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Identification of Bacteriophage T4D Gene Products 26 and 51 as Baseplate Hub Structural Components

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Products of two bacteriophage T4D genes, 26 and 51, both known to be essential for the formation of the central hub of the phage tail baseplate, have been partially characterized chemically, and their biological role has been examined. The gene 26 product was found to be a protein with a molecular size of 41,000 daltons and the gene 51 product a protein of 16,500 daltons. The earlier proposal (L. M. Kozloff and J. Zorzopulos, J. Virol. 40:635–644), from observations of a 40,000-dalton protein in labeled hubs, that the gene 26 product is a structural component of the baseplate, has been confirmed. The gene 51 product, not yet detected in phage particles, appears from indirect evidence also to be a structural component of the baseplate hub. These current conclusions about the gene 26 and 51 products are based on properties of T4 mutant particles containing altered gene 26 or 51 products and include (i) changes in heat lability, (ii) changes in adsorption rates, and (iii) changes in plating efficiencies on different hosts, and with the results of previous isotope incorporation experiments indicate that T4 particles contain three copies of the gene 26 product and possibly one or at most two copies of the gene 51 product. Properties of these mutant particles indicate that the gene 26 product, together with the other hub components such as the gene 28 product, plays a critical role in phage DNA injection into the host cell, whereas the 51 product seems essential in initiating baseplate hub assembly.

The precise functions of the products of bacteriophage T4D genes 26 and 51 have been difficult to determine. Early results clearly implicated these gene products as essential in forming the terminal baseplate of the phage tail (2, 8, 9, 16, 17). In 1975, Kikuchi and King (5–7) showed that the baseplate was formed by the addition of six wedges around a central hub and that these two particular gene products, gP26 and gP51, were involved in forming the central hub structure. The methods used by Kikuchi and King did not detect either of these products in the final phage particle or in any of the baseplate hub precursors. Since these proteins were necessary but could not be detected, it was proposed that both performed some catalytic function in the assembly process. This view was not new, since Snustad (16) had earlier classified these two proteins as performing catalytic functions rather than functioning as stoichiometric components.

Since the tail hub plays a unique role in the baseplate conformational changes (15) which occur upon infection (the hexagon-to-star transition which leads to the formation of an opening for the DNA), it is of considerable interest to determine the role that these gene products play during assembly and host infection. In 1981, Kozloff and Zorzopulos (13) reported a great improvement in the sensitivity of the analytical methods for detecting hub components. Essentially, phage were prepared with only the hub component labeled with ^{14}C. These chase experiments led to the detection by polyacrylamide gel electrophoresis of small amounts of three additional hub components (called A, B, and C) which has not been detected previously. Component A had a molecular size of 40 kilodaltons (kDa), and the chase experiments suggested that this hub component was coded for by gene 26 since it was not present in phage particles when the gene 26 product was unlabeled in complementation experiments. Furthermore, the gel analysis indicated that there were only three copies of this protein component per hub, and this was a partial explanation of why it had been overlooked previously. Results presented in this report directly confirm that the gene 26 product has a molecular size of 41 kDa and offer a variety of other evidence that this gene product is a stoichiometric structural component. Whether it plays an additional catalytic role is still undetermined.

Component C has since been identified in many different experiments as a 24-kDa stoichiometric component coded for by T4D gene 28 (11, 13). It has been shown that, in addition to being a structural component (three copies per hub), this protein is a pteroyl polyglutamate carboxypeptidase or hydrolase whose activity is necessary to form the phage baseplate dihydropteroyl hexaglutamate (13).

Component B, which has a size of 29 kDa in denaturing gels, has been thought to be the monomer of the phage-induced thymidylate synthase (12), a known baseplate component. This early, tentative identification of component B assumed that it could not be the product of gene 51. Since the earlier chase experiments did not give any information about the gene 51 product, the minimum molecular size of the gene 51 product has now been determined to be 16.5 kDa. This small size explains why this component had been missed previously. Evidence has been obtained that the gene 51 product is also a hub structural component. Finally, a comparison of the properties of phage mutant particles containing the altered gene 26 product, gene 51 product, or gene 28 product in the baseplate hub led to some clarification of the role of these tail components during infection and assembly.

