

Involvement of the Bacterial *groM* Gene Product in Bacteriophage T7 Reproduction

I. Arrest at the Level of DNA Packaging

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The multiplication of bacteriophage T7 is blocked in *Escherichia coli* M. The genetic determinant of this ability (*groM*) to inhibit T7 growth was transferred to an *E. coli* K-12 recipient by means of conjugation. We determined at which precise step T7 maturation is blocked. Phage-directed protein and DNA synthesis as well as degradation of host DNA were not qualitatively affected. Instead of infective phages, only preheads were produced. These, however, were maturable in vitro. The newly synthesized phage DNA accumulated in a concatemeric form and matured from its tetrameric or longer forms (very fast sedimenting DNA) only into its dimeric form (fast-sedimenting DNA) or longer forms. The following step, i.e., the maturation of the dimeric to unit-length DNA, was not observed. Since the concatemeric form of T7 DNA accumulated in spite of the presence of maturable preheads, it is likely that the maturation process was blocked at the level of DNA packaging. As intermediates in the packaging process, we found some prehead-DNA complexes. We interpreted these as true assembly intermediates (or breakdown products thereof), since the attached DNA was still in its concatemeric form. This shows that the very first DNA packaging step, the binding of the progeny DNA to the preheads, was obviously not blocked. Rather, a later step, such as the filling of the preheads with T7 DNA or the stabilization of completely packaged particles (i.e., the final cutting of the concatemers into unit-size length), was inhibited.

The infection of certain bacterial strains with bacteriophages may lead to an "abortive" phage reproduction. The mechanisms of this inhibition of viral development are numerous. (i) Bacterial restriction enzymes may fragment the parental phage DNA. In this case, the vegetative cycle of the virus is interrupted at its very beginning (2). (ii) For a variety of reasons, an incompatibility may exist between the host bacterium and the phage (19). For example, a bacterial gene product necessary for phage maturation may be altered so that the host phage interaction is disturbed. Growth of the uninfected bacterium is not remarkably affected by this change. Additional dispensable genetic elements in the host, such as F factors, may also cause inhibition of phage multiplication, as in the case of T7-infected male strains of *Escherichia coli* (6). In this case, the early phase of the vegetative cycle of T7 proceeds normally but is followed, 10 min

postinfection (p.i.), by a rapid decrease of all metabolic processes.

A number of female strains of *E. coli* displaying disturbed development of phage T7 have been isolated and described by Hausmann (11). One of these (*E. coli* M) did not show any restriction of the parental phage DNA, but a reduced burst of 0.5 phage per cell. The question arises of whether the abortive phage reproduction is due to a host factor coded by the host chromosome or by a plasmid. That the defect was due to a host cell determinant was previously shown by the fact that the defect (*groM*) could be transferred to *E. coli* K-12 by conjugation with an F' *E. coli* M derivative (M. Reck, Ph.D. thesis, University of Freiburg im Breisgau, Freiburg, West Germany, 1976). The precise nature of *groM* is yet to be determined. In this paper, we investigated the step at which the morphogenetic pathway is stopped in the K-12 *groM* (K-12-M) strain after T7 infection. Particular attention was given to whether the interruption of the pathway leads to the accumulation of abortive

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phage particles. In a second paper (A. H. U. Kuhn, H. Tütte, and E. Kellenberger, manuscript in preparation), we will show that the level of intracellular K and Mg is lower in infected K-12-M than in K-12 and that the morphogenetic block is overcome by raising the outside concentration of Mg.

MATERIALS AND METHODS

Bacterial and phage strains. *E. coli* B^E (*sup*⁺), K-12 (W3110), and B40-1 (*supF*) were used as normal hosts. *E. coli* M was described by Hausmann (11). *E. coli* K-12-M, obtained by M. Reck, was cured of F' by acridine orange and was insensitive to the male-specific phages M12 and Q β but retained the GroM⁻ phenotype. Phage T7-Ha has recently been described and compared with other T7 strains (36). The T7 mutant in gene 5, *amH30*, was described by Hausmann and Gomez (13), and that in gene 10, *am13*, was a gift of F. W. Studier. M9 minimal medium (1) supplemented with amino acids (1% [M9A] or 0.1% [M9a]) was used.

SDS-polyacrylamide gel electrophoresis. The experimental equipment for polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) was similar to that described by Studier (34). Infection and the treatment of samples were essentially as described by Korsten et al. (18). Host bacteria (3×10^8 cells per ml) were UV irradiated to inhibit host protein synthesis, infected with a phage multiplicity of infection (MOI) of 5, and incubated at 37°C. At various times, 50 μ Ci of [³⁵S]methionine (Amersham Corp.) per ml was added. Two minutes later the cells were heated to 100°C in SDS, and the proteins were electrophoretically separated on a polyacrylamide gradient (10 to 18%) slab gel. Autoradiographs of the dried gels were densitometrically analyzed with a Joyce Loebel Chromoscan.

