Roles of Bacteriophage T4 Gene 5 and Gene *s* Products in Cell Lysis

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Previous studies indicated that (i) T4 gene *s* product (gps) protects infected cells from superinfection lysis from without, (ii) the absence of gps in infected cells also leads to lysis from within even when T4 *e* lysozyme is absent, (iii) T4 gene 5 product (gp5), a polypeptide of the virion baseplate, may be responsible for inducing lysis from without, and (iv) altered gp5 of the T4 mutant *5ts1* can replace *e* lysozyme to cause lysis from within. Results of this study showed that (i) wild-type gp5 in infected cells lacking *e* lysozyme was responsible for lysis from within in the absence of gps, and (ii) gps did not protect infected cells from superinfection lysis from without by *5ts1* phage. We propose that gps normally prevents functional expression of wild-type gp5 activity from either side of the cell wall, whereas the *5ts1* form of gp5 is insensitive to this gps barrier and induces lysis from either side of the cell wall.

Bacteriophage T4 can lyse bacterial cells by at least two different processes (3). One, termed lysis from within (LI), occurs at a characteristic time after infection to allow release of progeny phage. The other, termed lysis from without (LO), is the immediate decomposition of host cells upon high multiplicity of infection (MOI).

An enzyme coded by T4 gene *e*, the *e* lysozyme, is responsible for degradation of the peptidoglycan layer of the host cell envelope during LI (12); bacteria infected with T4 strains that lack active *e* lysozyme do not demonstrate LI (10). The other process, that of LO, is not dependent on *e* lysozyme because virions of mutants lacking this enzyme cause LO (7, 14).

Previously, two kinds of T4 mutants that permit LI in the absence of *e* lysozyme have been isolated. One kind carries a mutation in gene *s* (spackle), but it is unknown why the absence of this gene product (gps) leads to LI (6). The *s*⁻ mutants display another property related to lysis: whereas bacteria infected with wild-type *s*⁺ phage develop resistance to superinfection LO (13), those infected with *s*⁻ phage do not (6). It has been postulated that gps may be incorporated into the bacterial cell wall during infection; in mutants that lack such modification, LI occurs in the absence of *e* lysozyme because cell walls are now sensitive to another lytic enzyme (6). Whether this other lytic enzyme is normally present in the uninfected bacteria or is the product of a phage gene is not known.

The other kind of mutants that permit LI in the absence of *e* lysozyme are described in the accompanying paper (8); these mutants carry a specific temperature-sensitive mutation (*5ts1*) in gene 5, a gene whose polypeptide product (gp5)

is a structural component of the baseplate central plug (9). LI was shown to be accomplished by the altered gp5, and we postulated that gp5 has lytic activity, though such activity remains to be demonstrated directly. Since the T4 baseplate structure comes into contact with the cell wall at the onset of infection (11), we postulated that gp5 is a virion-associated lytic activity which locally digests the cell wall before penetration of the tail. At high MOI gp5 may also induce LO. Moreover, to account for the observation that only the mutant form of gp5 promotes LI, we postulated that the action of wild-type gp5 is somehow inhibited in infected cells.

Since gps protects infected bacteria from superinfection LO, which presumably results from the action of gp5 of the superinfecting virions, it seems that gps blocks the activity of wild-type gp5. Therefore, we think that wild-type gp5 produced in infected cells normally does not induce LI because its activity is masked by gps, whereas the *5ts1* form of gp5 induces LI because its activity is insensitive to the gps barrier. These considerations have led us to propose that LI (in the absence of gps and *e* lysozyme) is accomplished by wild-type gp5, and that bacteria protected by gps of the primary phage are nevertheless sensitive to superinfection LO by *5ts1* phage. Tests of these predictions are reported here.

MATERIALS AND METHODS

Bacteriophage T4 strains, *Escherichia coli* strains, and media are described in the text or in the accompanying paper (8). Methods are described in figure legends.

RESULTS

Participation of wild-type gp5 in LI. To determine whether gp5 activity is responsible for lysis of $e^- s^-$ phage-infected cells, we compared the turbidity of bacterial cultures infected with $e^- s^- 5^-$ and $e^- s^-$ phages. The phage strains also carried a 27^- mutation to preclude assembly of progeny phage (9), thus eliminating any complications that might arise as a result of superinfection by progeny particles. Bacteria infected with $e^- s^- 27^-$ phage lysed, whereas those infected with $e^- s^- 5^- 27^-$ phage did not (Fig. 1). In addition, bacteria infected with $e^- s^- 5^-$, a strain which allows synthesis of gp27, but not the assembly of progeny phage due to the absence of gp5 (9), did not lyse either (data not shown). Thus gp5 is essential for LI in the absence of gps and e lysozyme.

