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Baseplate Protein of Bacteriophage T4 with Both Structural and Lytic Functions

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Analyses of a new bacteriophage T4 mutant that permits lysis of infected cells in the absence of *e* lysozyme showed that the strain carried a suppressor mutation in gene *5*, a gene whose polypeptide product (gp5) is an integral component of the virion baseplate. Indirect experiments indicated that cell lysis was caused by the lytic action of mutant gp5. With regard to the physiological role of normal gp5, we speculate that it functions in the initiation of infection by catalyzing local cell wall digestion to facilitate penetration of the tail tube through the cell envelope. The proposed lytic activity of gp5 may also be responsible for the well-known phenomenon of lysis from without observed with T4.

The initial step in the infection of *Escherichia coli* by a T-even phage is the attachment of the virus to the bacterium, which then leads to the penetration of the phage tail tube through the cell envelope and injection of phage DNA into the cell (19). How the tail tube penetrates the cell envelope is not known; it may be solely the result of a mechanical force generated by the contraction of the tail sheath, or it may be assisted by a phage particle-associated enzyme which locally digests the cell wall.

Previous studies showed that saturation of purified *E. coli* B cell walls with phage T2 results in complete disintegration of cell walls (23), and that adsorption of T phages to isolated ^{15}N- or ^{32}P-labeled cell wall preparations causes the release of isotope (2). It has been suggested that such degradation reflects the action of an enzyme associated with the tail structures of these phages; physiologically, this enzyme would facilitate injection of DNA into the cell and also catalyze lysis from without (2, 24), a process that consists of immediate dissolution of host cells upon infection with high multiplicities of phage (4). For some time it was believed that T4 *e* lysozyme, which is responsible for degradation of the host cell wall at the end of the infection cycle (15), was also the tail-associated enzyme. Indeed, *e* lysozyme is present in virions and can be detected after freeze-thaw treatment of T4 particles (6, 26). However, it has also been shown that the association of *e* lysozyme with phage particles is apparently adventitious and is not essential for the initial steps of infection (6, 26). Nevertheless, wild-type T4 and e^- mutants (propagated without exogenously added lysozyme) do have a second particle-associated lytic activity, and these particles are capable of inducing lysis from without (6, 26). Furthermore, it was shown more recently that e^- mutants and wild-type T4 cause the release of similar cell wall materials from host cells in the course of adsorption (12). Thus, the degradation of isolated cell walls by the adsorbed e^+ phage observed earlier may not have been caused by the *e* lysozyme adventitiously associated with phage particles, but rather by another particle-associated lytic activity.

In this paper, we describe the isolation and characterization of a new class of T4 mutants that permit lysis of infected cells in the absence of *e* lysozyme. Our results indicate that the polypeptide product of gene *5* (gp5) may have a lytic role in inducing both lysis at the end of the infection cycle (with *5ts1* e^- strains) and lysis from without. Because gp5 is incorporated into the T4 baseplate (11), a structure that makes contact with the cell wall at the onset of infection (19), we believe that gp5 is a candidate for the second particle-associated enzyme mentioned above.

MATERIALS AND METHODS

Bacteriophage. Wild-type T4D and lysozyme-negative (e^-) mutants used have been described previously (3, 9). Mutants *e*L5G and *e*L1G *e*L5G also contain a deletion mutation, Δ119, which eliminates genes for all T4 tRNA's except that of $tRNA^{Arg}$ (9). Mutant *s*12 was obtained from J. (Emerich) Owen. Amber mutants in genes *5*, *6*, and *53*, as well as temperature-sensitive mutants in gene *5*, were from the collection of W. B. Wood. Other amber mutants used were from our collection.

Bacteria. The following *E. coli* strains were used: B (18) and B/5 (13) are nonpermissive hosts for nonsense mutants; CAJ64 carries a UGA suppressor (17) and is a permissive host for UGA mutants; CR63 (Su_1^+) is a permissive host for amber mutants (13); 011′ (Su_2^+), a permissive host for amber mutants (3),

was used on egg white lysozyme (Worthington Biochemicals Corp.)-supplemented plates because it gave the best plaque size; Bb, a nonpermissive host for amber mutants (11), was used to prepare ^{14}C-labeled phage lysates.

Media. Lysozyme-supplemented plates were the citrate plates described by Emerich (5). Except when specified, EHA top and bottom agar (20) were used. B broth (5), C broth (3), and M9 medium (3) were prepared as previously described.

Dominance tests. B/5 bacteria grown to a concentration of 5×10^7 cells/ml in C broth were concentrated by centrifugation and resuspended at 10^9 cells/ml in prewarmed (30 or 42°C) C broth containing 4×10^{-3} M KCN. Cultures were infected at 30 or 42°C with an equal volume of prewarmed phage at a multiplicity of infection (MOI) of 10 (5 for each parental phage in the case of mixed infections). Ten minutes later, the infected cultures were diluted 10^{-5} into prewarmed C broth. Chloroform was added to the cultures at 60 or 90 min after infection for tests done at 42 or 30°C, respectively. The total number of progeny was determined by plaque assay at 30°C with permissive indicators (CR63 for amber mutants and B/5 for am^+ strains); plaque counts were corrected for unadsorbed phage present at 10 min after infection. Burst size was calculated as phage per input cell.