Degradation of prelabeled host DNA. Five microliters of a fresh overnight culture of *E. coli* K-12-M, K-12, and B^E grown in M9a was diluted into 5 ml of fresh M9 medium. One microcurie of [³H]thymidine (19 mCi/ml; Amersham) was added, and the culture was shaken at 37°C until the cell concentration reached 2×10^8 cells/ml. The cultures were centrifuged (Sorvall type SS-34 rotor) at $2,000 \times g$ for 5 min, and the cell pellets were suspended in 5 ml of fresh, prewarmed M9a medium. Phage was added at an MOI of 10. At different times after infection, portions of 0.5 ml were taken, treated with egg white lysozyme for 20 min at 4°C (200 μ g/ml), and precipitated overnight in 10% cold trichloroacetic acid (TCA) containing 7% NaCl. Each sample was filtered through a GF/C filter (Whatman), and the filters were washed and dried. The filters were placed into Insta-Gel (Packard Instrument Co., Inc.) scintillation liquid, and the TCA-precipitated radioactivity retained by the filter was determined in a Packard-2425 scintillation counter.

Phage DNA [³H]thymidine labeling. Fresh overnight cultures of *E. coli* K-12-M and *E. coli* B^E were diluted 1/50 into M9 medium. The cultures were grown to a concentration of 2×10^8 cells/ml at 37°C. To inhibit host DNA replication, the cells were irradiated with UV light for 30 s, at a distance of 50 cm, with a Westinghouse Sterilamp 182-20 (main emission band at 254 nm).

After 2 min of aeration at 37°C, the culture was infected with T7 phage at an MOI of 5. Six minutes after infection, 0.1 μ Ci of [³H]thymidine per ml was added (Amersham; 50 Ci/mmol), and 5-ml portions were withdrawn at the times indicated. The isolation procedure was essentially as described by Center (3). The portions were diluted into 5 ml of an ice-cold Tris buffer (100 mM, pH 8) containing 15 mM EDTA and 150 mM NaCl and centrifuged at $12,000 \times g$ for 5 min at 4°C. Each pellet was suspended in 0.5 ml of 50 mM Tris-hydrochloride (pH 8) buffer with 5 mM EDTA and 100 μ g of egg white lysozyme per ml. After 20 min in ice, 0.1% *N*-lauroylsarcosine (Sarkosyl; Sigma Chemical Co.) was added, and the temperature was raised to 30°C. After 5 min of this treatment, 50 μ g of pronase per ml (Calbiochem) was added. After at least 50 min, 200 μ l of each sample was layered on top of a 13-ml linear 5 to 20% sucrose gradient and sedimented in an SB-283 rotor of an IEC B-60 centrifuge at $140,000 \times g$ for 2 h at 4°C. After centrifugation, 0.5-ml fractions were collected from the bottom of the tube, and 0.1 ml of each fraction was tested for TCA-precipitable radioactivity, as described above.

Isolation of preheads. For the isolation of preheads, we followed essentially the method described by Miyazaki et al. (26). Five-liter cultures of *E. coli* B^E and of K-12-M were grown to a density of 5×10^8 cells/ml at 37°C in M9a medium. The culture was infected with T7 phage at an MOI of 5. Cells were harvested at 13 and 15 min p.i., respectively. The infected cells were immediately spun down at $8,000 \times g$ in an IEC-DPR 6000 centrifuge for 20 min at 4°C. The pellets were suspended in 20 mM Tris-hydrochloride buffer (pH 8) containing 0.1 M NaCl and 2.5% glycerol. The culture was lysed by adding 500 μ g of egg white lysozyme per ml and a few drops of chloroform. After incubation at 37°C for 15 min, 50 μ g of DNase per ml (Calbiochem) was added, and the lysate was centrifuged $12,000 \times g$ for 20 min at 4°C. The supernatant fractions were put into polyallomer tubes and centrifuged at 4°C in an A-237 rotor of an IEC-B60 ultracentrifuge at $10,000 \times g$ for 2 h. Each pellet was suspended in 1 ml of 20 mM Tris-hydrochloride, pH 7.5, containing 0.5 M NaCl and 1 mM MgCl₂ and stored overnight, and 200 μ l of this extract was layered on top of a 12-ml 5 to 20% sucrose gradient. Gradients were spun in an SB-283 rotor in an IEC-B60 ultracentrifuge for 150 min at $140,000 \times g$, and 0.5-ml fractions were collected and analyzed by electron microscopy.