Since gp5 is not incorporated into the central plug of the baseplate in the absence of gp27 (9), the results in Fig. 1 demonstrate that the presence of nonassembled gp5 is sufficient for LI. We then tested the effect on LI of incorporation of gp5 into baseplate, using an $e^- s^- 48^-$ phage strain; the 48^- mutation blocks assembly at a step after gp5 and gp27 are incorporated into the baseplate (9). We found a small but reproducible decrease in lysis with $e^- s^- 48^-$ phage compared with $e^- s^- 27^-$ phage (Fig. 2). Thus, incorporation of gp5 into baseplates appears to result in a reduction in LI. Since the fraction of total gp5 assembled into baseplates in the 48^- state is not known (i.e., how much gp5 remains nonassembled in 48^- infections?), we cannot assess the capacity of assembled gp5 to cause LI.

FIG. 1. LI in the absence of gps and e lysozyme. B/5 bacteria grown to a concentration of 2×10^8 cells/ml in B broth were concentrated to 2×10^9 cells/ml by centrifugation and infected at 37°C with phage at an MOI of 5. Ten minutes later, the infected cultures were diluted 10-fold and incubated at 37°C with aeration. Turbidity was measured at 420 nm with a Bausch & Lomb Spec-20 spectrophotometer. Symbols: ○, $e^- s^- 27^-$; ●, $e^- s^- 5^- 27^-$.

FIG. 2. Effect of incorporation of gp5 into baseplates on LI. Lysis of infected cultures was measured as described in the legend to Fig. 1. Symbols: ○, $e^- s^- 27^-$; ▲, $e^- s^- 48^-$.

Nevertheless, the observed effect on LI of incorporation of gp5 into baseplates indicates that the free gp5 is more active.

These results support the hypothesis that gps precludes expression of the activity of wild-type gp5; just as gps prevents gp5 in superinfecting phage from inducing LO, it also prevents gp5 in infected cells from inducing LI.

Since we previously attributed lytic activity to wild-type gp5 in inducing LO and to the *5ts1* form of gp5 in achieving LI (8), we think that wild-type gp5 also acts in this capacity to cause LI when gps is absent. However, we emphasize that the lytic activity of gp5 remains to be demonstrated directly.

LO by superinfecting *5ts1* phage. To test our hypothesis that gp5 in *5ts1* virions causes superinfection LO of infected bacteria containing wild-type gps, we followed the turbidity of bacterial cultures superinfected at an MOI of 100. Bacteria primarily infected with wild-type T4 were resistant to LO by superinfecting wild-type T4, but not by superinfecting *5ts1* phage (Fig. 3). These results also confirm an earlier report that s^- phage-infected cells are sensitive to LO by superinfecting wild-type T4 (6). We also observed that bacteria infected with *5ts1* phage were not sensitive to superinfection LO by wild-type T4 (data not shown). Thus, gps of primary phage, which normally protects infected cells from superinfection LO by wild-type T4, does not afford protection against *5ts1* phage. Furthermore, strain *5ts1* is not deficient in rendering infected cells resistant to superinfection LO.

These observations further support our proposal that gp5 in phage particles is responsible for LO, and are consistent with our hypothesis that the activity of the *5ts1* form of gp5 is insensitive to the inhibitory effect of gps. Just as mutant gp5 in infected cells is capable of causing

FIG. 3. Superinfection LO. *B/5* bacteria grown to a concentration of 2×10^8 cells/ml in *B* broth were infected at 37°C with primary phage at an MOI of 5. Seven minutes later, cultures were infected with secondary phage at an MOI of 100. Turbidity was measured with a Bausch & Lomb Spec-20 spectrophotometer. The arrow indicates time of superinfection. Symbols: $\square$, Wild-type T4-infected cells superinfected with wild-type T4; Δ , wild-type T4-infected cells superinfected with 5ts1 phage; $\circ$, s^- phage-infected cells superinfected with wild-type T4. Revertant strain 5ts1R behaved like wild-type T4 when used as the superinfecting phage (data not shown).

LI irrespective of the presence of gps, mutant gp5 in superinfecting virions is capable of inducing superinfection LO in bacteria containing wild-type gps.