Crosses. Crosses were performed at 30°C by growing *E. coli* CR63 to a concentration of 5×10^7 cells/ml in C broth, centrifuging, resuspending at 10^9 cells/ml in prewarmed C broth containing 4×10^{-3} M KCN, infecting with an equal volume of prewarmed phage at MOI of 7.5 for each parental phage, and diluting 10^{-5} into fresh C broth 10 min later. The infected cultures were lysed with chloroform 75 min after infection. Appropriate platings were done to assay for total progeny and recombinant-type progeny. Plaque counts obtained from different host indicator strains were corrected for any differences in plating efficiencies observed with wild-type T4. Less than 2% of the added phage remained unadsorbed when measured at 10 min after infection; therefore, less than 0.1% of the progeny phage represented unadsorbed parental phage. Each parent contributed approximately equally to the crosses as ascertained, whenever possible, by plating under conditions that distinguished markers carried by the two parental phages. Recombination frequency was calculated as 200% × (measured wild-type recombinant progeny/total progeny).

Complementation tests. B/5 bacteria grown to a concentration of 5×10^7 cells/ml in C broth were concentrated to 10^9 cells/ml in prewarmed (42°C) C broth and infected at 42°C with an equal volume of prewarmed phage at a total MOI of 10 (5 for each parental phage in the case of mixed infection). The infected cultures were diluted 10^{-5} in prewarmed C broth at 10 min after infection and incubated at 42°C with aeration. At 60 min after infection, the cultures were lysed with chloroform. The total number of progeny phage was measured by plaque assay at 30°C with CR63 as indicator and corrected for the number of unadsorbed phage present at 10 min after infection, which was less than 2% of the added phage. Burst size was calculated as progeny phage per input cell.

One-step growth experiments. B/5 bacteria grown to a concentration of 5×10^7 cells/ml in C broth were concentrated to 5×10^8 cells/ml in prewarmed (42°C) C broth, and KCN was added to a final concentration of 4×10^{-3} M. Cultures were then infected at 42°C with equal volumes of prewarmed (42°C) *5ts1* or wild-type T4 at an MOI of 2. Eight minutes later, anti-T4 serum was added to a final concentration of $k = 1.5$/min. Thirteen minutes after infection, cultures were diluted at least 10^{-4} into fresh medium at 42°C. Infective centers were measured periodically by plating appropriate dilutions on plates with B/5 as indicator at 30°C. Total progeny phages, including intracellular phages, were also measured periodically by shaking portions with chloroform before plating appropriate dilutions for plaque assay.

Preparation of ^{14}C-labeled lysates. An overnight culture of *E. coli* Bb in vitamin B1 (2 μg/ml; Sigma Chemical Co.)-supplemented M9 medium was diluted 50-fold into fresh medium and grown to a concentration of 4×10^8 cells/ml at 30°C. Immediately before infection, tryptophan (Sigma) was added to a final concentration of 10 μg/ml. Bacterial cultures of 2.5 ml were then infected at 30°C with phage at an MOI of 7.5 and superinfected with the same phage at the same MOI 8 min later. Five microcuries of uniformly labeled ^{14}C-amino acid mixture (New England Nuclear Corp.) was added at 18 min after primary infection. At 40 min after primary infection, Casamino Acids (Difco Laboratories) was added to a final concentration of 0.27% (wt/vol), and 5 min later the infected cells were collected by centrifugation in a Sorvall SS-34 rotor at 4°C (8 min at 10,000 rpm). The pellets were drained and resuspended in 0.1 ml of 0.01 M Tris-hydrochloride buffer at pH 7.4 supplemented with 5 mM $MgCl_2$. DNase (Worthington) was added to a final concentration of 100 μg/ml. The infected cells were lysed by freezing and thawing alternately in an acetone/Dry Ice bath and in a 30°C water bath five times. The lysates were then incubated at 30°C for 8 min. RNase A (Worthington) was added to a final concentration of 100 μg/ml; the lysates were further incubated at 30°C for 20 min. Solid urea (Schwarz/Mann, ultrapure grade) was added after incubation to bring the concentration to 9 M urea. An equal volume of NEPHGE (see below) lysis buffer was added, and samples were kept frozen at −20°C until dispensing onto the gels.

Two-dimensional gel electrophoresis of proteins and autoradiography. The two-dimensional electrophoresis procedure described by O'Farrell et al. (16) was used, except that Nonidet P-40 was replaced by Triton X-100 (Research Product International). Nonequilibrium pH gradient electrophoresis (NEPHGE) with pH 3.5 to 10 ampholines (LKB) was followed by a second-dimension fractionation via sodium dodecyl sulfate (Bio-Rad Laboratories, electrophoresis purity)-slab gel electrophoresis with a 4% stacking gel and 10% running gel. Immediately after fractionation, gels were dried in a gel dryer (Hoefer Scientific Instruments, SE540) and then autoradiographed with No-Screen Kodak medical X-ray films.

