Attachment of Tail Fibers in Bacteriophage T4 Assembly

PURIFICATION, PROPERTIES, AND SITE OF ACTION OF THE ACCESSORY PROTEIN CODED BY GENE 63*

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The attachment of tail fibers to tail-fiberless T4 particles in extracts of mutant-infected cells proceeds slowly in the absence of gene product gp63, but can be stimulated up to 50-fold by its presence, supporting the view that gp63 acts catalytically to increase the rate of tail fiber attachment. The active protein has been purified about 100-fold to yield a nearly homogeneous preparation. Polyacrylamide gel electrophoresis of the purified material in the presence of sodium dodecyl sulfate gives a single major component of molecular weight about 42,000. Production of infectious phage proceeds efficiently in the presence of purified gp63, tail fibers, and tail-fiberless particles, suggesting that no additional cofactors or unidentified gene products are required for the attachment reaction.

Tail fiber attachment involves transient interaction of the distal half of the tail fiber with the collar region of the particle as well as the joining of the proximal end of the fiber with the phage baseplate. To determine which of these interactions involves gp63, the slow and probably nonphysiological attachment of free proximal half-fibers to the baseplate was investigated and shown to be stimulated about 5-fold by gp63. Subsequent conversion of particles with attached proximal half-fibers to infectious phage by treatment with free distal half-fibers was stimulated less than 1.5-fold by gp63. We conclude that gp63 acts to promote the noncovalent association of the proximal end of the tail fiber with the phage baseplate. We cannot yet conclude whether or not its action represents a true catalysis.

An intriguing feature of bacteriophage assembly has been the finding of nonstructural accessory proteins that promote assembly, in addition to the structural proteins that self-assemble (reviewed in Refs. 2 and 3). The accessory proteins may be grouped into several classes. Enzymes involved in proteolytic cleavages have been implicated in both head and tail assembly of several complex phages (reviewed in Refs. 4 and 5). A scaffolding protein, required during head assembly in phage P22, is eliminated during maturation of the capsid and then reutilized for further capsid assembly (6). A number of other nonstructural T4 proteins of unknown function are required in tail fiber assembly (7) and head assembly (reviewed in Ref. 3).

One of the more accessible of such proteins for study is the product of T4 gene 63 (gp63), which promotes the attachment of tail fibers, the primary adsorption organelles of the virus, to the baseplate at the distal end of the contractile tail. We have shown previously that tail fiber attachment is dependent on the presence of functional gp63, in vitro as well as in vivo (1). The rate of the in vitro attachment reaction is proportional to gp63 concentration and dependent on temperature. The reaction shows a nonspecific requirement for either divalent or monovalent cation, a pH optimum near pH 7, and no requirement for dialyzable cofactors. Neither particles nor tail fibers can be activated for subsequent attachment by preincubation with gp63, and the final extent of reaction is independent of gp63 concentration. Vanderslice and Yegian (8) identified gp63 by polyacrylamide gel electrophoresis of infected cell lysates, but did not detect this protein in purified phage particles. These findings suggest that gp63 acts somehow as an accessory protein to promote tail fiber attachment, but they shed no light on its mechanism or site of action.

On the basis of kinetic studies of tail fiber attachment in vitro, Terzaghi (9) suggested that the reaction also involves interaction of the distal half of the tail fiber with sites on the bacteriophage head. We have confirmed Terzaghi's findings, and also obtained serological evidence that supports this notion. Recent studies on the whiskers, slender 40-nm filaments that extend outward from the phage collar, have suggested that these organelles interact with tail fibers (10–12), and it has now been shown clearly that the whiskers are required for efficient tail fiber attachment during assembly.

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1 The abbreviations used are: gp, gene product; Tes, N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; TFA, tail fiber attachment; am, amber (referring to suppressor-sensitive nonsense mutations); SDS, sodium dodecyl sulfate.

2 W. B. Wood and M. P. Conley, manuscript in preparation.

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These findings raise the possibility that gp63 might promote interaction either between a baseplate site and the tail fiber proximal tip, or between whiskers and the distal portion of the tail fiber, or both. In this paper we present further evidence on the nature of gp63 action. We describe a purification procedure for gp63 and also is responsible for RNA ligase activity in T4-infected cells provide evidence that it acts to promote the interaction of the portion of the tail fiber, or both.