MATERIALS AND METHODS

Bacteriophage and bacterial strains. We used the standard method for phage assays (1), except for those experiments in which the NaCl concentration in the plating media was changed. The bacterial strains used included *Escherichia coli* B and two *E. coli* K-12 strains, CR63, which was permissive for T4 amber mutants since it contained an Su+ (serine) gene, and AB259 (furnished by A. L. Taylor), which did not contain any suppressor genes and was nonpermissive

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for the T4 amber strains. In addition, *Shigella sonnei* 6, a strain susceptible to T4 (4), was obtained from Margaret A. Fraher, University of California, San Francisco. The bacteriophage strains used have been described (18); these included wild-type T4D and amber mutants in gene 5 (N135), gene 7 (B16), gene 26 (N131), gene 29 (B7), and gene 51 (A529). We also used three T4D temperature-sensitive mutants, 28*ts* (A61, characterized previously), 26*ts* (P68), and 51*ts* (A82). Since the 26*ts* and 51*ts* mutants were of critical importance, we picked single plaques from the original phage strains received from the California Institute of Technology, Pasadena, and characterized them by their temperature sensitivity and host range as described below. Phenotypic revertants of the 26*ts* and 51*ts* phage strains were selected by plating these strains on *E. coli* B at 44°C. Both temperature-sensitive strains had a plating efficiency of less than 10^{-4} at this temperature. Phage strains obtained at this nonpermissive temperature were characterized as described below and are referred to for convenience as revertants rather than, more correctly, as pseudorevertants. It is likely that the revertant mutations causing the change in heat sensitivity are not at the original site of the mutation responsible for the heat sensitivity. However, all the properties which change simultaneously in the revertant indicate that the phenotypic change is most likely due to an alteration in the same gene (see Tables 1–3).

Preparation and measurement of gP26 and gP51 from infected cell extracts. Extracts for the characterization of gP26 and gP51 were prepared by modification of the method described by Kikuchi and King (5). For characterization of gP51, *E. coli* B was multiply infected with T4D 29*am*. After incubation for 30 min, the cells were chilled, centrifuged, and then frozen and thawed twice. The broken cell suspension was clarified by centrifugation at 40,000 × *g* for 60 min. Triton X-100 (final concentration, 0.23%) was added to the supernatant solution to liberate free gene 51 product. This treatment usually increased the complementation two- to threefold against gene 51^- extracts. After a 1-h incubation at room temperature, the solution was dialyzed against 0.15 M NaCl plus 0.001 M phosphate buffer at pH 7.4. The solution in the dialysis bag was concentrated 10-fold by placing the dialysis bag on a bed of solid polyethylene glycol.

The material was then added to a calibrated Sephadex G-75 column, which was developed with 0.002 M phosphate buffer, pH 7.4. Each fraction was then assayed for the presence of gene 51 product by complementation with a gene 51^- extract. The highest degree of complementation was usually obtained by mixing 3 volumes of the column fraction with 1 volume of the gene 51^- extract and incubating this suspension for 4 h at room temperature.

Sephadex columns were calibrated with known protein markers before and after each run. The void volume was determined with high-molecular-weight blue dextran. Mixtures of blue dextran plus hemoglobin (68 kDa), ovalalbumin (43 kDa), chymotrypsinogen) (25 kDa), and cytochrome *c* (13.5 kDa), all at a final concentration of ca. 1 mg/ml, were run through both the G-100 and the G-75 columns. The column flow rate was set by a peristaltic pump. The logarithm of the molecular size was always proportional to the elution volume. When concentrated cell extracts were chromatographed, only the two extreme markers, hemoglobin and cytochrome *c*, were used to decrease the possibility of any protein interaction with components in the extracts. It was found that with a constant flow rate for a given column, the number of fractions between the hemoglobin and the cytochrome *c* markers was always the same and was within the range of the effective fractionation of each column.