RESULTS

Mutant isolation. In an attempt to isolate tRNA suppressors of UGA mutations, we iso-

lated revertants from T4 strain *e*L5G, which carries a UGA mutation in the *e* lysozyme gene. One class of revertants formed small plaques without added lysozyme on plates with *E. coli* B as indicator at 30°C, but not at 42°C; such revertants appeared at a frequency of roughly 10^{-6}. They did not have back mutations at the site of the original mutation since the plaques did not produce e^+-like halos when exposed to chloroform (21). One of these revertants was used for further analysis.

The possibility of a tRNA suppressor was eliminated because we found the new suppressor mutation was gene specific; i.e., it suppressed only mutations within the *e* gene, where it was active with deletion and nonsense mutations. The revertant was also different from the previously described s^-e^- mutants (5), which carry a suppressor mutation for e^- lesions in the spackle (*s*) gene and which generally grow on plates without added lysozyme only at 42°C. When the revertant was crossed with double mutant *s*12 *e*G19, a strain which bears an *s* mutation and a deletion of the entire *e* gene, the frequency of phenotypic e^- recombinants was about 22%. Thus, the new suppressor mutation is genetically distant from gene *s*.

We then extracted the suppressor mutation for further study by crossing the revertant to wild-type T4. Two recombinant types appeared in the progeny at a total frequency of 18%. One type had the original *e*L5G mutation. The other, which contained the suppressor mutation, formed wild-type-sized plaques at 30°C, but minute plaques at 42°C; the plaques produced e^+-like halos upon exposure to chloroform. Table 1 lists the distinctive phenotypes of the strains. The new mutation was eventually named *5ts*1; *ts* refers to the temperature-sensitive phenotype of strains carrying this mutation, and *5* refers to the gene location (see below).

Reversion of e^- mutations through a *5ts*1-type suppressor mutation does not appear to be a rare event. Revertants similar to *e*L5G *5ts*1 appeared at the frequency of 10^{-6} among *e*L1G *e*L5G phages, a strain which carries two UGA mutations in gene *e*. One of these revertants was analyzed, and the temperature-sensitive suppressor mutation carried by it was designated *ts*2. After segregation, strain *ts*2 was crossed with *5ts*1; the frequency of wild-type progeny was about the same as the combined frequencies of reversion of *5ts*1 and *ts*2 phages. We therefore conclude that *5ts*1 and *ts*2 mutations are very closely linked. The *ts*2 strain was not analyzed further.

Characterization of the *5ts*1 mutation. To determine whether the suppressor activity and the temperature sensitivity of *5ts*1 phage were caused by the same mutation, we isolated a revertant (*5ts*1R) from strain *5ts*1 by plating at 42°C. Strain *5ts*1R formed wild-type-like plaques at this temperature and appeared at the frequency of 10^{-4}. When we crossed *5ts*1R to e^- mutations (i.e., *e*G19), we found that the revertant had also lost the ability to suppress e^- lesions. Thus, a single mutation seems to be responsible for both the suppressor activity and the temperature sensitivity in *5ts*1 e^- phage.

TABLE 1. *Properties of T4 strains*

Strain[a]	Growth[b]		Halo formation[c]
	30°C	42°C	
*5ts*1[d]	+	±[e]	+
*5ts*1 e^{-d}	+	−	−
*5ts*1R[f]	+	+	+
*5ts*1R e^-	−	−	−
e^-	−	−	−
Wild type	+	+	+

[a] e^- mutations included nonsense and deletion mutations.

[b] Growth was measured by plaque assay under conditions that are nonpermissive for e^- strains.

[c] After plates are exposed to chloroform, halos are formed around plaques producing *e* lysozyme (21).

[d] Plaques of strains carrying the *5ts*1 mutation had *r*-like sharp edges.

[e] "±" indicates minute plaques (ranging in size from invisible to 1/10 that of the wild type).

[f] *5ts*1R is a wild-type revertant of *5ts*1.

We have not determined whether there are e^- suppressor mutations in gene *5* that are not temperature sensitive. Therefore we do not know whether temperature sensitivity is a prerequisite for suppressor activity. However, we noted that a temperature-sensitive mutation in gene *5* per se was not sufficient for suppressor activity because nine previously isolated temperature-sensitive mutations in gene *5* (including A28, A39, A40, B44, B48, B49, B53, B65, and N51) did not suppress the *e* gene amber mutation *e*L1a (i.e., did not yield suppressed double mutants in crosses). Therefore, *5ts*1 is distinctive among gene *5* temperature-sensitive mutants in its ability to suppress e^- lesions.

We then used dominance tests to characterize the functional consequences of the *5ts*1 mutation. First, the suppressor activity for e^- lesions was examined by infecting bacteria with a mixture of *5ts*1 e^- and e^- phages at 30°C. The suppressor activity was expressed (Table 2). Next, temperature sensitivity was examined by infecting bacteria with a mixture of *5ts*1 and wild-type T4 at 42°C. The temperature sensitivity was recessive (Table 3). Thus, the presence

of a novel product of the gene containing *5ts*1 mutation generated suppressor activity for e^- lesions, whereas the absence of the normal product caused temperature sensitivity. Furthermore, since the *5ts*1 phage is temperature sensitive for growth even though it produces *e* lysozyme upon infection, it seemed that temperature sensitivity of this phage strain was due to a defect in a function of the altered gene product not related to suppression of e^- lesions. We therefore conclude that temperature sensitivity and suppressor activity in strain *5ts*1 reflect changes in two distinct functions of the same gene product.