**EXPERIMENTAL PROCEDURES**

**Metha**

H-broth, used for phage and bacterial growth, and EHA top and bottom agar for plating were prepared as described by Steinberg and Edgar (16). A defined glucose-salts medium, M9 (17), containing 0.06% casamino acids (w/v), was used for large scale preparation of phage-infected cells for gp63 purification. The same medium containing 0.4% casamino acids was used for smaller scale preparation of infected cell lysates.

**Reagents**

Crystalline DNase I and pancreatic RNase A were obtained from Sigma. Purified bovine serum albumin, gallinaceous ovalbumin, human y-globulin, and bovine liver catalase were obtained from Mann. A-grade dithiothreitol, Tes and EDTA were obtained from Calbiochem. Constituents of buffers are listed in Table I.

**Bacterial Host Strains and Bacteriophage T4 Mutants**

The following Escherichia coli strains were used as indicated: K12 strain CR63 (permissive for am mutants) for plaque assays and for preparation of phage stocks; B/5, Bb, and K12 strain 504 Sm<sup>e</sup> (nonpermissive) for preparation of defective extracts and lysates;

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.01 M Tris, pH 7.4, 0.005 M MgSO&lt;sub&gt;4&lt;/sub&gt;</td>
</tr>
<tr>
<td>B</td>
<td>Buffer A containing 0.001 M Na&lt;sub&gt;2&lt;/sub&gt;EDTA and 0.001 M dithiothreitol&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>0.01 M potassium phosphates pH 6.5, 0.005 M β-mercaptoethanol, 0.0001 M EDTA</td>
</tr>
<tr>
<td>D</td>
<td>0.025 M Tris base and 0.192 M glycine (pH 8.3)</td>
</tr>
<tr>
<td>E</td>
<td>0.049 M Na&lt;sub&gt;2&lt;/sub&gt;HPO&lt;sub&gt;4&lt;/sub&gt;, 0.022 M KH&lt;sub&gt;2&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;, 0.068 M NaCl, 0.001 M MgSO&lt;sub&gt;4&lt;/sub&gt;, 0.00% gelatin (pH 7.1)</td>
</tr>
<tr>
<td>F</td>
<td>0.01 M potassium phosphates, pH 7.0, 0.01 M MgSO&lt;sub&gt;4&lt;/sub&gt;, 0.2 M NaCl</td>
</tr>
</tbody>
</table>

<sup>a</sup> Buffers containing dithiothreitol were prepared fresh just prior to use.

**Table II**

<table>
<thead>
<tr>
<th>Mutant designation</th>
<th>Defective genes (and amber mutations)</th>
<th>Defective component(s) or function(s)</th>
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<tr>
<td>A455</td>
<td>34</td>
<td>Proximal half-fiber</td>
</tr>
<tr>
<td>X1</td>
<td>41(N81), 42(N122), 43(B22), 44(N82), 45(E10)</td>
<td>DNA synthesis</td>
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<td>X46</td>
<td>34(B25, A455), 37(N52), 38(B262), 35(B252)</td>
<td>Proximal and distal half-fibers</td>
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<td>X76</td>
<td>37(D620, N52)</td>
<td>Distal half-fiber</td>
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<tr>
<td>X372</td>
<td>37(B280, N52), 63(M69)</td>
<td>Distal half-fiber, gp63</td>
</tr>
<tr>
<td>X376</td>
<td>18(E18), 23(B17), 27(N120), 34(A455), rII(rfd41)</td>
<td>Sheath, head, baseplate, proximal half-fiber, rII</td>
</tr>
<tr>
<td>X379</td>
<td>23(B17), 63(M69), rII(rfd41)</td>
<td>Head, gp63, rII</td>
</tr>
<tr>
<td>X389</td>
<td>18(E18), 23(B17), 27(N120), 37(N52), rII(rfd41)</td>
<td>Sheath, head, baseplate, distal half-fiber, rII</td>
</tr>
<tr>
<td>X473</td>
<td>34(A455), 63(M69)</td>
<td>Proximal half-fiber, gp63</td>
</tr>
<tr>
<td>X671</td>
<td>18(E18), 28(B17), 27(N120), 37(N52)</td>
<td>Sheath, head, baseplate, distal half-fiber</td>
</tr>
</tbody>
</table>

Defective extracts of mutant-infected cells were prepared as described previously (1) by freezing and thawing of cell pellets to give concentrated preparations of 20 to 30 mg of protein/ml. Defective lysates of mutant-infected cells also were prepared as described previously by treatment of infected cultures with chloroform after 50 min of incubation at 30° (1). Extracts and lysates are referred to in the text by the number(s) of the defective genes in the infecting phage: e.g., 33:83-defective extract.