The preparation of other extracts, usually a gene 29^- extract, for characterization of the gene 26 product differed in some respects from that used for the gene 51 product. The addition of Triton X-100 was not required, and the gene 26 product was characterized on a calibrated G-100 Sephadex column instead of a G-75 Sephadex column. The complementation reaction to detect the gene 26 product used 2 volumes of the column extract to 1 volume of the gene 26^- extract.

Other methods. Heat inactivation rates, adsorption rates, and gene rescue experiments were carried out as described previously (13). The heat inactivation rate and adsorption rates were calculated as first-order rate constants. Since

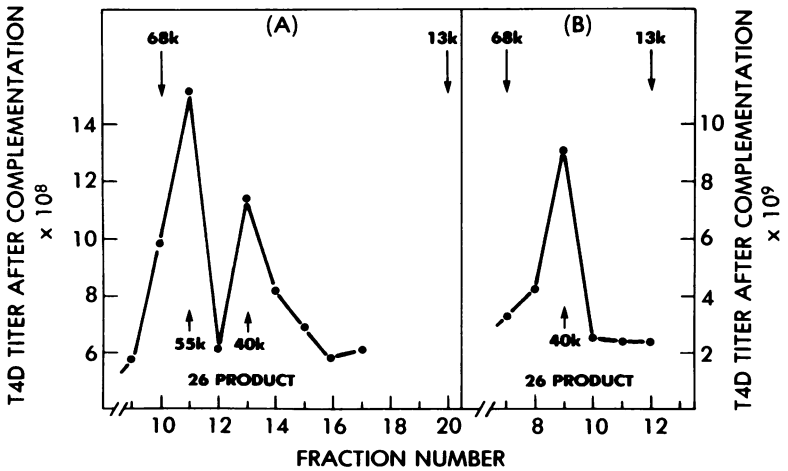

FIG. 1. Chromatography of gene 26 product on Sephadex G-100. An extract was obtained from 1.5 liters of broth culture of *E. coli* B infected with T4D 5*am*. This extract was then chromatographed with molecular weight markers at 68 and 13 kDa on a 210-ml Sephadex G-100 column (A). The fraction containing most of the lowest-molecular-weight gene 26 product activity was found by complementation with a gene 26^- extract. This fraction was concentrated and rechromatographed on an 18-ml G-100 column (B). Samples from these fractions were incubated with a gene 26^- extract for 3 h at 30°, and the complementation mixtures were assayed.

wild-type T4 was always included in the experiment, the relative rate was the ratio of the rate for the mutant compared with that for T4.

RESULTS

Molecular size of T4D gene 26 and gene 51 products. The molecular sizes of these two gene products were determined by gel exclusion chromotography. The behavior of gene 26 product on Sephadex after infection with T4D 5*am* (or T4D 29*am*) is shown in Fig. 1. T4D 5*am* and T4D 29*am* mutants do not make tail baseplate hubs, and the gene 26 product activity is found in these cell extracts. The use of a complementation assay to detect the product in column fractions gave reasonably good information about the molecular size of the phage gene products. In five separate experiments, the gene 26 product was shown to have a minimum molecular size of 40 kDa, which chromatographed reproducibly. Given the earlier indication of this size from isotope chase polyacrylamide experiments, the gel exclusion column data provided confirmation. The nature of the larger component at 55 kDa with gene 26 product activity (Fig. 1) is not certain but one major possibility is that it is a complex of the gene 26 product (40 kDa) and the gene 51 product (16.5 kDa). Any other complex of the gene 26 product (40 kDa) with another hub component, such as the gene 28 product (24 kDa), the gene 5 product (55 kDa), the gene 29 product (77 kDa), or the gene 27 product (49 kDa), could not have a molecular size of 55 kDa (7).