Gene identification. We located the *5ts*1 mutation between markers in genes *5* and *6* by a series of two-factor crosses (Fig. 1). Figure 2 shows how this location was further defined by three-factor crosses.

We then used complementation tests to determine whether the *5ts*1 mutation was in gene *5*, gene *6*, or an unidentified gene. *5ts*1 complemented a gene *6* amber mutant, but not gene *5* amber mutants, (Table 4); by this criterion, the *5ts*1 mutation resides in gene *5*.

These genetic data were confirmed biochemically by two-dimensional polyacrylamide gel electrophoresis of ^{14}C-labeled phage proteins. The resulting gel patterns in Fig. 3 show that strain *5ts*1 produced an altered gene *5* polypeptide (gp5). Thus, *5ts*1 is probably a missense mutation in gene *5* that specifies an amino acid substitution with a charge change. For the revertant, *5ts*1R, the spot corresponding to gp5 returned to its normal position.

Temperature sensitivity. The results of dominance tests at 42°C (Table 3) imply that *5ts*1 virions inject their DNA into cells at 42°C. One-step growth experiments performed at 42°C also showed that the *5ts*1 virions were capable of initiating infection. But in contrast to a wild-type infection where the infective centers per infected bacterium rose dramatically from 1 to 97 at the end of latent period, the *5ts*1 infection showed a decrease from 1 to less than 0.1. The measurement of intracellular phages further confirmed the lack of viable progeny in *5ts*1 phage-infected cells after the normal eclipse period (data not shown). Thus, the most prominent defect in formation of viable progeny (at high temperature) in *5ts*1 infections is an impairment at a step subsequent to DNA injection. The process of DNA injection may be affected too, but this was not revealed by our superficial measurement.

Since gp5 is an essential component of the T4 baseplate (11), temperature sensitivity of *5ts*1 phage may result from a defect in one or more of the multiple processes associated with assembly or function of the progeny baseplate. Further experiments such as in vitro assembly and electron microscopy study of defective lysates are necessary for defining the temperature-sensitive step(s).

Mode of phage release. Phage release can occur through cell lysis as in the case of wild-type T4, or by extrusion without cell lysis as in the case of the filamentous phages (7). Therefore, we determined whether release of progeny phage from *5ts*1 e^- phage-infected cells was accompanied by cell lysis.

The turbidity of B/5 cultures infected with *5ts*1 e^-, e^+, or e^- phages was compared. These phage strains also carried amber mutations in genes *27* (N120) and *28* (A452); when infecting B/5 cells, such multiple mutants produce no viable progeny phage (11) and thereby preclude possible complications caused by superinfection. Cultures infected with *5ts*1 e^- or e^+ phages lysed, whereas that infected with e^- did not (Fig. 4).

Since the absence of gp27 or gp28 blocks the incorporation of gp5 into the baseplate (11), Fig. 4 also demonstrates that the presence of nonassembled mutant gp5 is sufficient for lysis. We then determined whether lysis also occurred when gp5 was incorporated into the baseplate. This experiment involved following the turbidity of cultures infected with *48*$^-$ *5ts*1 e^- or *27*$^-$ *5ts*1 e^- phages. The absence of gp48 does not block

TABLE 2. *Dominance tests for suppressor activity*[a]

Infecting phage		Burst size (%)
Parent 1	Parent 2	
*5ts*1 *e*L1a	*e*L1a[b]	43.4[c]
*e*L1a		3.5
*5ts*1 *e*L1a		74.7
Wild type		100.0[d]

[a] Dominance tests were performed at 30°C.
[b] *e*L1a is a gene *e* amber mutant.
[c] Ratio of progeny phage types was similar to the input.
[d] 100.0% = 132 phages/input cell.

TABLE 3. *Dominance tests for temperature sensitivity*[a]

Infecting phage		Burst size (%)
Parent 1	Parent 2	
*5ts*1	Wild type	112.4[b]
*5ts*1		2.1
Wild type		100.0[c]

[a] Dominance tests were performed at 42°C.
[b] Ratio of progeny phage types was similar to the input.
[c] 100.0% = 82 phages/input cell.

