JOURNAL OF VIROLOGY, Jan. 1987, p. 113–118
0022-538X/87/010113-06$02.00/0

Vol. 61, No. 1

Prohead Core of Bacteriophage T4 Can Act as an Intermediate in the T4 Head Assembly Pathway

ANDREAS KUHN,* BEAT KELLER,† MARLIES MAEDER, AND FRANZ TRAUB‡
Department of Microbiology, Biozentrum, University of Basel, CH-4056 Basel, Switzerland

Received 9 May 1986/Accepted 18 September 1986

Bacteriophage T4 assembly was impaired in *Escherichia coli* hdB3-1 at an incubation temperature below 30°C. Naked prohead cores (head scaffold) bound to the inner surface of the plasma membrane accumulated, and the major shell protein (gp23) precipitated into visible intracellular aggregates in the cytoplasm. Shifting the temperature to 42°C allowed newly synthesized gp23 to assemble around the accumulated cores. We conclude that synchronous assembly of the scaffold and shell is not obligatory and that naked cores can serve as intermediates in the T4 assembly pathway.

The assembly of bacteriophage capsids is preceded by the formation of a prohead consisting of a shell and a proteinaceous core (3). For bacteriophage T4, the major components of the shell are gene products 23 and 24. The core is composed of at least seven gene products, namely proteins gp21, gp67, gp68, IPI, IPII, and IPIII and its major component, gp22 (17). After prohead assembly is complete, the scaffolding core structure is proteolytically removed (12). In this process the core proteins are either totally or partially degraded, and the degradation products of some core proteins remain inside the mature head (13). Only the internal proteins (IPI, IPII, and IPIII) are found in comparable amounts in the prohead and in their processed form (IP*) in the phage. We have previously shown the formation of naked scaffolding cores in vivo when shell proteins are absent (17, 18). These naked cores consisted of the T4 internal head proteins and were morphologically similar to cores found in the prohead, i.e., they had a prolate shape and a defined size. This suggests that the scaffolding core controls the head size and directs the shell into its prolate shape.

The detection of naked cores allows us to ask whether the core is a possible intermediate in prohead formation, i.e., whether the shell can still be formed after the core is completed. To determine whether naked cores can serve as true intermediates in T4 head assembly, we used an *Escherichia coli* strain that shows a block in an early stage of the T4 assembly pathway. In this strain, the assembly of the tail or of the tail fibers is not affected, while the assembly of the head is nearly completely impaired at an incubation temperature of 28°C (15). We have recently shown that under these conditions naked prohead cores are formed and the major shell protein (gp23) precipitates into "lumps" as observed in thin-sectioned cells (18). A shift of the incubation temperature from 28 to 41°C during infection results in the production of phage progeny (15). The progeny might result either from de novo assembly of head structures or from maturation of the accumulated scaffolds. To distinguish between these possibilities, we used a mutant of T4 carrying two mutations in gene *40*. Since gp40 is only required for phage growth at high temperature (6, 7), de novo assembly after the shift should be excluded. We show here that T4 40*am* 40*oc* infections in *E. coli* hdB3-1, when shifted from 28 to 42°C, yield viable phage. These results suggest that the accumulated cores can be matured to functional phage heads and can act as true intermediates in the T4 assembly pathway.

MATERIALS AND METHODS

Bacterial and phage strains. *E. coli* B (*sup*$^+$) and CR63 (*supD*) were used as standard hosts. *E. coli* hdB3-1 is a mutant strain with an altered fatty acid and phospholipid composition (15) and was kindly provided by L. D. Simon. Phage T4D and its mutants 10(*am*B255), 18(*am*E18), 19 (*cs*542), 20(*am*E481), 20(*cs*1041), 23(*am*H11), 40(*am*A142), and 40(*oc*L84) were from our collection. The double and multiple mutants were made by crossing the single mutants.

Media and phage infections. Cultures of host cells and infected cells were grown in M9 minimal medium (1) supplemented with 1% (M9A) or 0.1% (M9a) Casamino Acids (Difco Laboratories). The host cells were grown to a concentration of 2×10^8 cells per ml and then infected at a multiplicity of infection of 5, followed by superinfection at the same multiplicity 8 min after infection to obtain lysis inhibition.

Electron microscopy. In situ lysis, negative staining, embedding, and thin sectioning were performed as described previously (18). Micrographs were made on Kodalith film (Eastman Kodak Co.) with a Zeiss EM10 or a Philips EM300 electron microscope at an acceleration voltage of 80 kV.

