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Evidence for an Internal Component of the Bacteriophage T4D Tail Core: a Possible Length-Determining Template

ROBERT L. DUDA AND FREDERICK A. EISERLING*

Molecular Biology Institute and Department of Microbiology, University of California, Los Angeles, California 90024*

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The length of the T4 tail is precisely regulated in vivo at the time of polymerization of the tail core protein onto the baseplate. Since no mutations which alter tail length have been identified, a study of in vivo-assembled tail cores was begun to determine whether the structural properties of assembled cores would reveal the mechanism of length regulation. An assembly intermediate consisting of a core attached to a baseplate (core-baseplate) was purified from cells infected with a T4 mutant in gene 15. When core-baseplates were treated with guanidine hydrochloride, cores were released from baseplates. The released cores had the same mean length as cores attached to baseplates. Electron micrographs of these cores showed partial penetration of negative stain into one end, and, at the opposite end, a modified tip which often appeared as a short fiber projecting from the core. When cores were purified and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, two minor proteins and the major core protein were detected. One minor protein, the product of gene 48 (gp48), was present in at least 72% of the amount found in core-baseplates, relative to the amount of the major core protein. These findings suggest that cores contain a fibrous structure, possibly composed of gp48, which may form a "ruler" that specifies the length of the T4 tail.

The bacteriophage T4 tail is composed of a baseplate, a contractile sheath, and a core or tail tube. The core is a rigid tube, 100 nm long, composed mainly of the 20,000-molecular-weight protein gp19 (gp19 refers to the protein *g*ene *p*roduct of gene 19; most T4 proteins are named in this manner) arranged in a helix of 24 annuli of six subunits each (16). The core is surrounded by 144 copies of sheath protein gp18, arranged in a helix that probably matches the symmetry of the core (19, 21). Both the sheath and the core are attached to the baseplate, a multifunctional structure that contains at least one copy each of 15 different proteins and requires 19 genes for its assembly (12–14).

The pathway of T4 tail assembly, established primarily by King and co-workers (for a review, see reference 15) begins with the formation of the baseplate. After the formation of a morphologically identifiable baseplate, two proteins, gp48 and gp54, must be added sequentially to the structure before the 20,000-dalton tail core protein, gp19, can initiate polymerization (1, 17, 20). After initiation, gp19 molecules assemble into a 100-nm-long core, and gp18 polymerizes around the core to form the sheath. Upon the addition of gp3 and gp15, the tail is complete and competent to join to a head.

In vivo, gp19 remains soluble until the baseplate is complete. Once polymerization begins, assembly proceeds to the "correct" length and stops, but the mechanism by which this length is controlled is unknown. Since no mutants which produce phage with abnormal tail lengths have been identified, we do not even know which gene(s) specifies tail length. In the absence of such mutants, a detailed study of assembled cores was started to determine whether features of core structure suggest how the length is determined.

MATERIALS AND METHODS

Bacterial and phage strains. *Escherichia coli* B^e and B40 *supD* were used as nonpermissive and permissive hosts, respectively (2). A triple amber (*am*) mutant of $T4D^+$, *am*N133 (gene 15) *am*H11 (gene 23) *am*B25 (gene 34), was used for these studies.

Media and buffers. EHA top and bottom agars used for assaying phage were prepared as described by Steinberg and Edgar (24). Cells were grown in Hershey broth for plating (24) or in M9 medium (11) supplemented as described previously. The 3× TYE medium contained (per liter) 25 g of tryptone, 17.5 g of yeast extract, and 7.5 g of sodium chloride. Phosphate buffer (16) was supplemented with 1 mM $MgSO_4$ (BUM1) and sometimes with 1 mM sodium azide. BUM1 with 0.001% gelatin was used for diluting phage suspensions.

Enzymes and chemicals. Acrylamide and methylene

bisacrylamide were purchased from Kodak Laboratory and Specialty Chemicals, Rochester, N.Y. Sodium dodecyl sulfate (SDS) was obtained from BDH, Poole, England. Bovine pancreatic DNase I was obtained from Worthington Diagnostics, Freehold, N.J.; guanidine hydrochloride (GuHCl) was from Schwarz/Mann, Orangeburg, N.Y. The GuHCl concentration was found to be a critical factor in reproducing the results reported below. In addition, different batches of GuHCl from other suppliers were found to produce the same results as reported here, but at approximately 10% lower concentrations. All other chemicals were reagent grade.