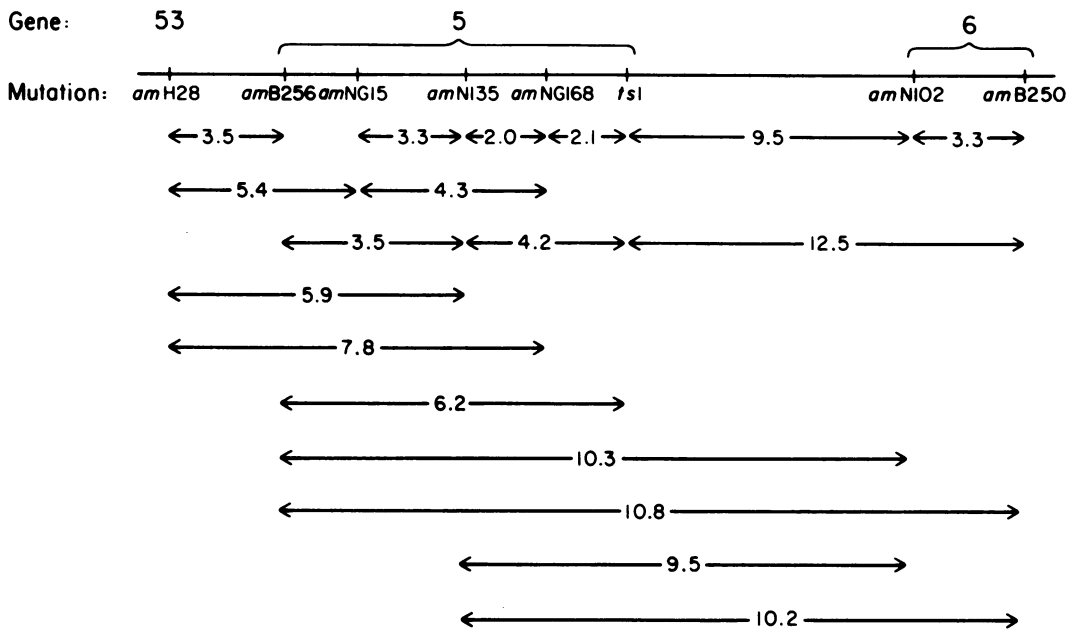

FIG. 1. *Genetic map in the region of the 5ts1 mutation.*

the assembly of gp5 into baseplates since gp48 acts after baseplate assembly is completed (11). We found that lysis of *48⁻ 5ts1 e⁻* phage-infected cultures occurred reproducibly at a slower rate than the *27⁻ 5ts1 e⁻* infection (Fig. 5). Since we do not know the fraction of total gp5 that was assembled into baseplate structures, we do not know which form of gp5 (assembled or unassembled, or both) was responsible for the observed lysis. Nevertheless, the rate difference may indicate that the free gp5 is more active. Aside from these uncertainties, our data do show that lysis promoted by the *5ts1* form of gp5 was not absolutely dependent upon progeny assembly or the incorporation of gp5 into baseplates.

Temperature sensitivity of lysis. Since *5ts1* phage formed minute plaques at 42°C, whereas *5ts1 e⁻* phage formed no plaques at all (Table 1), we speculated that lysis of *5ts1 e⁻* phage-infected cells is temperature sensitive. Indeed, when *5ts1 e⁻* phages were incubated at 42°C on plates supplemented with eggwhite lysozyme, minute plaques appeared. We then examined lysis of cultures infected with *27⁻ 5ts1 e⁻* phage at 30°C, 37, and 42°C and found that it was temperature sensitive (Fig. 6). In a control experiment where cultures were infected with the single mutant, *27⁻* (a strain that produces *e* lysozyme), we found that the time of the onset of lysis was influenced by the three temperatures, but neither the rate nor the extent was significantly affected (data not shown).

To exclude the possibility that temperature sensitivity of lysis of the *5ts1 e⁻* infection resulted from failure to synthesize gp5 at the high temperature, we analyzed phage proteins synthesized at 42°C by two-dimensional gel electrophoresis. We found that the ratio of gp5 to a reference protein (wild-type gp48) was the same for *5ts1* and wild-type T4, as judged visually by spot intensity (data not shown). Thus, *5ts1* phage is not deficient in synthesis of gp5 at 42°C.

Since lysis of *5ts1 e⁻* phage-infected cells was due to the presence of the *5ts1* form of gp5 (Fig. 4 and Table 2), its temperature sensitivity suggested to us that lysis was accomplished by mutant gp5 (i.e., the *5ts1* form of gp5 had lytic activity). Confirmation of this proposal will require biochemical purification and in vitro assay, and until this is performed we cannot exclude the possibility that lysis is caused indirectly by gp5.

Role of gp5 at the onset of infection. Because it is unlikely that a single mutation creates an entirely new function for a gene product, we believe that the wild-type form of gp5 also has lytic capacity.

With regard to the physiological function of gp5, we note that it is part of the central plug of the T4 baseplate (11), a structure which presumably remains at the tip of the tail tube after contraction of the tail sheath upon infection. Thus, given its proximity to the cell wall and its proposed lytic activity, we speculate that gp5