Analysis of the radiochemical composition of isolated phage particles. One milliliter of a fresh overnight culture of *E. coli* hdB3-1 grown at 28°C in M9 medium was diluted into 100 ml of M9 medium and shaken at 28°C until the culture reached a density of 2×10^8 cells per ml. Phage T4 40 (*am*A142) 40(*oc*L84) was added at a multiplicity of infection of 5. Thirty seconds later 150 μCi of ^{14}C-amino acids (Amersham Corp.; 50 μCi/ml) and in some cases 300 μCi of ^{3}H-amino acids (Amersham; 1 mCi/ml) were added. At 6 min postinfection, the culture was superinfected by the same phage mutant at a multiplicity of infection of 5. At 12 min postinfection, the culture was shifted to 24.2°C and shaken for 48 min. The cells were harvested at 6,000 × *g* for 5 min (Sorvall type GSA rotor) at room temperature and suspended in warm (30°C) M9 medium. The culture was then shifted to 42°C, and 30s later 150 μCi of ^{14}C-amino acids and in some cases 300 μCi of ^{3}H-amino acids were added. Incubation of the culture was continued until 2 h after the primary infec-

* Corresponding author.
† Present address: The Salk Institute, San Diego, CA 92138.
‡ Present address: Gesundheitsamt, CH-9001 St. Gallen, Switzerland.