Preparation of infected cells. A 10-liter culture of *E. coli* B^e in M9 medium supplemented with 1% Casamino Acids and 5% 3X TYE medium was grown with vigorous aeration at 37°C in a 14-liter Microferm fermentor (New Brunswick Scientific Co., New Brunswick, N.J.). When the cell density reached 4×10^8 cells per ml, a cooling cycle was initiated to bring the culture to 30°C, and the cells were infected with five phage per bacterium. The culture was superinfected 9 min later and harvested at 150 min. Infected cells were sedimented in two batches at 3,800 rpm for 22 min at 4°C in the 4.2 rotor of a Beckman J-6 centrifuge (Beckman Instruments, Inc., Fullerton, Calif.).

Purification of core-baseplates. Infected cells from 10 liters of culture were suspended in 250 ml of BUM1 and lysed by stirring with 4 ml of $CHCl_3$. Crystalline DNase I (ca. 0.5 mg) was added to reduce the viscosity, and the suspension was stirred for 1 h at room temperature. Cell debris was removed by centrifugation at 5,000 rpm for 10 min at 4°C in a Sorvall GSA rotor (Ivan Sorvall, Inc., Norwalk, Conn.). The supernatant was stored overnight in the cold and concentrated by ultrafiltration under N_2 gas, using an Amicon XM300 membrane in a stirred pressure cell (Amicon Corp., Lexington, Mass.).

The concentrate was layered onto a 1,400-ml 5 to 30% (wt/vol) sucrose gradient in a BXV zonal rotor (Beckman). At 2,500 rpm, the gradient was loaded from the edge of the rotor, and the 50-ml sample, followed by an overlay of BUM1 (100 ml), was injected at the center. The rotor was accelerated to 20,000 rpm for 6 h at 20°C, and the gradient was unloaded at 2,500 rpm by pumping 50% sucrose in at the edge of the rotor and collecting 18- to 20-ml fractions from the rotor center. The absorbance at 280 nm of each fraction was measured, and a small peak of rapidly sedimenting material was found and identified as core-baseplates (desheathed tails) by electron microscopy. The peak fractions were pooled and dialyzed extensively at 4°C against BUM1 containing 1 mM NaN_3. The dialyzed sample was then concentrated by ultrafiltration at 0°C, using an XM-300 membrane as described above. The purity of the preparation was judged to be relatively high by SDS-polyacrylamide gel analysis and electron microscopy. Some gp18 was present (in the form of polysheath), and small amounts of bacterial cell envelope fragments were also seen by microscopy.

Treatment of core-baseplates with GuHCl. Pilot experiments were conducted with small volumes (0.2 ml) of dilute (80 μg of protein per ml) suspensions of core-baseplates. These concentrations and volumes were sufficient for analysis of results by microscopy. A sample of purified core-baseplates in BUM1 was dialyzed into 100 volumes of GuHCl (2.0 to 4.0 M) for 12 h at 22°C. For some experiments, small samples were removed at this stage and examined by negative-contrast electron microscopy. The samples were then dialyzed extensively against BUM1 at 4 to 8°C.

Sucrose gradient purification of structures surviving GuHCl treatment. A sample (0.5 to 0.8 ml) was layered onto 5 to 30% (wt/vol) linear sucrose gradients made in BUM1 containing 1 mM NaN_3 and centrifuged at 40,000 rpm for 2 h at 4°C in an SW41 rotor. Gradients were fractionated with a piston device (4) into 18 approximately equal fractions. Samples were taken directly for electron microscope analysis and for SDS-polyacrylamide gel electrophoresis.

SDS-polyacrylamide gel electrophoresis. The methods and conditions of SDS-gel electrophoresis were essentially those described previously (2), with only minor modifications. Separating gels (2 mm thick) were composed of a linear gradient of the following: 7.5 to 12.5% (wt/vol) acrylamide, 0.4 to 0.67% (wt/vol) methylenebisacrylamide, and 0 to 10% (vol/vol) glycerol. Gels were stained in a solution containing 0.04% (wt/vol) Coomassie brilliant blue (R-250), 25% (vol/vol) isopropanol, and 10% (vol/vol) acetic acid for 1 to 2 h and destained in 10% (vol/vol) acetic acid. The stain-destain procedure was repeated to ensure complete staining.