**Large Scale Preparation of amX1-infected Cells for gp63 Purification**

A large fermentor was charged with 275 liters of sterile medium at 30° and inoculated with 15 liters of an overnight culture of B/5 cells. After growth with forced aeration to about 1.5 × 10<sup>7</sup> cells/ml, the bacteria were infected with amX1 phage at a multiplicity of 2, and superinfected 20 min later at a multiplicity of one phage per cell. The amX1-infected cells, which are defective in several early phage functions (Table II), were used because they produce gp63 (1) but few other late gene products and no phage structural components. Also, because they produce no lysozyme or become fragile, so that harvesting is facilitated. After 2 h of forced aeration at 30° the fermentor was cooled and the culture harvested at a rate of about 4 liters/min in a Sharples continuous flow centrifuge. The packed cells were divided into 40- to 60-μ portions, frozen with dry ice, and stored at −30°. About 800 g of packed cells were obtained from the 300 liters of infected cell culture. Cells were used for gp63 purification within 1 year of preparation.

**Purification of gp63**

All purification procedures were carried out at 0-4° unless otherwise specified.

**Extract Preparation** — Frozen amX1-infected cells (250 to 300 g) were suspended in 350 ml of Buffer B containing 10 μg/ml of DNase and diluted to a total volume of 600 ml with the same buffer. The suspended cells were broken by sonication in 50-ml portions with six bursts of 10 s each, using a Braun sonifier fitted with a large tip, and taking precautions that the temperature of the extract during sonication never exceeded 15°. The extract was centrifuged at 27,000 × g for 40 min, and the supernatant fraction (crude extract) was then centrifuged at 60,000 rpm for 60 min in the 60 Ti rotor of a Beckman L2-65B ultracentrifuge. The high speed supernatant fraction was stored at 0° overnight after addition of RNase to a concentration of 1 μg/ml (Fraction I).

**Ammonium Sulfate Fractionation** — Fraction I was diluted with Buffer B to a concentration of 9 mg/ml of protein (total volume 656 ml) and brought to 35% saturation with ammonium sulfate by dropwise addition, with constant stirring, of 0.54 volume (354 ml) saturated ammonium sulfate solution at 0°, pH 7.4. After an additional 15 min of stirring at 0°, the suspension was centrifuged at 16,000 × g for 10 min. The supernatant solution was brought to 0° and inoculated with 15 liters of an overnight culture of B/5 cells. After growth with forced aeration to about 1.5 × 10<sup>7</sup> cells/ml, the bacteria were infected with amX1 phage at a multiplicity of 2, and superinfected 20 min later at a multiplicity of one phage per cell. The amX1-infected cells, which are defective in several early phage functions (Table II), were used because they produce gp63 (1) but few other late gene products and no phage structural components. Also, because they produce no lysozyme or become fragile, so that harvesting is facilitated. After 2 h of forced aeration at 30° the fermentor was cooled and the culture harvested at a rate of about 4 liters/min in a Sharples continuous flow centrifuge. The packed cells were divided into 40- to 60-μ portions, frozen with dry ice, and stored at −30°. About 800 g of packed cells were obtained from the 300 liters of infected cell culture. Cells were used for gp63 purification within 1 year of preparation.
satisfaction by addition, as before, of another 0.69 volume (451 ml) of saturated ammonium sulfate solution. After 30 more min of stirring, the suspension was centrifuged as before. The drained pellets were dissolved slowly in Buffer B to give a total volume of 200 ml and stored at 0°C overnight (Fraction II).

Chromatography on DEAE-cellulose and Reprecipitation with Ammonium Sulfate—A column (2.6 × 25 cm) of DEAE-cellulose (Cellulose D, Bio-Rad) was washed overnight with 3 liters of Buffer C containing 0.65 M NaCl. A 200-ml portion of Fraction II was diluted with an equal volume of the same buffer and applied to the column at a flow rate of 7 ml/min. The column was washed first with 200 ml of Buffer C containing 0.05 M NaCl and then with about 800 ml of Buffer C containing 0.18 M NaCl at a flow rate of 4 ml/min, until the optical density at 280 nm of the eluate was less than 0.10 relative to a sample of the same buffer. The gp63 activity then was eluted with a 1,200-ml linear gradient of 0.18 to 0.35 M NaCl in Buffer C and collected in 10-ml fractions at a flow rate of 1 ml/min. The fractions that contained the bulk of the gp63 activity, which elutes at about 0.25 M NaCl, were pooled (about 240 ml), and stored overnight.