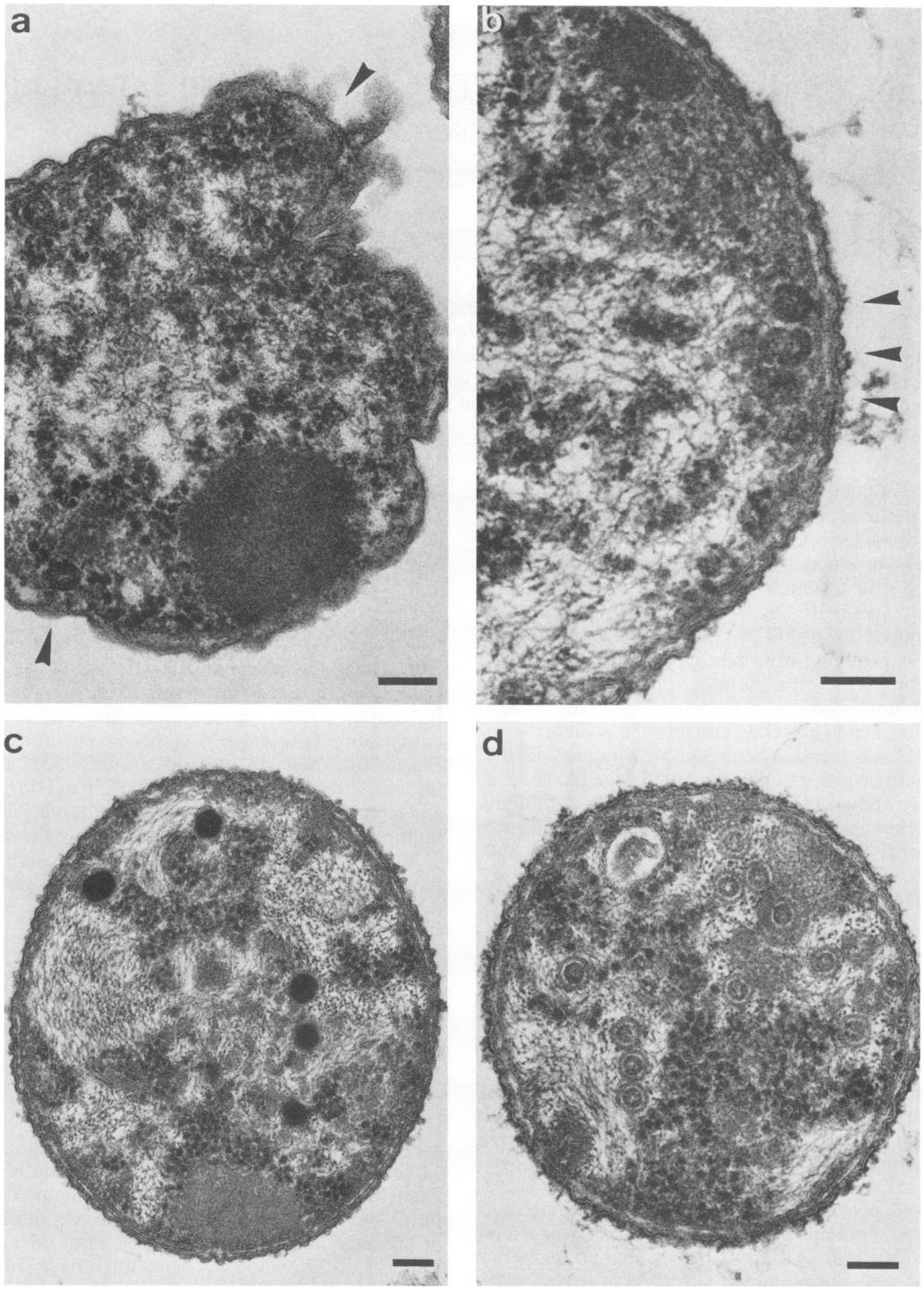

FIG. 1. Thin sections of *E. coli* hdB3-1 cells infected with T4 40(*am*A142) 40(*oc*L84). (a and b) Incubation temperature was 28°C, and fixation was done at 75 min (a) or 120 min (b) postinfection. Arrows point to naked core particles. (c) Incubation temperature was held at 28°C for 75 min postinfection and then shifted to 42°C until 120 min postinfection. (d) Incubation temperature was held at 28°C until 8 min postinfection and then shifted to 42°C until 120 min postinfection. Bars, 100 nm.

tion. The cells were then sedimented at 6,000 × *g* for 5 min and suspended in 1 ml of phosphate buffer (150 mM, pH 7.0) containing 1 mM $MgSO_4$ and 10 μg of DNase (Serva) per ml. This suspension was incubated at 60°C and continuously mixed with a CsCl solution to a final density of 1.50 g/liter. Density gradients were formed by centrifugation for 72 h at 40,000 × *g* in an SB283 rotor of an IEC-B60 ultracentrifuge at 20°C. The visible bands were harvested, and the material was slowly dialyzed against sodium citrate buffer.

SDS gel electrophoresis and autoradiography. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) was performed as described by Laemmli (10). The gels were fixed and stained with Coomassie blue. After drying, the gels were exposed to Fuji RX medical X-ray film,

and the autoradiograph was compared with the gels. The identified protein bands were then cut out of the gel. The gel strips were put into 0.1 ml of concentrated nitric acid and 50 μl of 30% hydrogen peroxide. The mixture was heated in a boiling water bath, and the strips were dissolved completely after three subsequent additions of 50 μl of 30% hydrogen peroxide. The dissolved protein bands were mixed with 5 ml of Instagel scintillation fluid (Packard) and counted in a Packard Tricarb 460C liquid scintillation spectrometer. The counts obtained were substracted from spillover and background noise, and the $^{14}C/^{3}H$ ratio was determined.

In vitro experiments with isolated prohead cores and shell proteins. T4 scaffolding cores were isolated from T4 10 (*am*B255) 18(*am*E18) 20 (*am*E481) 23 (*am*H11)-infected cells as described previously (17). The purified shell proteins were kindly provided by R. van Driel (gp23 and gp24) and J. Caldentey (gp20). The assembly mixture contained 10 mM $MgSO_4$ and 10 mM phosphate (pH 6.0) as described by van Driel and Couture (19). The proteins were added at a concentration of 5 to 10 mg/ml for gp23, 0.2 to 1 mg/ml for gp24, and 0.1 to 0.2 mg/ml for gp20. The mixture was held at room temperature for 1 to 3 h, and then the samples were negatively stained with 5% sodium phosphotungstate and examined in the electron microscope.

RESULTS

Conversion of naked cores to head-related structures. We used electron microscopy to investigate whether prohead cores, which accumulate in T4-infected *E. coli* hdB3-1, can mature to "black particles" (the appearance of DNA-containing phage on thin sections). Naked cores were attached to the inner membrane of thin-sectioned cells when the incubation temperature was held below 30°C (18). In addition, large precipitates (lumps) were visible in the cytoplasm, often in contact with the membrane. These lumps consist of precipitated gp23, as demonstrated by immunogold labeling of sections (M. Dürrenberger, personal communication). After a shift to higher incubation temperatures (>37°C), the number of intracellular phage particles increased substantially. Similarly, cells, infected with the phage mutant T4 40(*am*A142) 40(*oc*L84) at 28°C also accumulated intracellular naked cores and lumps of precipitated gp23 (Fig. 1a and b). However, when the temperature was shifted to 42°C at 75 min postinfection and incubation was continued for 45 min, the number of core particles decreased, and black particles were formed (Table 1, Fig. 1c). It is unlikely that these black particles were assembled de novo because T4 requires gp40 for its assembly at 42°C (7). When the culture was shifted to 42°C immediately after infection and incubated for 120 min at the higher temperature, only a few cores and some black particles were seen. In addition, aberrant tubular structures (polyheads) were formed (Table 1, Fig. 1d) as in T4 40 *am*-infected wild-type cells. Therefore, these results suggest that the accumulated naked cores were converted to black particles.