Quantification of stained proteins in polyacrylamide gels. The relative quantities of proteins electrophoresed in polyacrylamide gels were determined from measurements of the amount of dye eluted from individual stained protein bands as described previously (8). Individual bands were cut from gels, placed in calibrated tubes, and brought to a constant volume (0.5 ml) with 25% (vol/vol) pyridine in water. The tubes were sealed and shaken overnight at 37°C. The eluted Coomassie brilliant blue (R-250) dye was measured for absorbance at 605 nm.

Electron microscopy. Samples were applied to carbon-coated Parlodion films for 1 to 10 min, rinsed with three to six drops of distilled water (depending on the solute concentration), negatively contrasted with 1% aqueous uranyl acetate, blotted, and air dried. Specimens were examined in a JEOL JEM 100B electron microscope operated with an anti-contamination device. Images were recorded at 80 kV on Kodak 4463 electron image film. Magnifications were determined relative to measurements of spacings of a carbon replica of a precision-ruled grating (no. 607; Polaron Instruments, Inc., Doylestown, Pa.).

Measurement of particle lengths. Selected electron micrographs were projected onto the surface of a 9864A digitizer platen interfaced to a Hewlett-Packard 9825A calculator programmed to measure lengths. The particles were measured, and measurements were scaled with the appropriate magnification factors.

RESULTS

Effects of GuHCl on core-baseplates. GuHCl was shown by To et al. (25) to degrade baseplates at concentrations that did not disrupt tail cores, when mixtures of T2 and T4 structural parts derived from chemically degraded phage were treated with denaturants. Purified core-baseplates (Fig. 1A) were treated with GuHCl at