Similar gel exclusion measurements of the molecular size of the gene 51 product are shown in Fig. 2. The minimum molecular size of the active gene 51 product is 16.5 kDa. This value for the size of the gene 51 product was obtained in four separate experiments by using extracts obtained from *E. coli* infected with either T4D 29*am* or T4D 26*am*. In addition to the 16.5-kDa component, there was always a 33-kDa component and a 68-kDa component in these extracts. This 33-kDa component most probably represents a dimer of the gene 51 product. The nature of the 68-kDa component has not been determined; it could well be a complex of a number of hub components.

The low molecular size of the gene 51 product explains why this baseplate hub component (see experiments described below) has been missed previously. Even in the highly sensitive labeled hub chase experiment, the presence of one or two copies (from the dimer) of the gene 51 product per hub on denaturating polyacrylamide gels would have been too diffused and small to be detected as a distinct band. On the other hand, three copies of the gene 51 product probably would have formed a barely detectable band.

Heat sensitivity and absorption rates of T4 particles containing altered baseplate hub proteins. It was shown several years ago that the tail baseplate is the most heat-sensitive substructure of the phage particle (12) and that structural changes in the baseplate changed the rate of heat inactivation at 60°C. This has been established for baseplate dihydrofolate reductase (12), hub thymidylate synthase (13), hub gene product 5 (3), and hub gene product 28 (12). Since changes in baseplate wedge components such as gene products gP6, gP7, gP8, and gP10 had little or no effect on phage heat lability, it seems likely that the lability of the hub itself determines the rate of heat inactivation. Dihydrofolate reductase, although largely a baseplate wedge component (14), is presumed to be an element linking the hub to the wedge (10) and can be thought of as a quasi-hub component.

These observations suggested that an examination of the rate of heat inactivation of phage particles presumed to contain altered gP26 or gP51 proteins would give evidence about the possible presence of gP51 in the hub and would add additional confirming evidence for the presence of gP26.

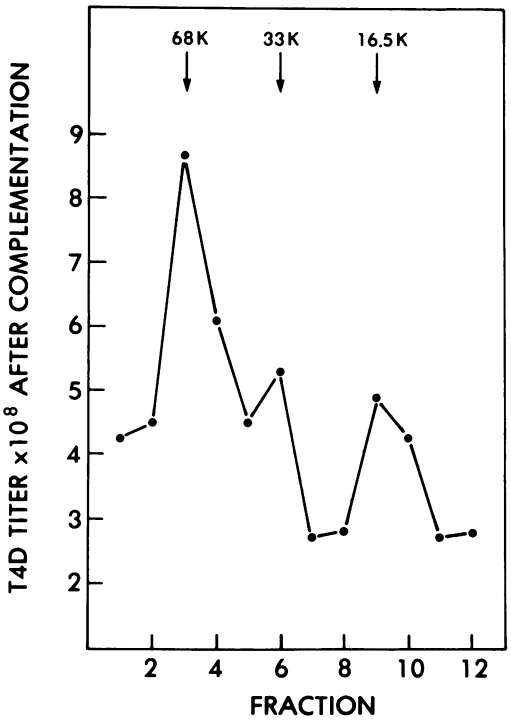

FIG. 2. Chromatography of gene 51 product on Sephadex G-75. An extract was obtained from 250 ml of *E. coli* B infected with T4D 29*am*. The infected cells were collected by centrifugation, frozen and thawed twice, and clarified by centrifugation at 40,000 × *g* for 1 h. Triton X-100 was added to the supernatant solution to a final concentration of 0.23%. This solution was dialyzed against 0.15 M NaCl–6 mM potassium phosphate, pH 7.4. After dialysis, the solution was concentrated to ca. 0.5 ml by covering the dialysis bag with solid polyethylene glycol (20 kDa). The whole sample was put on the G-75 column (51 by 0.9 cm), and the gene 51 product contained in the various fractions was found by complementation with a gene 51$^-$ extract after 4 h of incubation.