TABLE 1. Particles produced before and after temperature shift of T4 40*am* 40*oc*-infected *E. coli* hdB3-1

Growth conditions	No. of head-related particles on thin sections[a]			
	Cores	Black particles	Proheads	Tubular structures
28°C, 75 min	30	29	30	31
28°C, 75 min; 42°C, 45 min	1	311	15	14
42°C, 120 min	3	121	5	173

[a] The profiles of 100 thin-sectioned cells were analyzed.

TABLE 2. Phage titer in strain hdB3-1 before and after temperature shift[a]

T4 mutant	Growth conditions	No. of PFU/10^7 cells
T4 40*am* 40*oc*	28°C, 75 min	50
	28°C, 75 min; 42°C, 45 min	560
	28°C, 120 min	100
	42°C, 120 min	6
T4 19*cs* 20*cs* 40*am* 40*oc*	28°C, 75 min	2
	28°C, 75 min; 42°C, 45 min	80
	28°C, 120 min	5
	42°C, 120 min	3

[a] Exponentially growing hdB3-1 cells were infected at 2×10^8 cells per ml at a multiplicity of 5, followed by superinfection at the same multiplicity 8 min after the initial infection.

Maturation of naked cores into viable phage. Since our analysis of thin-sectioned cells suggested that cores can mature to black particles, we investigated whether naked cores can be converted to infective phage. To do this, we analyzed the number of PFU from lysed cells before and after the temperature shift. T4-infected cultures of *E. coli* hdB3-1 were grown at either 28 or 42°C or were shifted from the low incubation temperature to the high temperature. The infecting phage was defective for gp40, which is required for phage growth at high temperature (6, 7). Due to the phage-related defect, the number of PFU from infections performed at 42°C was only 6×10^7/ml and, due to the host-related defect, only 1×10^9/ml at 28°C (Table 2). For the shifted culture, however, the number of PFU was increased to 6×10^9/ml. This increase is probably not due to de novo assembly after the temperature shift but is readily explained by the maturation of accumulated intermediates. As shown in Table 2, a similar result was obtained with T4 19(*cs*542) 20(*cs*1041) 40(*am*A142) 40(*oc*L84)-infected cells. We had introduced the cold-sensitive mutations to lower the background at 28°C.

Composition of pulse-labeled head proteins suggests the maturability of naked cores. To analyze whether cores accumulated in strain hdB1-3 cells matured to phage after the temperature shift, we pulse-labeled T4-infected cells and analyzed the radiochemical protein composition of isolated phage. By incorporating ^{3}H-and ^{14}C-labeled amino acids into phage, we determined the times that phage head proteins were originally synthesized. *E. coli* hdB3-1 cells were infected with T4 40(*am*A142) 40(*oc*L84) at 28°C. Thirty seconds after infection, a mixture of 150 μCi of ^{14}C- and 300 μCi of ^{3}H-labeled amino acids was added to the culture. Consequently, proteins synthesized and assembled during the first time period should have a 1:2 ratio of the two isotopes. At 60 min postinfection the culture was shifted to 42°C and pulsed with 150 μCi of ^{14}C-labeled amino acids. Any proteins synthesized after the temperature shift should contain mainly the ^{14}C isotope. This allowed us to distinguish whether the core had matured or was synthesized de novo. To analyze the protein composition of the phage, the culture was harvested and lysed after 1 h of incubation at 42°C. Phage were isolated and purified on a CsCl gradient. Phage proteins were analyzed on an SDS-polyacrylamide gel and stained with Coomassie blue. The proteins were identified by their banding pattern, the radioactivity of the isolated bands

TABLE 3. Radiochemical composition of various head proteins to indicate synthesis before or after temperature shift