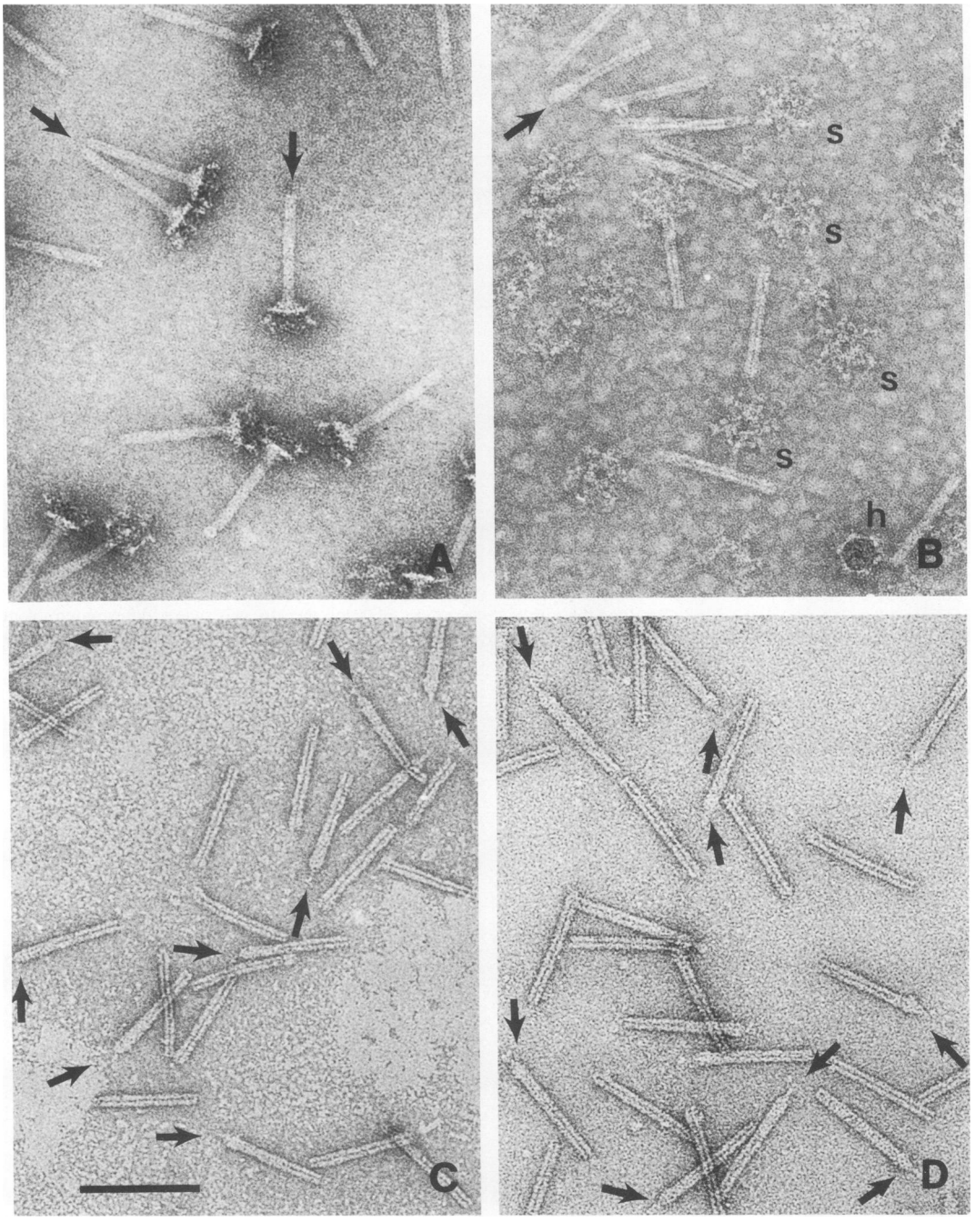

FIG. 1. Electron micrographs of phage T4 tail tubes after various treatments. (A) Purified tail tubes with attached baseplates used as starting material. The arrows indicate tail tubes which show evidence for an internal component, best seen by viewing the micrograph at a low angle along the tube axis. (B) Result of treatment with 3.0 M GuHCl, showing both hexagonal (h) and star-shaped (s) detached baseplates. (C) Same sample as in (B) after dialysis into BUM1. The arrows indicate tubes with tapered tips which sometimes appear as a short fiber. (D) Same structures after isolation on a sucrose gradient, showing that the modified tips persist (arrows). Bar, 100 nm.

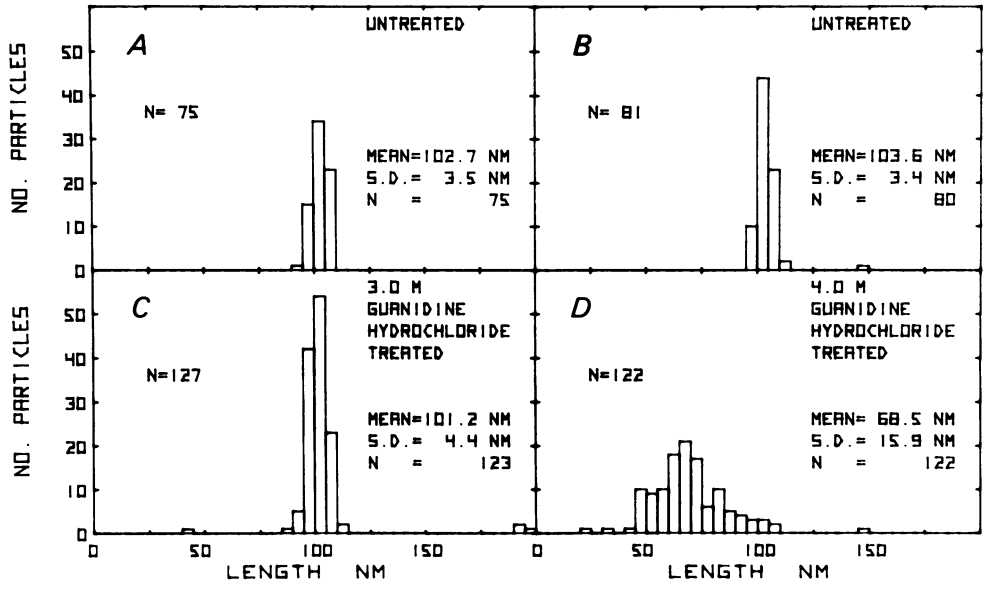

FIG. 2. Distributions of tail core lengths measured from micrographs of core-baseplates and cores surviving GuHCl treatment. Core-baseplates (80 μg) were treated with GuHCl and sedimented in a sucrose gradient as described in the text. Samples of peak fractions containing cores were prepared for electron microscopy, and particle lengths were measured from the resulting micrographs as described in the text. (A and B) Measurements made from two different micrographs of untreated core-baseplates. (C and D) Measurements made from electron micrographs of purified cores that have survived the indicated treatment. The number (N) of cores measured is indicated on the left in each panel. The mean length and standard deviation (SD) were calculated for each set of measurements and appear in the appropriate panel. Note that in (B) and (C), respectively, one and four measurements, which were significantly far from the mean, were excluded from statistical computations.