The gp63 activity was precipitated from the pooled DEAE-cellulose fractions by slow addition of 30% saturated ammonium sulfate with stirring to 70% saturation (0.5 g/ml of original volume). After an additional 20 min of stirring, the suspension was centrifuged at 27,000 × g for 30 min, and the drained pellets were resuspended in a total of 500 ml of Buffer C containing 0.25 M NaCl; Fraction III. Fraction III was quick-frozen in 2-ml portions by immersion in liquid nitrogen, and stored in a Linde liquid nitrogen refrigerator for up to 1 year before use.

**Size Fractionation on Bio-Gel P-150**—A 2-ml sample of Fraction III was pumped into the bottom of a column (2.5 × 100 cm) of Bio-Gel P-150 (Bio-Rad) equilibrated with Buffer D containing 0.05 M NaCl, and eluted in a Pharmacia column equipped with flow adapters and a peristaltic pump. Following sample application, buffer was pumped upward through the column at a flow rate of 0.2 ml/min, and 5-ml fractions were collected from the top. Fractions in the A280 peak at the expected elution position of gp63 were assayed for activity and analyzed by polyacrylamide gel electrophoresis (see below). Fractions that contained activity were pooled in such a way as to maximize recovery and minimize the number of protein species present. The pooled fractions (generally about 20 ml) were scaled into a large piece of dialysis tubing (Union Carbide), concentrated by application of dry Sephadex G-75 to the outside of the bag until the sample was reduced to less than 5 ml, and then dialyzed overnight against fresh Buffer B. The dialyzed solution (Fraction IV) was stored at 0°C for up to a week before use.

**Electrophoresis in Polyacrylamide Gels**

Analysis of purified TFA protein under denaturing conditions was carried out in the presence of SDS as described for stained gels by Laemmli (22). Stained gels were photographed using a Polaroid MP-3 Land camera with Polaroid Type 55 film.

**Electrophoresis in Polyacrylamide Gels**

The protein compositions of purified or partially purified components were analyzed by polyacrylamide gel electrophoresis under either of two conditions. Nondenaturing gels at pH 9.5 were prepared, loaded, run, stained, and destained as described previously (19) except that the amide concentration in all gels was 7.2%. Analysis of purified TFA protein under denaturing conditions was carried out in the presence of SDS as described for stained gels by Dickson (20), using a procedure slightly modified from Laemmli (22). Protein concentrations were estimated by the method of Lowry et al. (21), using bovine serum albumin as a standard. Optical densities were determined with a Zeiss PMQ II spectrophotometer.

**RESULTS**

**Tail Fiber Attachment to Fibreless Particles Proceeds Slowly in Absence of gp63**—Although the rate of tail fiber attachment to tail-fibercless particles in crude extracts is greatly enhanced by the presence of functional gp63, a slow but reproducible attachment is still observed when the source of tail fibers is a gp63-defective extract, as shown in Fig. 1. The first-order rate constants for tail fiber attachment, computed as described previously (1) are about 0.01 min⁻¹ in the absence of added gp63 and about 0.23 min⁻¹ in its presence. The slow rate of reaction observed with gp63-defective extracts could be explained in one of two ways: either the gp63 mutation in the gene 63 mutant used for extract preparation is "leaky" so that the extract in fact contains a low level of functional gp63 activity, or the gp63-defective extract contains very low levels of a partially active gp63 protein that can mediate tail fiber attachment. This possibility is ruled out by the results shown in Table I, where the tail fiber attachment activity of tail-fiber-depleted extracts (extracts containing less than 10% tail fibers) is measured as a function of the incubation time with an excess of tail-fiberless particles. The reaction rate is seen to increase with increasing incubation time, consistent with the presence of a partially active gp63 protein in the extract.
Accessory Protein in T4 Tail Fiber Attachment

Fig. 1. Kinetics of tail-fiberless particle activation in the presence and absence of TFA protein. Tail-fiberless particles were incubated with excess 23:63:rII-defective extract under standard conditions for tail fiber attachment. Samples were diluted and plated for infectious plaque at the times indicated. O, no protein added; ⨁, 0.2 unit of Fraction III TFA protein added. Error bars indicate ± 10%, the mean standard deviation observed in replicate experiments. The left- and right-hand panels show the same data plotted on linear and logarithmic coordinates, respectively.

gp63, or alternatively, the attachment reaction is not entirely dependent on gp63. To distinguish between these alternatives, tail fibers were purified from a 63-defective lysate by the method of Ward et al. (19), which removes more than 99% of the TFA activity when carried out on lysates that contain functional gp63. These purified tail fibers then were compared with the original 63-defective extract at equivalent tail fiber concentrations for their rates of attachment in the presence and absence of added gp63. As shown in Table III, the gp63-independent rate of attachment actually is increased slightly after purification. This result suggests strongly that tail fiber attachment is not entirely dependent on gp63, and adds support to the notion that the function of gp63 is to enhance the rate of an otherwise slow reaction.