The relative rates of heat inactivation at 60°C of the different phage preparations (Table 1) were determined as reported previously (13). The relative rates are the ratios of the first-order rate constants for heat inactivation of the different types of phage particles (measured at least twice) to the rate of heat inactivation of wild-type T4 measured at the same time. The larger the ratio the more rapidly the phage was inactivated compared with wild-type T4. All the gene 26 mutants were found to be markedly less sensitive to heat inactivation than was wild-type T4D. The 26*ts* mutant was only 30% as labile, and the 26*ts* phenotypic revertant was even less heat sensitive, whereas 26*am* was 80% as sensitive. These properties clearly support the view that the gene 26 product is a baseplate hub component.

Similarly, the changes in heat lability varied with mutations in the gene 51 product and are evidence that it is also a phage hub component (Table 1). The three gene 51 mutant particles examined were 30 to 60% more heat resistant than the wild-type particles. Previous measurements of the changes in heat lability with changes in hub 28 protein are also included in this table (13). These measurements for three hub components clearly show that the structural changes which increase the heat lability of the free protein

TABLE 1. Heat lability and adsorption rates of T4 particles containing altered baseplate hub proteins

Phage	Relative rate of heat inactivation at 60°C	Relative rate of adsorption to heat-killed *E. coli* K-12
T4D	1.0	1.0
T4D gene 26 mutants		
26*ts*	0.3	2.4
26*ts* revertant	0.15	2.2
26*am*	0.8	1.5
T4D gene 51 mutants		
51*ts*	0.4	1.0
51*ts* revertant	0.7	1.2
51*am*	0.4	1.5
T4D gene 28 mutants		
28*ts*	1.8	1.6
28*ts* revertant	0.9	
28*am*		2.6

may increase (e.g., 28*ts*) or decrease (e.g., 26*ts* or 51*ts*) the heat lability of the phage particle.

Table 1 also gives information on the relative adsorption rates of these three groups of baseplate hub mutants to heat-killed *E. coli* K-12 CR63 compared with that of wild-type T4. Similar results were obtained with adsorption to *E. coli* B, but the data on the heat-killed K-12 strain are presented because of the host strain specificity of these hub mutants (described below). All hub mutants adsorbed considerably faster to both heat-killed K-12 and B strains than did wild-type T4D. Although the gene 51 mutants adsorbed only slightly faster than did wild-type phage, the gene 26 and gene 28 mutants adsorbed considerably faster, and the adsorption data clearly indicate that changes in any of these three hub genes result in particles which are in some way physically altered. Indeed, one suspects that any change in hub structure probably results in a phage particle with its long attachment tail fibers more extended than they are in wild-type phage.

Plating efficiencies of T4D hub mutants on various bacterial host strains. One unexpected property of a gene 28*ts* hub mutant reported earlier was its failure at permissive temperatures to form plaques on all *E. coli* K-12 strains tested (13). The plating efficiencies of gene 26 and gene 51 mutants, as well as more recent and earlier data on the plating efficiencies of gene 28 mutants (13) on *E. coli* K-12 strains and on another T4-susceptible enterobacter strain, *S. sonnei* 6 (4), are shown in Tables 2 and 3 and in Fig. 3. The data in Table 2 and Fig. 3 compare the plating efficiencies of these phage strains on *E. coli* K-12 strains with the plating efficiencies on *E. coli* B. The gene 26*ts* mutant plated reasonably well on K-12 strains under usual plating conditions, but when the salt content of the plating agar was varied (Fig. 3) there was a considerable difference in the plating efficiency of the gene 26 mutant compared with that of wild-type T4. Since the variation in salt concentration affects only the infection process (and not protein or DNA synthesis or phage assembly), it seems likely that the T4 26*ts* mutants injects less efficiently into K-12 strains than does wild-type phage. Similarly, changes in the plating efficiency on the *S. sonnei* strain with changes in the gene 26 protein again support the view that the gene 26 hub protein is involved in viral DNA injection.