2.0, 2.5, 3.0, 3.5, and 4.0 M concentrations, as described above, to determine whether a particular concentration of the denaturant would separate cores from baseplates without degrading the cores. The concentration of GuHCl which produced the desired effect was found to be 3.0 M, as described below. When samples of treated preparations were applied directly to specimen support films in the presence of GuHCl and then negatively stained as described above, different structures were observed in various amounts in the electron microscope. Figure 1B shows an electron micrograph of one sample in this experiment—core-baseplates treated with 3.0 M GuHCl. At or below 3.0 M GuHCl, core-baseplates were present either in their normal form (Fig. 1A) or as "core-stars"—cores attached to baseplates in the star form (5). Baseplates appeared predominantly in the star configuration, and the few hexagons which were seen usually lacked the central hub portion, normally seen as a stain-excluding spot or ring in the center of the baseplates (e.g., the hexagon marked "h" in Fig. 1B). At 3.0 M GuHCl, the separation of cores from baseplates was nearly complete. Lower concentrations of GuHCl were less efficient in removing cores from baseplates and produced more hexagonal baseplates. At GuHCl concentrations above 3.0 M, cores and baseplates were completely separated, and no baseplates were seen in the hexagonal configuration. When samples were applied to specimen support films after dialysis into buffer, as described above, few free baseplates were observed (Fig. 1C), and in many cases large aggregates, believed to be composed of degraded baseplates, were seen. No attempt was made to quantify the proportions of the structure types present, since the technique used for sample preparation is unreliable for comparing relative concentrations of different kinds of particles (7).

Length of cores after GuHCl treatment. Histograms of core length measurements before and after GuHCl treatment are shown in Fig. 2. Cores released from baseplates at 3.0 M GuHCl had a very narrow distribution of lengths, suggesting that the entire core structure was removed intact from the baseplate as a unit. The fact that the mean length of the isolated cores is nearly identical to the mean length of cores on untreated core-baseplates (Fig. 2) suggests that little or no degradation (or depolymerization) of the ends of the cores took place. However, if the GuHCl concentration was raised to 4.0 M, the

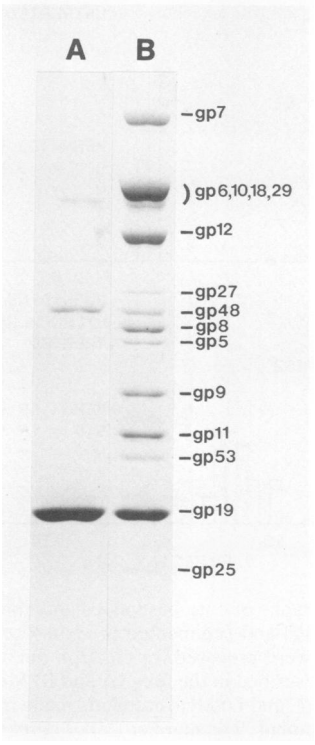

FIG. 3. Protein composition of purified cores. Samples of sucrose gradient-purified cores (fraction no. 5 from Fig. 4) and untreated core-baseplates were denatured, electrophoresed, and stained as described in the text. Lane A contained 0.11 ml of the gradient peak fraction, and lane B contained 24 μg of untreated core-baseplates.

cores began to depolymerize. The cores treated with 4.0 M GuHCl were all shorter and more broadly distributed in length than intact cores (Fig. 2D).

