R17 Bacteriophage Replicase: Association with the Inhibition of Qβ, fd Bacteriophage and β-Galactosidase Production

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Superinfection of Escherichia coli with amber mutants of R17 phage which produce R17 replicase inhibits production of the RNA phage Qβ, the DNA phage fd, and the host enzyme β-galactosidase. Inhibition required R17 replicase production and was related to the amount of replicase produced.

With high-resolution autoradiographic techniques, Granboulan and Franklin (6) demonstrated the subjugation of Escherichia coli macromolecular synthesis during infection with R17 bacteriophage. An altered host RNA metabolism results from an inhibition of ribosomal RNA synthesis (7). This effect requires the synthesis of R17 replicase, and the level of inhibition correlates with the amount of replicase produced but is independent of the synthesis of the other phage proteins (15). Infection with R17 phage also causes an alteration in the polyribosome distribution. The altered polyribosome distribution is dependent on the production of viral replicase (12) and results in a shift from the synthesis of host proteins to the synthesis of viral proteins (15). Because R17 phage protein synthesis occurs during the inhibition of host protein synthesis, it was of interest to know if the inhibition associated with R17 phage infection was specifically directed against cellular macromolecular synthesis, or macromolecular synthesis in general. This was examined by infecting cells with R17 and an heterologous phage. In this paper, we show that superinfection with amber mutants of R17 phage inhibit production of the RNA containing Qβ and the DNA containing fd phages, and that this inhibition is also associated with R17 replicase production.

Amber mutants of R17 phage (amA31, amB22, amB24, and amC16) were replicated in the permissive strain of E. coli S26 RIE according to the procedure of Tooze and Weber (17).

The effect of superinfection with the amber mutants of R17 on Qβ or fd phage production was determined in E. coli S26 cultures which are nonpermissive for the R17 mutants. E. coli S26 was grown to 2 x 10⁸ cells/ml in MS broth (15) and then infected with Qβ or fd phage (10 PFU/cell). After 5 min of incubation the cultures were superinfected with the various amber mutants of R17 phage (10 PFU/cell). At 15 min postinfection a sample of cells was withdrawn from each culture and washed with cold MS broth. The number of infectious centers was determined by titering the infected cells with the standard agar overlay technique using the nonpermissive strain of E. coli S26 as the indicator strain. At 120 min postinfection the cultures were harvested by shaking with 5% chloroform to free cell-associated virus. The final yield of Qβ or fd phage was determined by plaque assay on E. coli S26. Qβ or fd phage burst size was calculated as the average number of phage produced per infectious center.

Production of β-galactosidase was assayed in nonpermissive E. coli C3000 cultures infected with amber mutants of R17 phage. Cells were grown to 2 x 10⁸ cells/ml in TCG1-t medium (12) and then infected with the amber mutants (10 PFU/cell). At 30 min postinfection β-galactosidase synthesis was induced with isopropyl-β-D-galactoside at 5 x 10⁻⁴ M. Samples were withdrawn at various times after induction and assayed for β-galactosidase production as described by Pardee et al. (11).

In studying interference during mixed infection it is important to distinguish between competition for adsorption sites and intracellular interference. In the case of the sex-specific coliphage, multiple adsorption sites exist on F pilus (3). The fd phage adsorbs to the tip of the pilus whereas the RNA coliphage adsorbs to multiple sites along its length (3). Studies of host mutants suggest the sites along the side of the F pilus may be distinct for different RNA coliphage (14) and that preinfection of cells by one type of RNA phage does not affect subse-
quent infection by another type of RNA phage (9). Nevertheless, to minimize the possibility that our amber mutants were inhibiting heterologous phage production at an extracellular stage of infection, cells were infected with Qβ or fd phage for 5 min before superinfecting with the amber mutants of R17 phage. Under these conditions the majority of the initial infecting phage is adsorbed and eclipsed within 5 min of infection (10). Secondly, the average burst size of Qβ or fd phage was determined as a function of the number of phage produced per productively infected cell. These precautions appear satisfactory since superinfection of Qβ- or fd-infected cells with amA16, which is able to adsorb to cells but lacks replicase production, did not alter the burst size of Qβ- or fd-infected cells as compared to control cultures (Table 1).

Inhibition of Qβ and fd phage production was related to the amount of R17 replicase produced (Table 1). The mutant amB22, which produces an abnormally large amount of replicase, resulted in a substantially greater inhibition than superinfection with amA31 which produces the same amount of replicase as infection with wild-type R17 (15). The mutant lacking the ability to synthesize replicase, amC16, had no effect on Qβ or fd phage production. Moreover, the inhibition of Qβ or fd phage production was independent of R17 coat protein or assembly protein synthesis since the inhibition occurred upon superinfection with amB22 and amA31 which lack the ability to synthesize coat and assembly protein, respectively. Thus the degree of inhibition of Qβ and fd phage production was only dependent on the amount of R17 replicase synthesized.