Purification of gp63—To further investigate the nature of gp63 action, the activity was purified from sonic extracts of amX1-infected cells as described under "Experimental Procedures." The results of a typical purification procedure are summarized in Table IV. The Fraction IV material represents a purification of about 100-fold over the crude extract, with about 10% overall recovery of TFA activity. Electrophoretic analysis of the preparation at this stage of purification shows that several protein species still are present, even in the Bio-Gel column fractions that contain peak gp63 activity (Fig. 2, Panel b). Preparative gel electrophoresis of Fraction IV was successful in producing a nearly homogeneous preparation that was estimated to be greater than 95% pure by electrophoretic analysis (Fig. 2, Panel c). Unfortunately, however, the specific activity of the preparation decreased during this procedure, suggesting that TFA activity is unstable under the conditions employed for preparative electrophoresis. (It is unlikely that this decrease in specific activity is due to removal of a stimulatory protein component because homogeneous gp63 preparations purified as RNA ligase, by a different procedure (26) showed specific activities in the TFA reaction of around 1000 units/mg (15).)†

Fraction III showed no appreciable loss of activity for more than a year when stored in liquid nitrogen. Fractions IV and V lost activity with a half-life of approximately 1 week when stored at 0–1°. Conditions for preserving the activity of these fractions over longer periods were not investigated. In general, however, the activity is protected by the presence of dithiothreitol and, as discovered subsequent to these experiments, by the presence of ATP (15).

Subunit Molecular Weight of TFA Protein Is about 42,000—To estimate the subunit molecular weight of TFA protein, a sample of Fraction V was heated to 100° for 1 min in Buffer D containing 1% β-mercaptoethanol and 1% SDS. As shown in Fig. 2, Panels d and e, the denatured material exhibited a single major band when subjected to electrophoresis in the presence of 1% β-mercaptoethanol and 1% SDS on gels of 7.5% and 10% polyacrylamide, respectively. The 10% gel was calibrated by electrophoresis of a set of four proteins of known molecular weight (ovalbumin, M, = 43,000, catalase, M, = 60,000, γ-globulin heavy chain, M, = 50,000, and γ-
globulin light chain, $M_r = 23,000$) as standards under the same conditions. Good proportionality was obtained between distances migrated by the various standards and the logarithms of their molecular weights. The major band on the gels of Fraction V, assumed to be the TFA protein subunit, migrated at approximately the same rate as the ovalbumin standard (Fig. 2, Panel f), and was estimated to have a molecular weight of 42,000. This value is in good agreement with that estimated for the gp63 band identified on SDS gels of T4-infected cell lysates on the basis of its elimination by am mutations in gene 63 (8).

We have shown previously that the TFA activity in preparations corresponding to Fraction I of the purification procedure chromatographs on calibrated Sephadex columns with an apparent molecular weight of about 80,000 (1). These results together suggest that the TFA protein can exist as a dimer of identical 42,000-dalton subunits.

**gp63 Promotes Slow Attachment of Free Proximal (A) Half-fibers to Tail-fiberless Particles**—The question of whether gp63 acts at the site of interaction between the baseplate and proximal half-fibers or elsewhere was investigated by taking advantage of the finding that free proximal half-fibers will attach slowly to the baseplates of fiberless particles in the absence of distal half-fibers. Particles with attached proximal half-fibers are unlikely to be normal intermediates in morphogenesis, because particles purified from 37-defective lysates (which contain no distal half-fibers) exhibit much less than 1 phage eq/particle of the A-antigen characteristic of proximal half-fibers (23). However, the actual amount of attached A-antigen was not measured in these previous experiments. As shown in Table V, Column 1, the particles produced by a 37-defective mutant in lysis-inhibited cells do carry a measurable level of A-antigen that is far too high to be accounted for by the low background of infectious phage that failed to adsorb during the initial infection. By contrast, in a similar experiment using 34-defective mutant, which produces distal half-fibers (BC-antigen) but no proximal half-fibers, no associated BC-antigen was observed over the level predicted from unadsorbed infectious phage (Table V, Column 3). When the same experiment was performed with a 37:63-defective mutant (Column 2), the level of particle-associated A antigen was reduced about 5-fold compared to the value obtained with the 37-defective mutant, suggesting that the inefficient attachment of free proximal half-fibers to baseplates is at least partially dependent on the presence of functional gp63.