Protein composition of isolated cores. Free cores released with 3.0 M GuHCl were purified by sedimentation in a sucrose gradient, and their protein composition was compared with that of whole core-baseplates by SDS-polyacrylamide gel electrophoresis as described above (Fig. 3). The major protein of isolated cores was gp19, as expected (17). Two baseplate proteins apparently remained associated with cores after their separation from intact core baseplates (Fig. 3A). The molecular weight of the upper band is about 80,000; its size corresponds to that of gp29 (18), a protein of the central plug of the baseplate (14), but any assignment of identity is still speculative. The protein gp48 is clearly present in high yield in the purified cores. Other baseplate proteins were found at the top and bottom of the sucrose gradient shown in Fig. 4 (data not shown), indicating that many baseplate proteins had either been solubilized or aggregated after GuHCl treatment.

The stoichiometry of gp48 relative to gp19 in different samples was determined from measurements of the amount of dye bound to protein bands in stained gels after SDS-polyacrylamide gel electrophoresis, as described above. The ratio of gp48-bound dye to gp19-bound dye was computed for each gel track. Values of 0.1 to 0.092 were obtained for core-baseplates; values of 0.072 to 0.095 were obtained from isolated cores. These data indicated that approximately 72 to 100% of the gp48 (relative to gp19) present in core-baseplates remained associated with detached cores. If both proteins bound dye equally per mass unit, and if the molecular weight estimates for each were correct, one can calculate stoichiometries from these data. Under these assumptions, and also assuming 144 copies of gp19 per core, our data predict that there are 6 to 7 copies of gp48 per core-baseplate and 5 to 7 copies per purified core. Six copies of gp48 seems to be a reasonable estimate, given the sixfold symmetry of the entire tail (5).

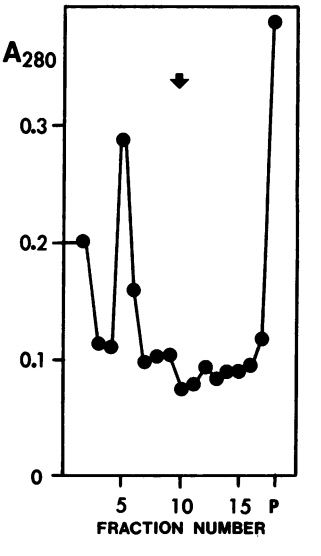

FIG. 4. Sucrose gradient purification of cores surviving 3.0 M GuHCl treatment. A 1-ml sample of purified core-baseplates (0.8 mg/ml) was dialyzed first into 3.0 M GuHCl and then back into buffer as described in the text. A 0.7-ml sample of this preparation was layered onto a sucrose gradient and centrifuged. The absorbance at 280 nm (A_{280}) of each fraction was measured. Sedimentation was from left to right. A small pellet was suspended in the bottom fraction marked "P." The arrow marks the position at which untreated core-baseplates sedimented. The peak of material found in fraction 5 was identified by electron microscopy as cores free of baseplates.

Core structure after removal from baseplate. Close inspection of micrographs of separated T4 tail cores reveals two classes of structures, as judged by stain penetration into the tube: hollow tubes and partially filled tubes (Fig. 1C). Hollow tubes have a line of stain penetrating the entire length of the structure. The partially filled cores have stain penetrating partway into the hollow center of the tube and often display a modified tip at the opposite end. A surprising feature of the modified tip is a short, fibrous extension often protruding from the end of the core. The appearance of the modified tips suggests the adherence of an additional structural component to a tube composed of gp19. The modified tip is only seen on partially filled tubes, suggesting that it arises from the partial extrusion of an internal component whose loss was arrested by incomplete dissociation. The modified tips could be an artifact of the separation of cores from baseplates during specimen preparation, but the structure persists when cores are purified (Fig. 1D). Since gp48 remains associated with purified cores, it is possible that the modified tips are composed of gp48 partly extruded from the hollow center of the core.

