exclusion, and little if any phenotypic mixing, might be taken to mean that they are not related. This conclusion must be tempered, however, by the fact that about 10% of the mixedly infected cells do yield both kinds of phage. Careful experiments with T1 and T2 (3), T1 and T7 (2), and λ and T5 (12) have shown that dual yielders, if they occur at all, are much less frequent than 10%. Dual yielders were observed (13) when P1c1-infected cells were superinfected with T1 late in the P1 latent period, but the suspicion was that this was occurring in cells in which mature P1 were already present in the cell before T1 superinfection. That can scarcely be true in the present instance, at least in the experiments in which superinfection was carried out immediately following induction.

Furthermore, closely related phages can show extensive mutual exclusion even in simultaneous infection. Hausmann *et al.* have reported (5) that only 2 to 5% of cells mixedly infected with T3 and T7 are dual yielders, despite the fact that genetic recombination and phenotypic mixing for serological properties were found.

TABLE 5. Exclusion of λ accompanied by complementation of T1am23

Phage added ^a	Infectious centers per cell	
	λc_{60}	T1am23
λc_{60}	0.88	
λc_{60} and T1am23	0.06	0.67
T1am23		0.09

^a Infection procedure as in Table 4.

Since none of the T1 mutants tested were unable to exclude λ , these experiments do not identify the T1 gene or genes that are required for exclusion. No doubt further T1 genes remain to be discovered, since there are only 18 complementation groups in a phage with the same amount of DNA as λ . In any case, the ability of T1 to exclude λ decreases with time after induction. T4, on the other hand, can effectively exclude λ in a large majority of UV-induced lysogens even if superinfection is delayed until 60 min after induction (10).

Interestingly, λ can provide a substitute for an essential T1 function (gene 4). In a nonsuppressing host, gene 4 mutants have the following phenotype (4): the synthesis of phage DNA is prematurely arrested, lysis of the cells occurs at the normal time, and phage tails are released. No λ mutant has been reported to have a similar phenotype, and the ability of λ to substitute for this function may be the result of a fortuitous analogy rather than being evidence for homology. Further investigation, both of the function of T1 gene 4 and of the λ gene responsible for this complementation, are underway.

ACKNOWLEDGMENTS

This investigation was supported by a Public Health Service grant RR 05403 from the National Institute for General Medical Sciences to the University of Rochester.

LITERATURE CITED

- Christensen, J. R., and J. M. Geiman. 1973. A new effect of the *rex* gene of phage λ : premature lysis after infection by phage T1. *Virology* **56**:285-290.
- Delbruck, M. 1945. Interference between bacterial viruses. III. The mutual exclusion effect and the depressor effect. *J. Bacteriol.* **50**:151-170.
- Delbruck, M., and S. E. Luria. 1942. Interference between bacterial viruses. I. Interference between two bacterial viruses acting upon the same host, and the mechanism of virus growth. *Arch. Biochem.* **1**:111-141.
- Figurski, D. H., and J. R. Christensen. 1974. Functional characterization of the genes of bacteriophage T1. *Virology* **59**:397-407.
- Hausmann, R. L., E. P. Almeida Magalhães, and C. Araugo. 1961. Isolation and characterization of hybrids between bacteriophages T3 and T7. *Anais Microbiol.* **9**:511-526.
- Labaw, L. 1953. The origin of phosphorous in the T1, T5, T6, and T7 bacteriophage of *Escherichia coli*. *J. Bacteriol.* **66**:429-436.
- MacHattie, L. A., M. Rhoades, and C. A. Thomas. 1972. Large repetition in the non-permuted nucleotide sequence of bacteriophage T1 DNA. *J. Mol. Biol.* **72**:645-656.
- Michalke, W. 1967. Erhöhte Rekombinationshäufigkeit an den Enden des T1-Chromosoms. *Mol. Gen. Genet.* **99**:12-33.
- Puck, T. T., A. Garen, and J. Cline. 1951. The mechanism of virus attachment to host cells. I. The role of ions in the primary reaction. *J. Exp. Med.* **93**:65-88.
- Soller, A., and H. T. Epstein. 1965. Biochemical and immunological aspects of the exclusion of lambda by superinfection with T4. *Virology* **26**:715-726.
- Streisinger, G. 1956. Phenotypic mixing of host-range and serological specificities in bacteriophages T1 and T4. *Virology* **2**:388-398.
- Weigle, J. J., and M. Delbruck. 1951. Mutual exclusion between an infecting phage and a carried phage. *J. Bacteriol.* **62**:301-318.
- Werner, E. R., and J. R. Christensen. 1969. Infection by bacteriophage P1 and development of host-controlled restriction and modification and of lysogenic immunity. *J. Virol.* **3**:363-368.
- Wu, R., and E. Taylor. 1971. Nucleotide sequence analysis of DNA. II. Complete nucleotide sequence of the cohesive ends of bacteriophage λ DNA. *J. Mol. Biol.* **57**:491-511.