These results were corroborated by SDS-gel electrophoresis of purified standard-type T4D phage and the particles pro-

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**TABLE V**

<table>
<thead>
<tr>
<th>am mutant</th>
<th>X76</th>
<th>X372</th>
<th>A455</th>
<th>X473</th>
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<tr>
<td>Gene defect(s)</td>
<td>37</td>
<td>37:63</td>
<td>34</td>
<td>34:63</td>
</tr>
<tr>
<td>Half-fiber produced</td>
<td>Proximal</td>
<td>Proximal</td>
<td>Distal</td>
<td>Distal</td>
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<tr>
<td>Tail fiber antigens</td>
<td>A</td>
<td>A</td>
<td>BC</td>
<td>BC</td>
</tr>
<tr>
<td>Phage equivalents</td>
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<td>of antigen/particle</td>
<td>maximum</td>
<td>predicted from infectivity</td>
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<td>Phage equivalents</td>
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<tr>
<td>of antigen/particle</td>
<td>observed</td>
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**FIG. 3. Comparison of purified T4 phage and three defective particles by electrophoresis in SDS-polyacrylamide gels.** Electrophoresis was carried out in 7.5% gels as described under "Experimental Procedures." T4 phage and defective particles were prepared and purified from defective lysates as described in Table V. The three defective particle gels were overloaded relative to the phage gel in order to better detect small quantities of gp34 and gp37.
duced following infection by 37-defective and 37:63-defective mutants under the conditions described in Table V. Fig. 3 shows the electrophoretic pattern obtained with standard-type (T4+) phage and indicates the bands corresponding to gp34, the polypeptide subunit of the proximal half-fiber, and gp37, the major subunit of the distal half-fiber, as well as gp7 and gp10, which are constituents of the baseplate. The 37-defective particles contain a subnormal amount of gp34, and 37:63-defective particles contain even less.

The effects shown in Fig. 3 were quantitated by scanning the gels for optical density of the protein stain, cutting out tracings of the gp34 and gp7 peaks (Fig. 4), and weighing them to obtain estimates of the amounts of gp34 present relative to gp7, which should be the same in all the particles. If the ratio of gp34 to gp7 from the standard-type T4D gel is taken to represent 1.0 phage eq of gp34/particle, then the 37- and 37:63- particles were found to carry 0.26 and 0.09 phage eq/particle, respectively, in reasonable agreement with the serological measurements in Table V.

Electron micrographs of the purified 37-defective particles (Fig. 5, upper panels) show that the associated proximal half-fibers were attached at one end to the baseplate in apparently normal fashion.

The attachment of free proximal half-fibers to tail-fiberless particles and the dependence of this reaction on gp63 also was demonstrated in vitro. Tail-fiberless particles incubated with an excess of purified proximal half-fibers under the standard conditions for tail fiber attachment were found to acquire attached A-antigen with the kinetics shown in Fig. 6. An attachment was approximately linear up to at least 0.5 phage eq of antigen/particle, and the reaction leveled off at approximately the expected saturation point of 1 phage eq/particle. However, the reaction was extremely slow, requiring about 30 h to reach completion under conditions where attachment of whole fibers to particles would be complete in less than 30 min.

Attachment of A is clearly dependent on gp63 as shown in Fig. 7. The initial rate of A-antigen attachment in the presence of 1 unit of gp63 is about 5 times faster than the rate observed when no gp63 was added. Electron micrographs of the particles produced in these and similar reactions (Fig. 5, lower panels) shows that the attachment of A-antigen in vitro leads to association of one end of the half-fiber with the phage baseplate, just as in the normal attachment of whole tail fibers. The particles shown in the lower panels of Fig. 5 have been treated with anti-A-antisera on the electron microscope grid to make the half-fibers more easily visible.