A similar dependence on R17 replicase production was associated with the inhibition of β-galactosidase synthesis (Fig. 1). A substantial inhibition of β-galactosidase synthesis occurred in cells infected with the mutant amB22 which lacks coat protein synthesis and synthesizes an abnormally large amount of replicase. However, no significant effect on β-galactosidase synthesis was noted when cells were infected with amC16 or amB24. The mutant amC16 lacks

![Graph showing β-Galactosidase activity](http://jvi.asm.org/)

**Fig. 1. β-galactosidase synthesis in E. coli during infection with various amber mutants of R17 bacteriophage.** E. coli 3000 was grown to 2 × 10⁸ cells/ml in TCG1-t medium (16) and infected with amber mutants of R17 phage: amB22 (coat protein mutant, ◆); amB24 (polar coat protein mutant which restricts replicase production, ■); and amC16 (replicase mutant, ▲) at 10 PFU/cell; control (○). Thirty minutes postinfection, the infected and control cultures were induced for β-galactosidase production with IPTG (isopropyl-thiogalactoside, 5 × 10⁻⁴ M). Samples were collected at various times postinfection and assayed for enzyme production. β-Galactosidase experiments were repeated five times. Inhibition of β-galactosidase was determined from the mean of triplicate samples from cultures infected with phage mutants compared to triplicate samples from uninfected control cultures. The average standard error of the mean is 0.36 × 10⁻¹ U/ml.

### Table 1. Average burst size of Qβ or fd phage-infected cells superinfected with amber mutants of R17 phage

<table>
<thead>
<tr>
<th>Superinfecting R17 mutant</th>
<th>Qβ burst size (PFU/infectious center)</th>
<th>% Inhibition of Qβ phage production</th>
<th>fd Burst size (PFU/infectious center)</th>
<th>% Inhibition of fd phage production</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>935 ± 60</td>
<td>0</td>
<td>506 ± 55</td>
<td>0</td>
</tr>
<tr>
<td>amA31</td>
<td>500 ± 45</td>
<td>46</td>
<td>348 ± 22</td>
<td>31</td>
</tr>
<tr>
<td>amB22</td>
<td>58 ± 22</td>
<td>94</td>
<td>160 ± 37</td>
<td>68</td>
</tr>
<tr>
<td>amC16</td>
<td>962 ± 81</td>
<td>0</td>
<td>535 ± 52</td>
<td>0</td>
</tr>
</tbody>
</table>

*E. coli S26 cultures (nonpermissive for amber mutants of R17 phage) were infected with the RNA phage Qβ or the DNA phage fd at 10 PFU/cell and then superinfected with an amber mutant of R17 phage 5 min later. Samples were removed at 15 min postinfection and washed with cold media, and the number of Qβ- or fd-infected cells was determined by infectious center assay on E. coli S26. Final Qβ or fd phage production was determined at 120 min postinfection by the standard agar overlay technique. Each value in the Table is the average obtained from two or more experiments performed with triplicate samples, with standard deviations indicated.
replicase production while amB24 is a polar coat protein mutant which is unable to synthesize coat protein and greatly restricts the translation of the adjacent replicase cistron (15). These results support an earlier study on the effect of R17 amber mutants on polyribosome distributions in infected cells (12).

Figure 2 shows the effect on the final Qβ phage titer when superinfected with amB22 at various time intervals after initial infection with Qβ phage. The maximum inhibition of Qβ phage replication occurred when the amB22 phage was added 1 min after Qβ phage infection. The degree of inhibition is similar to that shown in Table 1, suggesting that interference at the level of phage adsorption is not significant. As Qβ phage infection proceeds it becomes less sensitive to superinfection with amB22, suggesting an early function such as a defect in Qβ replicase production or function may be involved in lower yields of Qβ phage.

Inhibition of Qβ and fd phage production shares with the inhibition of host macromolecular synthesis a dependence on R17 replicase production. It seems unlikely that this similarity results from a dependence of Qβ phage production on the repressed host macromolecular synthesis since RNA phage biosynthesis is independent of the inhibition of host DNA, RNA, and protein synthesis (1, 2, 5, 12).

Because of the similarities in RNA coliphage gene function and biosynthesis, it seems likely that the composition of R17 replicase will resemble f2 replicase (4) and Qβ replicase (8) in containing a phage-specified peptide and several host peptides. However, subtle differences in the composition of the replicases of different RNA coliphages may eventually explain their widely different effects on host macromolecular synthesis (18). The present study provides further support for the involvement of R17 replicase in the inhibition of host macromolecular synthesis. Furthermore, our results indicate the mechanism of inhibition is not directed only against cellular biosynthesis but also against production of certain heterologous phage. Investigation of the block in Qβ and fd phage production in cells superinfected with amB22 should provide information on the mechanism of inhibition associated with R17 replicase production.

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**LITERATURE CITED**