Lysis inhibition of bacteria infected with 5ts1 phage. LI is delayed when infected bacteria are superinfected a few minutes after the initial infection. This phenomenon is known as lysis inhibition (4). However, bacteria primarily infected with s^- phage cannot be lysis inhibited (6), a finding which indicates that gps is required to establish normal lysis inhibition. Since gps normally prevents LI and superinfection LO by blocking the lytic activity of gp5, gps might be expected to participate in an analogous fashion in the establishment of lysis inhibition. Because gp5 of the 5ts1 mutant is insensitive to the inhibitory effect of gps, we tested the mutant for competence in lysis inhibition.

Turbidity of bacterial cultures primarily infected with various T4 strains and superinfected with wild-type T4 at low MOI was followed. Whereas lysis was not observed after 4 h with cells primarily infected with wild-type T4, lysis was observed with cells primarily infected with 5ts1 phage (Fig. 4). In addition, bacteria primarily infected with either s^- or rapid-lysis mutant

rI48 (5) phage also lysed. Note that each mutant manifests a unique lysis profile. The defect of 5ts1 phage in establishing lysis inhibition was further manifested by its *r*-like plaque morphology (8). Thus, either the presence of the 5ts1 form of gp5 or the absence of gps in infected cells results in a partial defect in lysis inhibition.

These observations lead us to hypothesize that gps participates in the establishment of lysis inhibition by preventing expression of the lytic activity of intracellular wild-type gp5; thus, in superinfected cells, either the absence of gps or an alteration of gp5 rendering it insensitive to gps accelerates the onset of cell lysis.

DISCUSSION

Roles of gp5 and gps in cell lysis. Although *e* lysozyme is normally responsible for LI (12), there are T4 mutations that permit such lysis in the absence of *e* lysozyme. Previous studies of strains harboring such mutations revealed that: (i) in the case of s^- mutations, the absence of gps not only leads to LI, but also renders the infected cells sensitive to superinfection LO (6); (ii) in the case of the 5ts1 mutation, gp5 is postulated to have lytic activity that is responsible for LI (8); and (iii) gp5, a structural component of the virion baseplate, may be responsible for LO (8). These observations suggest to us that gps may mask the lytic activity of wild-type gp5 in superinfecting virions and thereby protect the infected cells from LO; on the other hand, the intracellular gp5 of 5ts1 e^- phage is

FIG. 4. Lysis inhibition. Cultures of *E. coli* Bb grown at 30°C to 2×10^8 cells/ml in *B* broth supplemented with 10% M9 salts and 0.2% glucose were infected with primary phage at an MOI of 5 and superinfected with wild-type T4 at the same multiplicity 10 min later. Primary phages used were: $\square$, wild-type T4; Δ , 5ts1; $\circ$, s^- ; $\blacksquare$, rI48. Revertant 5ts1R behaved like wild-type T4 when used as the primary phage (data not shown).

insensitive to the masking of gps and thereby induces LI.

The results in this study showing that wild-type gp5 in infected cells is responsible for LI in the absence of *e* lysozyme and gps, and that gp5 of 5ts1 virions induces superinfection LO irrespective of the presence of intracellular gps, lend further support for the above proposal. Thus, gp5 in virions induces superinfection LO when intracellular gps is absent or when gp5 is in the mutant form. Analogously, gp5 in infected cells can cause LI when gps is absent or when gp5 is in the mutant form.

How gp5 acts (does it have lytic activity?) and how gps represses this activity, though not that of the mutant 5ts1, remain to be determined.

Lysis inhibition. Although little is known about the details of lysis inhibition, the phenomenon is known to be accompanied by prolonged integrity of the cytoplasmic membrane; disruptive changes of the membrane, such as ATP leakage, cessation of oxygen uptake, and exposure of the cell wall to the action of *e* lysozyme, which normally occur at the end of the characteristic latent period, are delayed during lysis inhibition (2, 10).

In superinfected cells, gp5 of the primary phage can accelerate the onset of lysis, either when gps is absent or when mutant gp5 is present (Fig. 4). How gp5 gains access to the appropriate region(s) of the cell is an interesting problem for future research. One possibility is that when the gps barrier is absent, intracellular gp5 acts at the cell wall-membrane adhesions, sites which are otherwise shielded by gps (see below).

There are approximately 300 such adhesion sites on the *E. coli* cell surface, and it is at these sites that T4 is believed to inject its DNA (1). Thus, during normal infections the lytic activity of virion-associated gp5 should be manifested at the adhesion sites to facilitate injection. Subsequent association of gps with adhesion sites would then protect infected cells from the lytic function of gp5 of superinfecting phage. With

regard to LI, it is possible that the structure of the adhesions is such that, in the absence of gps, the gp5 target is left accessible to intracellular gp5.

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