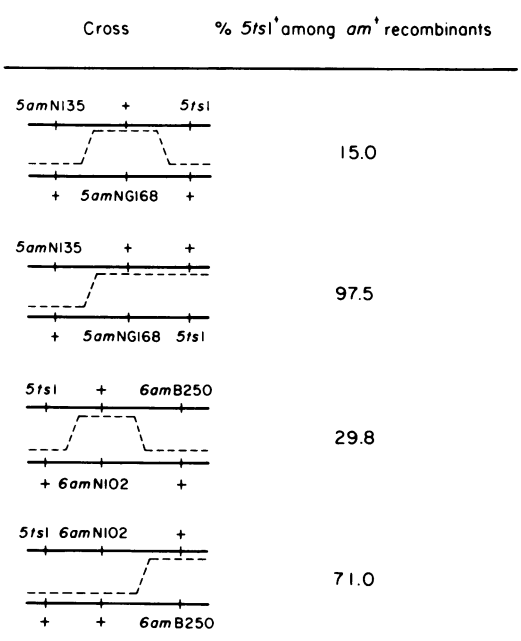

FIG. 2. *Three-factor crosses. Progeny am⁺ recombinants were selected on plates with B/5 as indicator at 30°C. The frequency of 5ts1⁺ among am⁺ recombinants was then determined for each cross by picking 200 to 1,000 am⁺ plaques, stabbing them onto two sets of plates preseeded with B/5 as indicator, and incubating one set of plates at 30°C and the other at 42°C. Two additional series of three-factor crosses showed that 5ts1 is to the right of markers 5amNG15 and 5amB256: the percentage of 5ts1⁺ among am⁺ recombinants was 18.4 for cross 5amB256 5ts1 × 5amN135 and 86.1 for cross 5amB256 × 5amN135 5ts1; 18.2 for cross 5amNG15 5ts1 × 5amN135 and 89.8 for cross 5amNG15 × 5amN135 5ts1.*

locally digests the cell wall to facilitate penetration of the tail tube at the onset of infection. Demonstration that particles lacking gp5 are unable to penetrate cells would support this speculation. However, since gp5 plays an essential role in the early steps of phage tail assembly (11), phage tails and, consequently, phage particles cannot be formed in the absence of gp5. Therefore, we cannot isolate phage particles lacking only gp5 to directly test our hypothesis.

Furthermore, although the intracellular *5ts*1 form of gp5 was temperature sensitive in promoting cell lysis (Fig. 6), *5ts*1 phages were capable of injecting their DNA into the host at 42°C (see "Temperature sensitivity" section above). We also found that *5ts*1 *e*G19 phages (propagated on plates without added egg white lysozyme) were capable of initiating infection at 42°C and were as efficient as wild-type T4 in inducing lysis from without at 42°C (data not shown). These findings leave some doubt as to the essentiality of virion gp5 activity in initiating infection; perhaps cell wall degradation can be directed by an auxiliary enzyme(s) of the virion. It is important to emphasize, however, that our data may be misleading because of the rudimentary nature of the measurements and the possibility that the incorporation of gp5 into baseplates might affect its thermostability. Aside from these uncertainties, the following experiments support our hypothesis.

During our analyses of the *5ts*1 mutant, we found that *5ts*1 virions are thermolabile; i.e., they are inactivated more rapidly than wild-type virions by heating at 60°C (Fig. 7). This observation suggested to us that the lytic activity of gp5 might be similarly thermolabile. Based on the assumption that lysis from without at high MOI involves the same particle-associated lytic factor as penetration of the tail tube at the onset of infection, we tested the thermolability of mutant gp5 by measuring lysis from without induced by virions that had been subjected to prior heat treatment.

Heating reduced the abilities of *5ts*1 and *5ts*1 e^- virions to cause lysis from without, but had little or no effect on wild-type and *5ts*1R virions (Table 5). Thus, particles containing altered gp5 were thermolabile in their capacity to induce lysis from without. The interpretation of these results consistent with our hypothesis is that gp5 is a particle-associated lytic enzyme. However, it should be noted that the proteins comprising the T4 baseplate, including gp5, function as an integral unit that undergoes a conformational change upon attachment of the virus to the host cell (1). Thus, an alternative though not necessarily mutually exclusive interpretation of the experiment reported in Table 5 is that the mu-

TABLE 4. *Complementation tests*

Strain 1	Strain 2	Burst size (%)
*5ts*1	*5amN*135[a]	1.1[b]
*5ts*1 *5amN*135	Wild type	42.9[b]
*5ts*1		1.6
*5amN*135		<0.1
*5ts*1	*6amN*102	25.3[b]
*5ts*1 *5amN*102	Wild type	65.3[b]
*6amN*102		<0.1
*5amN*135	*6amN*102	27.0
Wild type		100.0[c]

[a] Five other gene *5* amber mutants (including *5amB*256, *5amN*G15, *5amN*G168, *5amN*G326, and *5amN*G563) gave similar results.

[b] Approximately half of the progeny phage were *am*⁺.

[c] 100.0% = 105 progeny phages/input cell.