DISCUSSION

In electron micrographs, we have interpreted the absence of stain in the lumen of the core as being due to the presence of stain-excluding material, possibly protein. The hollow tubes we have observed are similar to those obtained by others from degraded phage (22, 25). In whole phage, complete tails, or core-baseplates, stain rarely penetrates into the core interior, suggesting that it is filled, or the ends are blocked. In extra-long T4 tails produced in vitro, stain is excluded from the baseplate end of the core up to the length of a normal tail, but it does penetrate to this level from the other end (26). The same phenomenom has been observed in extra-long tails of bacteriophage SPO1 produced in vivo (M. Parker, Ph.D. thesis, University of California, Los Angeles, 1979). Earlier studies have indicated that the core is composed of a single protein species, gp19, when the compositions of baseplates and core-baseplates are compared (19), but when cores are purified from chemically degraded phage, at least one additional protein is also found (6). The results presented in this paper show that gp48, which is clearly assembled onto the baseplate before the core (1), copurifies with cores that have been removed from baseplates. Since gp48 and gp54 are required for the assembly of gp19 onto the baseplate (17, 20), either one might be expected to copurify with the core, perhaps as the terminal annulus of the core. The proteins of the central plug of the baseplates (gp5, gp27, and gp29) could also be expected to be in the core due to structural considerations (14).

If gp48 is a protein that fills the interior of the core, then its role may be to form a template that determines the length of the phage tail. This type of length-regulating mechanism is the simplest of the models that have been proposed; these models have been discussed extensively elsewhere (3, 10, 27). King (17) has suggested that gp48 and gp54 might specify a long protein chain that projects from the baseplate and determines the core length. No such projecting fiber has been observed in the electron microscope when negative stain is used, but low-angle metal shadowing, recently used to allow visualization of fibrous molecules like spectrin (23), might allow detection of fibers protruding from baseplates. The addition of gp48 or gp54 or both to baseplates, required for core polymerization, does not change the appearance of baseplates (14), but the addition of gp48 (17) or gp54 (20) seems to influence the ability of baseplates to form dimers of baseplates paired together by their top faces. Wagenknecht and Bloomfield have purified baseplates and core protein (gp19) and assembled cores onto baseplates in vitro (28). Using their in vitro assembly system, they have noted that an unknown protease (present in commercial RNase) will inactivate baseplates, producing a distribution of core lengths shorter than on untreated baseplates, and that the same protease selectively cleaves three baseplate proteins, including gp54 and possibly gp48 (29). They suggest that baseplates may be inactivated by the cleavage of a length-determining molecule. Moody and Makowski have determined that the inside diameter of the core is 3.2 to 3.8 nm (21); this would be enough room for six alpha-helices, if a protein that filled the core took on this conformation. An alpha-helical protein 100 nm long would need to have a molecular weight of about 75,000 (about the size of gp29, molecular weight of 80,000, as opposed to gp48, molecular weight of 42,000), but other, more extended conformations are possible. As a fully extended chain or beta-sheet structure, a molecular weight of about 31,000 would be sufficient. There is one example known of a long extended chain in a biological structure; this is the "e" sequence in the interdigitating arms of tomato bushy stunt virus type C subunits (9). It is worth noting that temperature-sensitive mutants in gene 19, when grown at semipermissive temperatures, produce stable core-baseplates that have short cores. (Incorrectly folded gp19 molecules may bind to the growing core and stop polymerization.) The proposed internal fiber might be expected to protrude from the end of short core-baseplates, but this has not been observed. The short core-baseplates contain

gp48 and gp54 after partial purification at room temperature and tend to aggregate in twos and threes by material at the tips of the cores (unpublished data). Experiments to visualize protruding fibers on these structures are in progress.