TABLE 2. Relative efficiency of plating of T4 particles containing altered baseplate hub proteins on *E. coli* K-12 and on *S. sonnei* 6 compared with efficiencies on *E. coli* B

Phage	Relative plating efficiency	
	E. coli K-12 strain CR63/ *E. coli* B	*S. sonnei* 6/ *E. coli* B
T4D	1.0	0.6
T4D gene 26 mutants		
26*ts*	0.8	0.2
26*ts* revertant	1.0	0.1
T4D gene 51 mutants		
51*ts*	10^{-4}	10^{-4}
51*ts* revertant	0.5	0.6
T4D gene 28 mutants		
28*ts*	10^{-4}	10^{-4}
28*ts* revertant	0.9	

The data in Tables 2 and 3 show how changes in the gene 51 product dramatically change the plating efficiencies on K-12 strains. The 51*ts* mutant forms plaque with very low efficiency (10^{-4}) on the two K-12 strains tested, as well as on the *S. sonnei* strain. When phenotypic revertants of the 51*ts* mutant were examined, they had regained to some extent the ability to reproduce on the K-12 strains and on *S. sonnei*.

The older data on the host range of mutants containing alterations in the third minor hub component, the gene 28 product, are also included in Table 2. In addition to the low frequency of plating on *E. coli* K-12, the gene 28*ts* mutant did not plate on the *S. sonnei* strain.

DNA injection by the T4 51*ts* mutant. Earlier studies of the failure of the 28*ts* mutant to infect *E. coli* K-12 strains led to the conclusion that this change in the gene 28 product resulted in phage particles which could not inject their DNA into K-12 strains. This conclusion was based on observations that the 28*ts* mutant adsorbed rapidly to K-12 strains (Table 1) but that no 28*ts* DNA or 28*ts* gene products could be detected in the K-12 cells in gene rescue experiments (13). The T4D 51*ts* mutant had properties so similar to those of the 28*ts* mutant (i.e., rapid adsorption and extremely low plating efficiency on K-12 and *S. sonnei* strains), that one might have expected the 51*ts* mutant to be similarly defective in injecting its DNA into these hosts. A gene rescue experiment was carried out to examine this possibility (Table 4). *E. coli* K-12 strain AB259 cells, as well as *E. coli* B cells, were simultaneously infected with 51*ts* mutant and T4D 7*am*. A high proportion of both types of cells yielded wild-type

TABLE 3. Plating efficiencies of T4 gene 51 mutants on various bacterial strains

Phage	Efficiency at indicated temperature (°C) on strain:								
	E. coli B			*E. coli* K-12 strain CR63			*E. coli* K-12 strain AB259		
	25	37	44	25	37	44	25	37	44
T4D 51$^+$	1.0	1.0	1.0	1.0	1.0	1.0	0.4	1.0	0.4
T4D 51*ts*	1.0	1.0	$<10^{-3}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$
T4D 51*ts* revertant	1.0	1.0	1.0	0.4	0.5	0.3	1.0	1.7	1.2

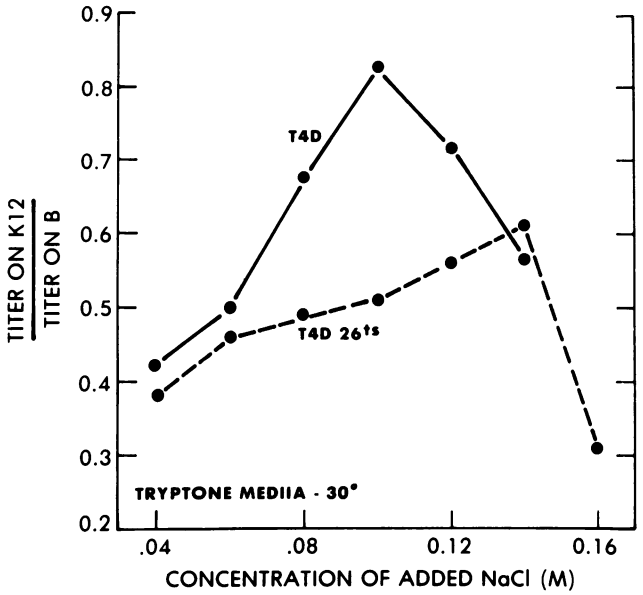

FIG. 3. Relative plating efficiencies of T4D and T4D 26*ts* on *E. coli* K-12 strain CR63 compared with their plating efficiencies on *E. coli* B as a function of the concentration of NaCl added to the assay plates. The phage stocks were diluted and assayed at 30°C on standard tryptone medium containing various concentrations of NaCl added to both the base layer and the top soft agar layer.