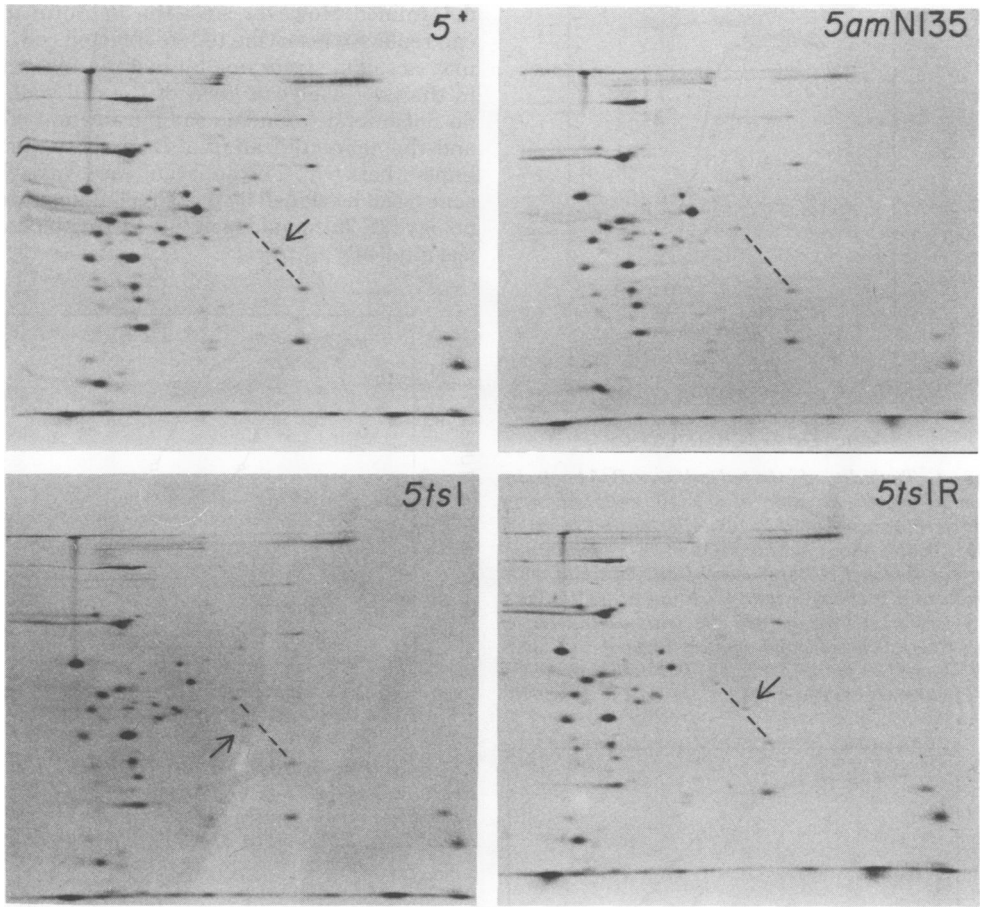

FIG. 3. *Resolution of T4 proteins synthesized in infected cells by two-dimensional gels. The arrow indicates the position of gp5. Notice that gp5 is: absent in the 5 amber mutant (5amN135), moved to a more acidic position in the 5ts1 mutant, and normal in the 5ts1 revertant (5ts1R). All strains contained amber mutation NO22 (gene 48) to allow the amber fragment of gp48 to serve as a reference point. The first dimension was left to right, and the second dimension was top to bottom.*

tant defect reflects an inability of gp5 to foster the necessary molecular rearrangements.

DISCUSSION

We have described a strain of T4 carrying a temperature-sensitive mutation (*5ts*1) that confers suppressor activity for nonsense and deletion-type mutations of the *e* lysozyme gene. An unusual feature of the suppressor mutation is that it resides in a gene (gene *5*) that codes for a polypeptide of the virion baseplate (11). We propose that suppression results from the lytic activity of mutant gp5.

The proposal that a polypeptide serves a structural role in the virion and also has lytic activity is not entirely without precedent. The tail spike protein of phage P22 is an endorhamnosidase, and this structure is believed to bind the phage to the surface of the host cell and then cleave the O antigen (8). Furthermore, a phage ϕ6-induced lysin associated with the virion membrane is believed to be necessary for the passage of the infecting core of the virion through the host cell wall at the onset of infection and for the liberation of progeny phage in the final lysis (14).

Our observations do not allow us to conclude confidently that gp5 acts as a lytic enzyme; it may act indirectly to activate a bacterial enzyme which in turn degrades the host cell wall. However, previous studies have demonstrated that degradation of isolated cell walls during adsorption of T phages is abolished by phenol or heat treatments of phage preparations, but is not affected by the same treatments of cell wall preparations (2). Thus, the responsible lytic ac-

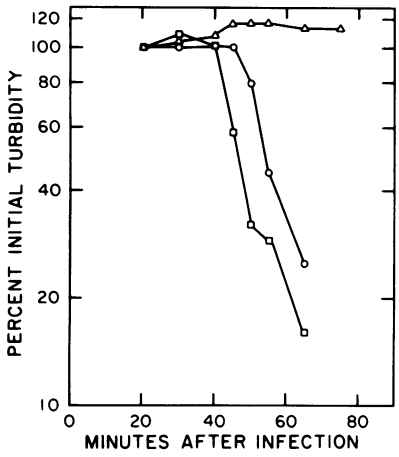

FIG. 4. *Turbidity of infected cultures. B/5 bacteria grown to a concentration of 5 × 10^7 cells/ml in B broth were concentrated to 10^9 cells/ml and infected at 30°C with phage at an MOI of 5. Ten minutes later, the infected cultures were diluted 10-fold with B broth and incubated with aeration at 30°C. Turbidity was measured at 420 nm with a Bausch & Lomb Spec-20 spectrophotometer. Symbols: ○, 5ts1 eG19 27amN120 28amA452; □, 27amN120 28amA452; △, eG19 27amN120 28amA452.*