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LITERATURE CITED

1. **Berget, P. B., and H. R. Warner.** 1975. Identification of P48 and P54 as components of bacteriophage T4 baseplates J. Virol. **16**:1669–1677.
2. **Brown, S. M., and F. A. Eiserling.** 1979. T4 gene 40 mutants. II. Phenotypic properties. Virology **97**:77–89.
3. **Caspar, D. L. D.** 1980. Movement and self-control in protein assemblies. Quasi-equivalence revisited. Biophys. J. **32**:103–135.
4. **Coombs, D. H.** 1975. Density gradient fractionation by piston displacement. Anal. Biochem. **68**:95–101.
5. **Crowther, R. A., E. V. Lenk, Y. Kikuchi, and J. King.** 1977. Molecular reorganization in the hexagon to star transition of the base-plate of bacteriophage T4. J. Mol. Biol. **116**:489–523.
6. **Cummings, D. J.** 1972. A remeasurement of the molecular weights of T-even bacteriophage substructural proteins. J. Virol. **9**:547–550.
7. **Dubochet, J., and E. Kellenberger.** 1972. Selective adsorption of particles to the supporting film and its consequences on particle counts in electron microscopy. Microsc. Acta **72**:119–130.
8. **Fenner, C., R. R. Trant, D. T. Mason, and J. Wikman-Coffelt.** 1975. Quantification of coomassie blue stained proteins in polyacrylamide gel based on analyses of eluted dye. Anal. Biochem. **63**:595–602.
9. **Harrison, S. C.** 1980. Protein interfaces and intersubunit bonding. The case of tomato bushy stunt virus. Biophys. J. **32**:139–151.
10. **Kellenberger, E.** 1972. Discussion, p. 295–299. *In* Polymerization in biological systems, Ciba Foundation Symposium, vol. 7. Elsevier, London.
11. **Kellenberger, E., and J. Séchaud.** 1957. Electron microscopical studies of phage multiplication. II. Production of phage related structures during multiplication of phages T2 and T4. Virology **3**:256–274.
12. **Kikuchi, Y., and J. King.** 1975. Genetic control of bacteriophage T4 base-plate morphogenesis. I. Sequential assembly of the major precursor, *in vivo* and *in vitro*. J. Mol. Biol. **99**:645–672.
13. **Kikuchi, Y., and J. King.** 1975. Genetic control of bacteriophage T4 baseplate morphogenesis. II. Mutants unable to form the central part of the baseplate. J. Mol. Biol. **99**:673–694.
14. **Kikuchi, Y., and J. King.** 1975. Genetic control of bacteriophage T4 baseplate morphogenesis. III. Formation of the central plug and overall assembly pathway. J. Mol. Biol. **99**:695–716.
15. **Kikuchi, Y., and J. King.** 1976. Assembly of the contractile tail of bacteriophage T4, p. 71–91. *In* R. D. Goldman, T. Pollard, and J. Rosenbaum (ed.), Cell motility, book A. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **King, J.** 1968. Assembly of the tail of bacteriophage T4. J. Mol. Biol. **32**:231–262.
17. **King, J.** 1971. Bacteriophage T4 tail assembly: four steps in core formation. J. Mol. Biol. **58**:693–709.
18. **King, J., and U. K. Laemmli.** 1973. Bacteriophage T4 tail assembly: structural proteins and their genetic identification. J. Mol. Biol. **75**:315–337.
19. **King, J., and N. Mykolajewycz.** 1973. Bacteriophage T4 tail assembly: proteins of the sheath, core, and base-plate. J. Mol. Biol. **75**:339–358.
20. **Meezan, E., and W. B. Wood.** 1971. The sequence of gene product interaction in bacteriophage T4 tail core assembly. J. Mol. Biol. **58**:685–692.
21. **Moody, M. F., and L. Makowski.** 1981. X-ray diffraction study of tail-tubes from bacteriophage T2L. J. Mol. Biol. **150**:217–244.
22. **Poglazov, B. F., and T. I. Nicolskaya.** 1969. Self-assembly of the protein of bacteriophage T2 tail cores. J. Mol. Biol. **43**:231–233.
23. **Shotton, D. M., B. E. Burke, and D. Branton.** 1979. Molecular structure of human erythrocyte spectrin, biophysical and electron microscopic studies. J. Mol. Biol. **131**:303–329.
24. **Steinberg, C. M., and R. S. Edgar.** 1962. A critical test of a current theory of genetic recombination in bacteriophages. Genetics **47**:187–208.
25. **To, C. M., E. Kellenberger, and A. Eisenstark.** 1969. Disassembly of T-even bacteriophage into structural parts and subunits. J. Mol. Biol. **46**:493–511.
26. **Tschopp, J., and P. R. Smith.** 1977. Extra long T4 tails produced in *in vitro* conditions. J. Mol. Biol. **114**:281–286.
27. **Wagenknecht, T., and V. A. Bloomfield.** 1975. Equilibrium mechanisms of length regulation in linear protein aggregates. Biopolymers **14**:2297–2309.
28. **Wagenknecht, T., and V. A. Bloomfield.** 1977. *In vitro* polymerization of bacteriophage T4D tail core subunits. J. Mol. Biol. **116**:347–359.
29. **Wagenknecht, T., and V. A. Bloomfield.** 1978. Bacteriophage T4 tail length is controlled by its base-plate. Biochem. Biophys. Res. Commun. **82**:1049–1055.