phage, indicating that the 51*ts* DNA, which was gene 7^+, was efficiently injected into this K-12 strain and participated in DNA recombination and production of the gene 7 product. In fact, the 51*ts* mutant appeared to inject even more efficiently into *E. coli* K-12 than into *E. coli* B. It may be concluded that failure of the T4D 51*ts* mutant to form plaques on K-12 strains at permissive temperatures cannot be due to a defect in DNA injection but most probably is due to a defect in phage assembly.

DISCUSSION

The results described in Tables 1–3 show that T4D gene products 26 and 51 are phage structural components. Gene 26 product was tentatively shown previously to be a baseplate hub component, and whereas the gene 51 product has not so far been identified in the baseplate hub, it also can be considered a hub component, given the earlier evidence that it is necessary for baseplate hub assembly. This location would be in agreement with the phenotypic properties of gene 51 (and 26) mutants. The explanation of why they were missed earlier seems clear: both are relatively small proteins and both are present in only a few copies per phage particle. There seem to be three copies of the gene 26 product per hub (13), but only one or two copies of the gene 51 product.

The exact function of each, however, has still not been defined, but reasonable possibilities can be proposed. For the gene 26 product, it appears that, like the gene 28 product, it plays a critical role in DNA injection. It is difficult to interpret the failure of some gene 26 mutants to efficiently infect *E. coli* K-12 or *S. sonnei* strains, depending on the external environmental conditions, such as salt concentrations, as being due to any other cause than an inefficiency in an early stage of infection. These particles adsorb well, and their external environment should not affect subsequent assembly internally in the cells. Whether the gene 26 product, like the gene 28 product (a known enzyme), also plays a catalytic role is uncertain, but this is still a possibility.

TABLE 4. Rescue of T4 51*ts* or T4D 51*am* genes by simultaneous infection with T4D 7*am* in *E. coli* AB259 or *E. coli* B[a]

Expt	Phage cross	No. of wild-type infectious centers produced in:		Cross in *E. coli* AB259/cross in *E. coli* B
		E. coli AB259	*E. coli* B	
1	T4D 51*ts* × T4D 7*am*	1.0×10^8	6.0×10^7	1.7
2	T4D 51*am* × T4D 7*am*	6.5×10^7	3.4×10^7	1.9

[a] *E. coli* AB259, a K-12 strain, or *E. coli* B at a final concentration of 2×10^8 cells per tube in separate tubes of broth were each multiply infected with the phage strains indicated. Each phage was added to a final concentration of 6×10^8 cells per tube. The cultures were incubated at 30°C for 30 min and then diluted with cold broth. The titer of wild-type phage produced in each cross was determined by measuring the phage titer under nonpermissive condition for both parents, i.e., on *E. coli* B at 44°C.

On the other hand, gene product 51 mutants inject extremely well (Table 4) but do not reproduce in *E. coli* K-12 or *S. sonnei* strains. Since DNA expression, which follows injection, does occur in K-12 cells, phage formation must be inhibited at some other step during formation. One clue is in the strong affinity of the gene 51 product for the host cell membrane. Reasonable amounts of the gene 51 product could be detected in cell extracts only when the cell was treated with a nonionic detergent such as Triton X-100. Given the report of Simon and Anderson (15), showing that phage assembly takes place on inner cell membrane with the baseplate in close proximity to this host cell structure, we can propose that the initiation of hub assembly is affected by the nature of the gene 51 product. *E. coli* K-12 cell walls are different from *E. coli* B cell walls (19), and the failure then of 51^{ts} mutant to reproduce in strain K-12 could reflect this change in the phage structure. Further work is needed to determine whether the gene 51 product does indeed perform this function.

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