Expt no.	$^{14}C/^{3}H$ ratio of isolated phage proteins			
	IPIII*	gp20	gp23	gp24
1	0.4	0.5	0.8	0.8
2	1.0	1.0	1.1	1.0
3	3.4	1.1	0.8	0.8

was measured, and the $^{14}C/^{3}H$ ratio was estimated (Table 3, experiment 1). The core protein remaining in the phage, IPIII*, had a ratio of about 1:2 for the two isotopes, suggesting that IPIII* derived from synthesis before the temperature shift. A similar ratio was obtained for gp20, which was probably partly linked to the core structures which accumulated. The shell proteins gp23 and gp24, however, had higher amounts of ^{14}C; protein synthesis during both time periods contributed about equally to the shell proteins incorporated into the phage. As a control experiment, a 1:1 ratio of the ^{3}H- and ^{14}C-labeled amino acids was added before and after the temperature shift (Table 3, experiment 2). For all the individual proteins, the two isotopes were equally incorporated. In a further experiment (Table 3, experiment 3), the first pulse at 30 s after infection contained solely 150 μCi of ^{14}C-labeled amino acids and in the second pulse, after the temperature shift to 42°C, 150 μCi of ^{14}C- and 300 μCi of ^{3}H-labeled amino acids were added. The proteins which were synthesized before the temperature shift should therefore contain only ^{14}C. As shown in Table 3, the core protein IPIII* showed a high $^{14}C/^{3}H$ ratio, supporting the conclusion that it was mainly derived from synthesis before the temperature shift, whereas the shell proteins mainly derived from synthesis after the shift. The results obtained for gp20 do not allow a clear conclusion, and it seems possible that gp20 can, at least partially, assemble around the accumulated cores.

Head-related particles found in absence of gp20. Infections with T4 double mutants carrying mutations in genes 20 and 23 result in the accumulation of naked cores (15). Accordingly, infection of *E. coli* hdB3-1 with T4 20(*am*E481) 40(*am*A142) 40(*oc*L84) at low temperature led to an accumulation of naked cores which lacked gp20 (Fig. 2a). To test whether these cores can mature to phage, the culture was shifted to 42°C and incubated for 1 h. Cell lysates were examined with the electron microscope for head-related particles. Core particles which were partially surrounded by shell structures could be detected (Fig. 2c to e). We should point out that in most cases, the proximal and distal vertices of the shells were missing. Besides these particles, we observed tubular structures which obviously represented shelled cores that were elongated by continuous shell growth. gp20 has been localized at the proximal vertex (11), and it therefore appears to be essential for correct initiation of shell assembly. However, even in the absence of gp20, gp23 was assembled and showed a strong affinity for the cores. The existence of these head-related particles underlines the idea that naked cores can undergo further steps in the head assembly pathway and are not simply degraded after the shift. The cores resulting from infections with the 20*am* 23*am* T4 mutant are either attached to the inner membrane or free in the cytoplasm, depending on the particular gene 20 mutant used (18). For the mutant used in this study, 20 (*am*E481), two of three of the naked cores were attached to the membrane of *E. coli* hdB3-1. Interest-