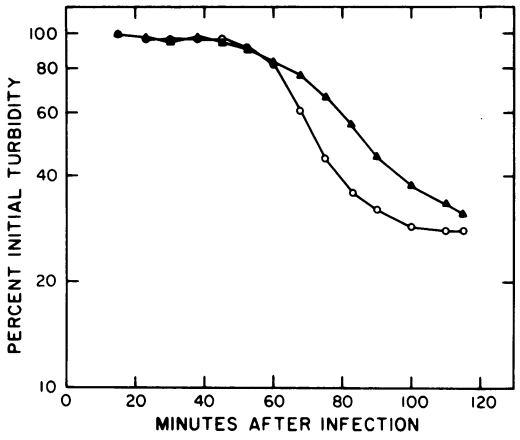

FIG. 5. *Effect of incorporation of gp5 into baseplate on cell lysis. Lysis was measured as described in the legend to Fig. 4. Symbols: ○, 27amN120 5ts1 eG19; ▲, 48amN022 5ts1 eG19.*

tivity is more likely associated with the phage. We therefore favor the notion that gp5 is a particle-associated lytic enzyme. Its activity is a candidate for: (i) limited digestion of the cell wall to facilitate penetration of the tail tube through the cell envelope in the initial step of infection, (ii) lysis from without at high MOI, and (iii) the final cell lysis of *5ts1* e^- infections.

The mode of action of gp5 remains to be determined. However, since the *5ts1* form of gp5 can replace *e* lysozyme to lyse infected cells, gp5 may act like *e* lysozyme by hydrolyzing a bond in the peptidoglycan layer of the cell wall. We do not intend to pursue the purification of gp5 and the characterization of its activity, but we hope others will. The apparent ease with which gene *5* can be cloned by using recombinant technology (22, 25) should facilitate further biochemical studies.

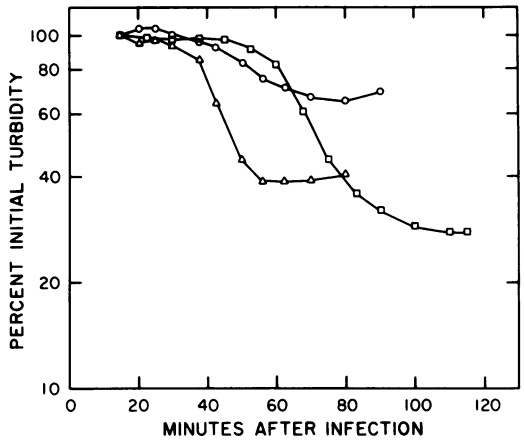

FIG. 6. *Temperature sensitivity of cell lysis by strain 5ts1 eG19 27amN120. Lysis was measured as described in the legend to Fig. 4. Symbols: □, infection at 30°C; △, infection at 37°C; ○, infection at 42°C.*

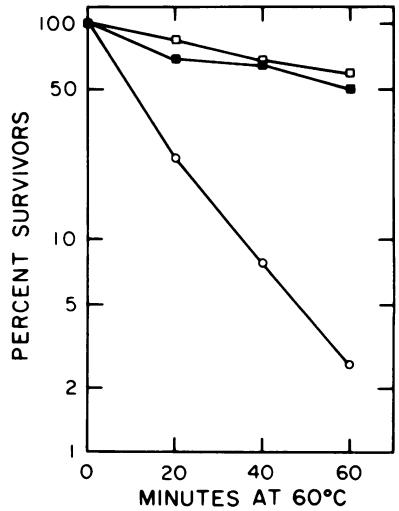

FIG. 7. *Heat inactivation of extracellular phage. Phage preparations at a concentration of 10^6 phage/ml in M9 medium (without glucose) were heated at 60°C. Portions were withdrawn at various times and plaque assayed at 30°C with E. coli B/5 as indicator. Symbols: ○, 5ts1; ■, 5ts1R; □, wild-type T4.*

TABLE 5. *Thermolability of lysis from without*[a]

Strain	Remaining turbidity (%)[b]		Thermolability (heated phage/unheated phage)
	Unheated phage	Heated phage[c]	
Wild type	36.6	26.7	0.73
*5ts*1 *e*G19	36.6	67.8	1.83
*5ts*1	36.6	71.4	1.93
*5ts*1R	36.6	34.5	0.94

[a] Lysis from without was measured as follows. B/5 bacteria grown to a concentration of 2×10^8 cells/ml in B broth were mixed with KCN to a final concentration of 2×10^{-3} M and infected with phage (an M9 stock) at an MOI of 100 at 37°C. Turbidity was measured at 12 min after infection at 420 nm with a Bausch & Lomb Spec-20 spectrophotometer.

[b] Calculated as 100% × (observed turbidity/turbidity of a sample to which M9 medium instead of phage was added). In control experiments using wild-type T4 at six MOIs ranging from 18 to 136, we confirmed the finding of Emerich and Streisinger (6) that the percentage of remaining turbidity was inversely proportional to MOI (data not shown).

[c] Phage preparation (in M9 medium) was heat treated at 60°C for 60 min before use.

Our results show that both wild-type gp5 and the *5ts*1 form of gp5 may have the ability to induce lysis from without, but only the mutant gp5 functions from within the cell. This leads us to speculate that one or more phage products preclude expression of the wild-type gp5 activity, but not the activity of mutant gp5. In the accompanying paper (10), we provide evidence that T4 gene *s* product is responsible for masking the activity of wild-type gp5, and that gp5 of the *5ts*1 phage is insensitive to such inhibitory effects.

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