Particles that Carry Proximal Half-fibers Can Be Converted to Infectious Phage by Addition of Distal Half-fibers—Although particles with attached proximal half-fibers are almost certainly not normal assembly intermediates in vivo (23), such particles can be converted efficiently in vitro to infectious.
Accessory Protein in T4 Tail Fiber Attachment

Fig. 6 (left). Attachment of proximal (A) half-fibers to tail-fiberless particles in vitro. A reaction mixture containing $4.2 \times 10^{11}$ tail-fiberless particles, $1.1 \times 10^{12}$ phage eq of purified proximal half-fibers, and excess Fraction III TFA protein (~1 unit) were incubated in a total volume of 300 μl under otherwise standard conditions for tail fiber attachment. At the times indicated, 50-μl aliquots were diluted into 2 ml of cold Buffer F. The particles were isolated by two cycles of differential centrifugation and resuspended in 0.5 ml of Buffer A. Isolated particle concentrations were determined by optical density measurement and A-antigen concentrations by serum-blocking assay (see “Experimental Procedures”). Error bars indicate ± 2σ, the mean standard deviation seen in replicate serum-blocking assays.

Fig. 7 (right). Effect of added TFA protein on attachment of proximal (A) half-fibers to tail-fiberless particles in vitro. Two reaction mixtures were made up, incubated, and assayed as in Fig. 6, except that TFA protein was omitted from one of them. ○, no TFA protein added; ●, excess TFA protein added.

Table VI

| Phage equivalents of A-antigen per X76 particle | 0.23 |
| Per cent infectivity of X76 particles before treatment | <0.01% |
| Predicted per cent infectivity of particles with 0.23 phage eq of complete tail fibers/particle (19) | 10 ± 2% |
| Observed per cent infectivity of X76 particles after incubation with excess 34-defective extract | 10% |
| Per cent infectivity of control X4E particles before treatment | 0.04% |
| Per cent infectivity of control X4E particles after incubation with excess 34-defective extract | 0.06% |
| Per cent infectivity of control X4E particles after incubation with excess 34-defective extract and 0.54 phage eq/particle of A half-fibers in the form of 37-defective extract | 10% |

A purified preparation of 37-defective particles obtained as described in Table V was assayed for A-antigen phage by incubation with 34-defective extracts, which contain active distal half-fibers. Such an experiment is presented in Table VI. A purified preparation of 37-defective particles was prepared (without centrifugation in CsCl) and characterized as described in Table V. These particles were incubated for 24 h with an excess of 34-defective extract under standard conditions for tail fiber attachment, and then diluted and plated to measure infectious phage. As a control, purified X4E tail-fiberless particles were incubated with excess 34-defective extract, or with excess 34-defective extract and an amount of 37-defective extract (prepared with amX389) corresponding to 0.24 phage eq of A-antigen/particle.

Fig. 8. Effect of TFA protein on addition of distal half-fibers to particles carrying proximal half-fibers. Standard tail fiber attachment reaction mixtures were made up to contain $5 \times 10^{10}$ 37-defective particles carrying 0.23 phage eq of attached A-antigen (see Table VI) and an amount of 34:63-defective extract corresponding to $1 \times 10^{11}$ phage eq of distal half-fibers (BC-antigen). Reaction mixtures were incubated at 30°C and sampled for assay of infectious phage at the times indicated (○, no TFA protein added; ●, ~1 unit of Fraction III TFA protein added). and found to carry 0.23 phage eq of A-antigen/particle. If each of the attached proximal half-fibers were converted to a whole fiber by addition of distal half-fibers, the resulting phage should carry an average of 0.23 phage eq of tail fibers or 1.4 tail fibers per particle. Assuming that these tail fibers are randomly distributed among the particle population, and that particles with less than six tail fibers can form plaques with reduced plating efficiencies, it can be predicted that about 10% of these particles should be able to form plaques (19). As
shown in Table VI, the observed level of plaques following incubation with 37-defective extracts was 10%, suggesting that most of the attached proximal half-fibers have been completed by addition of a distal half-fiber. The kinetics of this reaction and its dependence on the presence of gp63 were studied in the experiment depicted in Fig. 8. The source of distal half-fibers in this experiment was a 34-63-defective extract. In the lower curve, no gp63 was added to the reaction, in the upper curve a large excess of Fraction III gp63 was added. Addition of gp63 produces only a slight apparent increase in the rate of activation. With or without gp63, the reaction proceeds rapidly to completion in less than 30 min. This reaction is promoted strongly (30-fold effect) by interaction of the distal half-fibers with the collar region of the particles, as we have shown in experiments to be published elsewhere on the role of the whiskers in tail fiber attachment. Apparently, gp63 plays little or no role in this interaction.