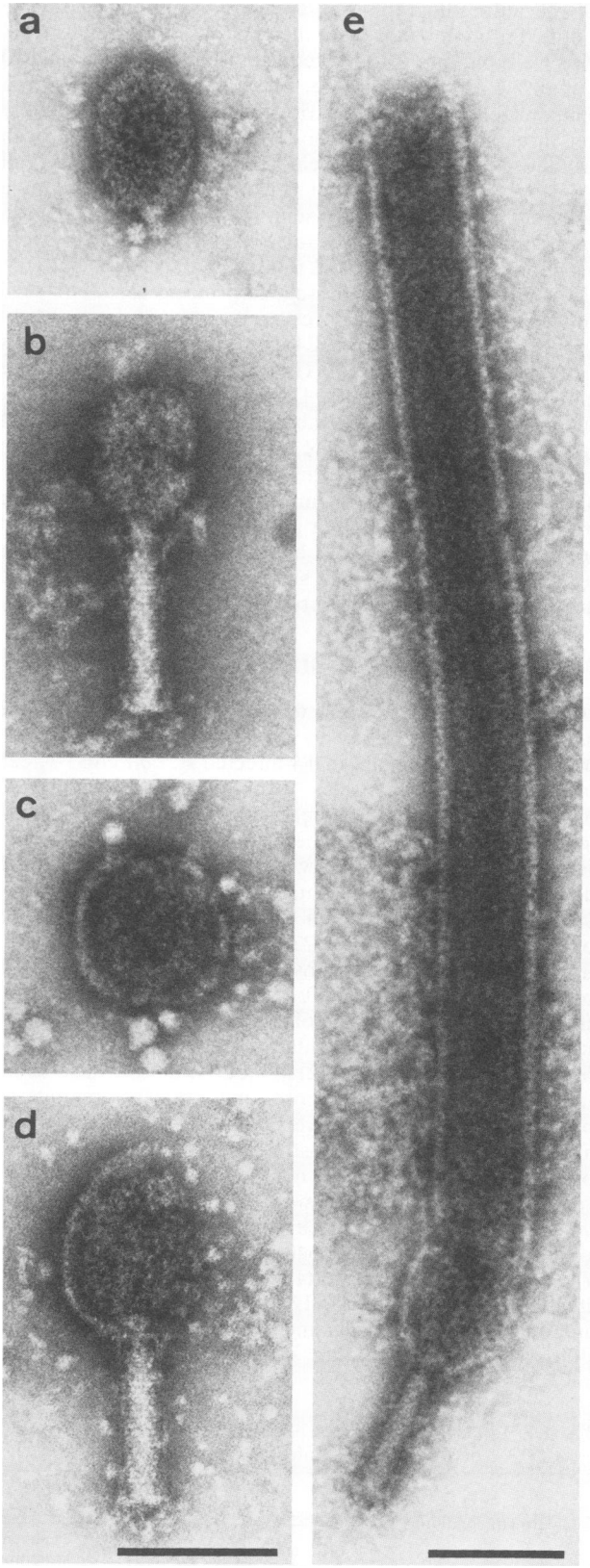

FIG. 2. T4 particles from T4 20(*am*E481) 40(*am*A142) 40(*oc*L84)-infected *E. coli* hdB3-1 released by in situ lysis. (a and b) Naked prohead core particles fixed after incubation at 28°C for 90 min. (c, d, and e) Partially shelled prohead core particles fixed after incubation at 28°C for 60 min and then shifted to 42°C for 30 min. Bars, 100 nm.

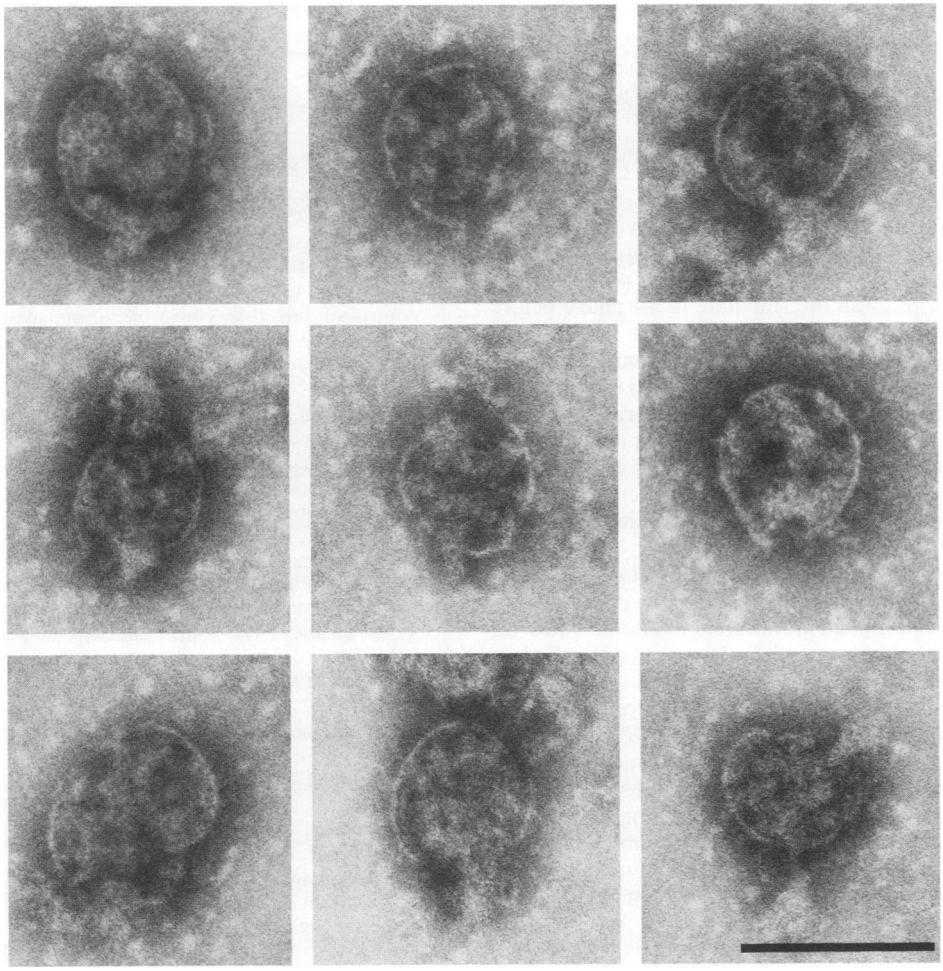

FIG. 3. Conversion of isolated naked cores to proheadlike structures. Isolated naked cores (17) were mixed with purified gp20, gp23, and gp24 for 50 min at room temperature and negatively stained with 5% sodium phosphotungstate. Bar, 100 nm.

ingly, we found that many of the free cores were linked to tail structures (Fig. 2b). This indicates that the core neck participates in head-tail joining.

