Exclusion of Bacteriophages by T2 Ghosts

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T2 ghosts do not exclude T4, T7, or lambda-induction in *Escherichia coli* which survive ghost infection. Latent periods are extended, probably by the temporary inhibition of protein synthesis.

We studied the attachment of deoxyribonucleic acid (DNA)-less T2 ghosts to *Escherichia coli* (6) as part of a study of virulence by T-even bacteriophages. At a multiplicity just sufficient to inhibit protein synthesis, ghost attachment killed most bacteria. However, it appeared that in the surviving cells transcription continued and the inhibition of protein synthesis reversed itself after about 30 min. T-even phages exclude development of other phages (4, 5), and we have examined exclusion by T2 ghosts to test the hypothesis that only the colony-forming cells are metabolically active soon after ghost infection. Early studies reported that ghosts do exclude superinfecting phages (3, 7-9), although Herriott and Barlow (9) observed less efficient exclusion by ghosts than by phage. French and Siminovitch (7) reported that exclusion was virtually complete if superinfection occurred during the first 100 min of ghost treatment, but, after that time, superinfecting phage were not excluded from the non-killed bacteria. We reinvestigated the problem by following the production of mature phage in one-step growth curves.

T2 ghosts were prepared by osmotic shock and purified in KBr gradients as described (6; In the published procedure [1] we failed to specify that the KBr density gradient centrifugation should be at 15 C. At lower temperature the KBr crystallizes and interferes with the banding. We are sorry for any inconvenience caused by this oversight). As in the previous experiments, a multiplicity of ghosts per cell (about 8) just sufficient to prevent induction of β -galactosidase (EC 3.2.1.23) was used (6). First, we investigated the exclusion of T4 and T7. Ghosts were added to *E. coli* B 5 min before superinfection. In each case, exclusion by ghosts was compared to complete exclusion by T2 as well as to the case of no exclusion. Two parameters of the one-step growth curves are affected by ghost attachment (Fig. 1). (i) The burst size is reduced but exclusion is not complete even though > 99% of the cells must have at least one ghost attached (β -galactosidase inducibility is <1% of normal). The reduction in average burst

size (to 6%) is approximately proportional to the reduction in number of viable cells at the time of superinfection (to 4%). (ii) In the ghost-treated cultures the latent periods of both T4 and T7 are extended by 20 to 30 min: approximately the interval in which protein synthesis is inhibited in ghost-treated bacteria (6).

FIG. 1. Exclusion of T4 (A) and T7 (B) in *Escherichia coli* B by T2 and T2 ghosts. Bacteria were grown at 37 C in M9 (ammonium [2]) medium supplemented with 0.2% glycerol rather than glucose. The mass doubling time was 60 min. At a cell density of 5×10^8 /ml, separate cultures were infected with T2, MOI = 20 ($\square$), or T2 ghosts, MOI $\approx$ 10 ($\circ$), or were not infected ($\bullet$). Five minutes later the cultures were infected with T4 (MOI = 0.5) or with T7 (MOI = 0.6). At indicated times, samples (0.1 ml) were shaken with 100 ml of phage-diluting fluid (P.D.F.) (6) containing 1 ml of chloroform. After appropriate dilutions, the samples were mixed with about 10^8 indicator bacteria (*E. coli* B/2) and plated for plaque-forming units (PFU).

Exclusion may be mediated by changes in the cell envelope which result from the primary infection (1). We asked if ghosts exclude development of a temperate phage from the lysogenic state. *E. coli* B, strain ER-46 (from Joseph Eigner), is sensitive to phage lambda. ER-46 was lysogenized

with λ C1857; this phage can be induced by raising the culture temperature to 40 C (10). The exclusion results parallel those observed with super-infecting T-phages (Fig. 2).

The parallel reduction in burst size and viability suggests that phage are *not* excluded in the cells that survive ghost attachment. However, this could be fortuitous, e.g., 100% of the bacteria might produce an average of 6% as many phage rather than 6% of the bacteria yielding a normal average burst. A comparison of viability and burst size to the number of cells that produce phage (productively infected) would test these alternatives directly. These parameters were measured after T7 superinfection of cultures infected with different multiplicities of T2 ghosts. In

all cases the reduction in burst size paralleled both the reduction in viable count and in productively infected cells (Table 1). As reported earlier (6, 7), β -galactosidase induction is inhibited much more severely. Similarly, in the experiment shown in Fig. 2, only 9% as many bacteria (compared to controls) gave lambda bursts at 1 min after ghost addition.

The close relationship between capacity to support phage immediately after ghost addition and to form a colony after overnight incubation confirms the earlier speculation (6, 7) that ghost infection results in viable and killed cells, with the former responsible for metabolic activities. The extension of the latent period probably results from the transient block in protein synthesis in the surviving cells. Thus, attachment by ghosts is not sufficient to exclude phage development permanently. This suggests that exclusion by whole phage is dependent on expression of the phage

FIG. 2. Exclusion of induced lambda phage by T2 and T2 ghosts in *Escherichia coli* B. Strain ER-46 (λ C1857) was grown in M9 plus glycerol (0.2%) at 30 C to a density of 5×10^8 bacteria/ml before transfer to 40 C. Three minutes after transfer, one culture was infected with T2 ($\square$), a second with T2 ghosts ($\circ$), and a third was not infected ($\bullet$). All cultures were brought to 34 C after 10 min at 40 C. Samples were chloroform-treated and diluted to measure free lambda phage using strain ER-46 indicator cells. Samples from the T2-infected culture were diluted into 5 ml of P.D.F. containing sufficient T2 antisera to inactivate >99.9% free T2. Plates were incubated at 40 C.

TABLE 1. Effects of T2 ghosts on *E. coli* and its capacity to support T7 superinfection^a

Viable cells/ml ^b (%)	Productively infected cells/ml ^c (%)	Avg burst size/ml ^d	β -galactosidase induction ^e (%)
100 ^b	100	100	100
45	37	50	13
23	17	17	2.2
10	10	8	1.6

^a Bacteria were infected with T2 ghosts at a given multiplicity or not infected (top line) and then superinfected with T7 (MOI ≈ 1) after 5 min. A value of 100% corresponds to about 7×10^8 bacteria/ml, 4×10^8 productively infected cells/ml, and an average burst size of 6×10^{10} phage/ml.

^b Viable cells per milliliter at time of T7 infection. In separate cultures (no T7) the number of viable cells decreased slightly (10 to 25%) between 5 min and 8 or 10 min (when productively infected cells were measured) after ghost infection. It is not clear which time should be used for the comparison, but the values at either time lead to the same conclusion.

^c Infected cells, each of which would give a plaque, were estimated at 3 to 5 min after T7 infection (total infective centers—free phage). The 5-min values were somewhat lower than the 3-min (about 20%), probably because the ghosts are continuing to kill cells during this interval even though the cells are infected with T7 (see footnote b).

^d Average burst sizes determined from 60 to 100 min after infection.

^e Bacteria induced with isopropyl thio- β -D-galactoside (5×10^{-4} M) simultaneously with ghosts. Rate determined by increase from 3 to 13 min later.

genome although the attachment process may be responsible for both exclusion and inhibition of nonphage protein synthesis very early after infection (6). These results emphasize the importance of recognizing the presence of both killed and non-killed cells in ghost-treated cultures in any analysis of the effects of ghosts on metabolism.

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