**Discussion**

Purification of TFA protein has revealed no new requirements for the TFA reaction, whose known characteristics now may be summarized as follows. The reaction appears to require no components other than tail-fiberless particles, tail fibers, TFA protein, and either a divalent cation at about 0.01 M (slow reaction) or a monovalent cation at about 1.0 M (fast reaction) (1). In the presence of 1.0 M (NH₄)₂SO₄ no divalent cation is required, based on the observation that EDTA does not inhibit the reaction (1). No other cofactors are required, although both dithiothreitol and ATP (15) stimulate the reaction by stabilizing the TFA protein.

The nature of the reaction remains unclear. It almost certainly does not involve formation of a covalent linkage between gp34 in the tail fiber and a baseplate protein, because the smallest protein in the baseplate (gp11) is about 24,000 (25), and there is no molecular weight difference between gp34 in free and attached tail fibers as measured by gel electrophoresis after denaturation in SDS with or without β-mercaptoethanol (20). The appreciable rate of tail fiber attachment in the absence of TFA protein also supports the view that the reaction involves formation of a noncovalent bond in a relatively low activation energy reaction that somehow is promoted by TFA protein. Somewhat puzzling with regard to this view is the recent finding that gp63 also is responsible for the T4 RNA ligase activity, which results in phosphodiester bond formation in an ATP-dependent reaction. The possible relationship of this reaction to tail fiber attachment has been discussed (15), but not fully resolved; further experiments are in progress to determine whether the protein promotes two unrelated reactions, or whether the RNA ligase and TFA activities are in fact related.

The question of whether TFA protein functions as a true catalyst in tail fiber attachment also remains open. Its role originally was assumed to be catalytic (1, 7), based on the observations that (a) TFA protein enhanced the rate but did not effect the final yield of attached tail fibers, and (b) gp63 is not found in the completed virion (8). We have presented evidence here confirming that TFA protein is not required for the TFA reaction to proceed at a slow rate. However, we cannot rule out a nonessential, but noncatalytic role for the protein because of the large molar amounts that must be added to see an effect on the reaction rate in vitro. Assuming a molecular weight of about 80,000 and a specific activity of about 1 unit/µg for the pure protein (based on the data in Table IV and on the results of Snopel et al., (15) then the molar amount of TFA protein that must be added to a standard in vitro reaction mixture to give a significant increase in rate is about an order of magnitude larger than the number of tail fibers in the mixture and several orders of magnitude larger than the number of attachment events generally measured. The situation probably is similar in vivo. Assuming from the purification data presented here for TFA protein and for RNA ligase by other investigators (26–28) that gp63 represents about 1% of the total soluble protein late in infection, then there are about 10,000 molecules of TFA protein/cell, approximately the same as the number of tail fibers present (19). Therefore, if the activity of TFA protein is truly catalytic it must be either intrinsically very low or, alternatively, it must reside in only a small fraction of the number of gp63 molecules present.

The experiments reported here bring to three the number of possible routes known for attachment of the two halves of the tail fiber to each other and to the phage particle to yield infectious virions. The two halves may be joined to each other and then attached to the baseplate as whole fibers (1, 19). Alternatively, the distal halves of the fibers can transiently bind to the tail-fiberless particle (9) in an association that involves the phage whiskers (12–14). The resulting complex then can react with proximal half-fibers, which attach to the distal halves and to the baseplate to form infectious phage. We demonstrate here a third route, whereby proximal half-fibers attach first to the baseplates of the tail-fiberless particle to form a stable noninfectious complex which can be made infectious by whisker-promoted addition of distal half-fibers. This route is almost certainly nonphysiological, based on the low level of particles with attached proximal half-fibers produced by distal half-fiber-defective mutants as well as on the extremely slow rate of proximal half-fiber attachment in vitro in the absence of distal half-fibers. The reason for this slow rate is presumably lack of interaction between the proximal half-fiber and the phage whiskers, which strongly promote normal tail fiber attachment by interacting with the distal half-fiber, as shown by Terzaghi et al. (14) and by us in experiments to be published elsewhere. Nevertheless, our finding that TFA protein increases the rate of proximal half-fiber attachment and has no significant effect on the subsequent addition of distal half-fibers strongly suggests that in the physiological attachment of tail fibers by either of the first two routes, TFA protein acts to promote joining of the proximal half-fiber to the baseplate.

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**References**

Accessory Protein in T4 Tail Fiber Attachment

Attachment of tail fibers in bacteriophage T4 assembly. Purification, properties, and site of action of the accessory protein coded by gene 63.

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