In vitro conversion of isolated naked cores with purified shell proteins. To test whether cores can be converted to proheadlike structures in vitro, naked cores were isolated as described by Traub et al. (17) and incubated with purified shell proteins (gp20, gp23, and gp24). After a short incubation time (1 to 3 h), we observed by electron microscopy that some cores were partially and some completely surrounded by shell-like structures (Fig. 3). When gp23 was omitted from the incubation mixtures, no such shelled particles were observed (data not shown). Longer incubation times (>3 h) resulted in the destruction of the particles. We conclude from these results that naked cores can be encapsidated by the purified shell proteins and give rise to prohead-related structures.

DISCUSSION

We have previously shown that the core of bacteriophage T4 can assemble in vivo without the shell, having a distinct size and architecture (18). In this paper we report that naked cores can potentially act as an intermediate structure and can mature to phage.

Three lines of evidence show that naked cores accumulated in the cold-sensitive host *E. coli* hdB3-1 can mature to phage by a temperature shift to 42°C. (i) Electron microscopic studies showed an increase in the number of black particles and a concomitant decrease of cores on thin-sectioned T4-infected cells, suggesting that the accumulated cores were converted into phage. (ii) Analysis of the phage titer showed an increase in PFU after the temperature shift, consistent with cores maturing to infectious progeny. (iii) Pulse-labeling experiments showed that the assembly of the core and the shell were not concomitant. We found that under these conditions the remaining core protein (IPIII) was derived mainly from synthesis before the shift, whereas the shell proteins (gp23 and gp24) were synthesized mainly after the shift. The maturability of the accumulated cores is underlined by the observation that they remained stable after the shift to high temperature, as shown by 20*am* infections. Naked cores are therefore not readily degraded but can mature to phage.

Further evidence that cores can mature was obtained from in vitro experiments with purified cores. Addition of purified shell proteins to isolated cores resulted in partially closed shells similar to those found in the 20*am* infections. The failure to assemble complete proheads might be ascribed to

gp20, which was not able to assemble onto core particles in vitro and initiate shell assembly from the vertex. Since the isolated cores had been fixed with cross-linking agents, some protein-protein interactions were probably inhibited. In addition, further protein components (such as groE and gp31) might be necessary to achieve complete shell assembly. Based on the partial assembly of shell-related structures onto cores, we conclude that cores are able to undergo further steps in the head assembly pathway in vitro.

We have shown here that the assembly of prohead core and shell is to a certain degree, an independent process and that the core can serve as an intermediate in T4 phage head assembly. Naked cores are thus not aberrant structures, such as the tubular forms of the shell protein, but are possible intermediates. Previous results revealed that gp20 is not a component of the core (17) and also not required for core assembly (17, 18). However, its involvement in shell formation is evident. The particles formed in vivo in the hdB3-1 host after a temperature shift in the absence of gp20 consisted of cores surrounded by incomplete shells. These particles were often elongated to tubular structures (polyheads), probably due to continuous growth of the shell on the distal vertex. Infections of *E. coli* B with mutants carrying three different amber mutations in gene 20 show a similar phenotype (M. Maeder and F. Traub, unpublished). Since gp20 is probably localized at the proximal vertex (11), it may assemble around the core neck and thereby anchor the prohead to the membrane. Indeed, mutant 20(*am*E481) 23 (*am*H11) infections of *E. coli* B show naked cores detached from the membrane (18). We propose that the initiation of shell assembly with a correct folding pattern of gp23 requires that gp20 be attached to the prominent neck structure seen at the vertex of naked cores. Further shell assembly then requires contact with gp22 during or after its integration into the assembling core structure. We believe that the interaction of gp23 with gp22 and gp20 is crucial for the folding pattern of the shell and determines the icosahedral symmetry of the prospective capsid, since mutations in genes 20 and 22 lead to the formation of tubular structures with a different folding pattern (16). Obviously, only a correct folding pattern allows the shell to close a cap at the distal vertex of the head.

Although our experiments allowed us to separate core and shell assembly, we assume that in the normal maturation pathway both assembly processes are rapid and nearly synchronous. It has been shown by gene dosage experiments that the balance of core and shell subunits might be crucial for correct head assembly (13). Furthermore, genetic studies have shown that mutations in genes 23 and 24, coding for shell proteins, can result in size variants of the phage (2, 4, 5). Similarly, mutations in genes 67 and 68, coding for core proteins, also lead to a variety of abnormal proheadlike structures and phage size variants (8, 9, 20). We conclude that the size and form of the T4 head are primarily determined by the core in a nearly synchronous assembly of core and shell.

ACKNOWLEDGMENTS

We thank L. D. Simon for the generous supply of the host strain hdB3-1 and J. Caldentey for the purified proteins. We also thank M. Zoller for photographic assistance and E. Amstutz for typing the manuscript.

This work was supported by grant 3.755.80 from the Swiss National Science Foundation.

LITERATURE CITED

1. **Adams, M. A.** 1959. The bacteriophages. Wiley Interscience, New York.
2. **Bijlenga, R. K. L., R. van den Broek, and E. Kellenberger.** 1974. The transformation of τ-particles into T4 heads. J. Supramol. Struct. **2**:45–59.
3. **Casjens, S., and J. King.** 1975. Virus assembly. Annu. Rev. Biochem. **44**:555–611.
4. **Doermann, A. H., F. A. Eiserling, and L. Boehner.** 1973. Genetic control of capsid length in bacteriophage T4. I. Isolation and preliminary description of four new mutants. J. Virol. **12**:374–385.
5. **Eiserling, F. A., E. P. Geiduschek, R. H. Epstein, and E. J. Metter.** 1970. Capsid size and deoxyribonucleic acid length: the petite variant of bacteriophage T4. J. Virol. **6**:865–876.
6. **Hsiao, C. L., and L. W. Black.** 1978. Head morphogenesis of bacteriophage T4. I. Isolation and characterization of gene 40 mutants. Virology **91**:1–14.
7. **Hsiao, C. L., and L. W. Black.** 1978. Head morphogenesis of bacteriophage T4. II. The role of gene 40 in initiating prehead assembly. Virology **91**:15–25.
8. **Keller, B., M. Maeder, C. Becker-Laburte, E. Kellenberger, and T. A. Bickle.** 1986. Amber mutants in gene 67 of phage T4. Effects on formation and shape determination of the head. J. Mol. Biol. **190**:83–95.
9. **Keller, B., C. Sengstag, E. Kellenberger, and T. A. Bickle.** 1984. Gene 68, a new bacteriophage T4 gene which codes for the 17K prohead core protein is involved in head size determination. J. Mol. Biol. **179**:415–430.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **Mueller-Salamin, L., L. Onorato, and M. K. Showe.** 1977. Localization of minor protein components of the head of bacteriophage T4. J. Virol. **24**:121–134.
12. **Onorato, L., and M. Showe.** 1975. Gene 21 protein-dependent proteolysis *in vitro* of purified gene 22 product of bacteriophage T4. J. Mol. Biol. **111**:459–485.
13. **Onorato, L., B. Stirmer, and M. K. Showe.** 1978. Isolation and characterization of bacteriophage T4 mutant preheads. J. Virol. **27**:409–426.
14. **Showe, M. K., and L. Onorato.** 1978. Kinetic factors and form determination of the head of bacteriophage T4. Proc. Natl. Acad. Sci. USA **75**:4165–4169.
15. **Simon, L. D., T. J. M. McLaughlin, D. Snover, J. Ou, C. Grishman, and M. Loeb.** 1975. *E. coli* membrane lipid alteration affecting T4 capsid morphogenesis. Nature (London) **256**:379–383.
16. **Steven, A. C., U. Aebi, and M. K. Showe.** 1976. Folding and capsomere morphology of the P23 surface shell of bacteriophage T4 polyheads from mutants in five different genes. J. Mol. Biol. **102**:373–407.
17. **Traub, F., B. Keller, A. Kuhn, and M. Maeder.** 1984. Isolation of the prohead core of bacteriophage T4 after cross-linking and determination of protein composition. J. Virol. **49**:902–908.
18. **Traub, F., and M. Maeder.** 1984. Formation of the prohead core of bacteriophage T4 in vivo. J. Virol. **49**:892–901.
19. **Van Driel, R., and E. Couture.** 1978. Assembly of the T4 head-related structures. II. *In vitro* assembly of prehead-like structures. J. Mol. Biol. **123**:115–128.
20. **Voelker, T. A., A. Kuhn, M. K. Showe, and T. A. Bickle.** 1982. Gene 67, a new essential bacteriophage T4 head gene codes for a prehead core component, PIP. II. The construction *in vitro* of unconditionally lethal mutants and their maintenance. J. Mol. Biol. **161**:491–504.