Isolation of possible packaging intermediates. Fresh overnight cultures of *E. coli* K-12-M and B^E were diluted 1/50 into M9a medium. Then 100-ml portions of these cultures were grown to a concentration of 3×10^8 cells/ml at 37°C. T7 phage was added at an MOI of 5; 4 min later, 0.1 μ Ci of [¹⁴C]-labeled amino acids per ml (Amersham; 58 mCi/matom) and 0.2 μ Ci of [³H]thymidine per ml (Amersham; 45 Ci/mmol) were added. At the times indicated, the cultures were harvested by centrifugation at $6,000 \times g$ for 20 min at 4°C, and the pellets were washed and suspended into 5 ml of 20 mM Tris-hydrochloride buffer, pH 7.9, containing 200 mM NaCl and 1 mM EDTA. The bacterial debris was removed by centrifugation at $12,000 \times g$ for 20 min at 4°C, and 3 ml of the supernatants was layered on top of a discontinuous sucrose-D₂O gradient according to Moncany et al. (27). The gradient was prepared by placing successive layers on top of one

another, as follows: 4 ml of a sucrose cushion with a density of 1.32, 4 ml with a density of 1.28, 5 ml with a density of 1.24, 5 ml with a density of 1.20, 6 ml with a density of 1.16, and 8 ml with a density of 1.12. Each of the sucrose solutions was in 20 mM Tris-hydrochloride, pH 7.8, 1 mM EDTA, 200 mM NaCl, 1 mM spermidine, and 1 mM dithiothreitol and was prepared with heavy water (Merck & Co.). The gradients were centrifuged at 4°C for 7 h in an SB-110 rotor of an IEC-B60 ultracentrifuge at $13,500 \times g$ at 4°C. Fractions of 0.6 ml were collected, assayed for acid-precipitable radioactivity, and analyzed by electron microscopy.

Electron microscopy. (i) **Embedding and thin sectioning.** Ten-milliliter cultures of *E. coli* B^E or K-12-M were allowed to grow in M9a medium at 37°C to a density of 3×10^8 cells/ml. T7 phage was then added at an MOI of 5. At various time intervals, 2-ml samples of the culture were prefixed by the method of Van Driel et al. (37) with 5% acrolein (Fluka) and 4% glutaraldehyde (Polysciences) at 0°C for 10 min. The prefixed cells were sedimented in a centrifuge, and the pellets were washed, agar embedded, osmified, postfixed, dehydrated by ethanol, and embedded in Epon 812 as described by Wunderli et al. (39). After polymerization at 50°C for 24 h and 70°C for 1 week, the embedded material was thin sectioned on an LKB-8800 A Ultratome III. The sections were collected on carbon-coated collodion films on 200-mesh copper grids (Balzers). The sections were stained with uranyl acetate (Fluka; 4%) for 1 min and lead citrate (Merck) for 1 min, according to Reynolds (28). Micrographs were taken in a Phillips EM 300 electron microscope at an accelerating voltage of 80 kV, using Kodak-LR film.

(ii) **Negative staining.** One drop of fractions from sucrose gradients was dialyzed against extraction buffer and then applied to collodion-carbon-coated grids for 1 min. After washing on a drop of distilled water for 10 s, the grid was put on a drop of 1% uranyl acetate, pH 4.6, for 1 min for negative stain. After the excess stain was removed, the grids were dried and examined with a Phillips EM 300 electron microscope.

(iii) **Observation of DNA-protein complexes.** The dialyzed fractions from sucrose gradients were prepared on pentylamine glow discharged carbon films (5). Then 15 μ l of the fractions was spread on the grids and stained with a 2% uranyl acetate solution and shadowed by platinum. The grids were examined with a Phillips EM 300 electron microscope at an accelerating voltage of 80 kV.

RESULTS

Effects of *groM* on the physiology of T7 multiplication. As a first step in determining the cause of the block of phage development in T7-infected *E. coli* K-12-M cells, we followed bacterial growth and lysis by the turbidity of the culture and by PFU. Turbidity stopped increasing after about 5 min p.i. and stayed constant for at least 2 h. Lysis did not occur, and only 0.5 phage/cell was detected after the cells were lysed by egg white lysozyme and chloroform at 20 min p.i.

The fate of host DNA was followed by monitoring the degradation of [³H]thymidine-prelabeled DNA in T7-infected *E. coli* K-12-M cells and compared with that in T7-infected K-12 and

B^E cells (Fig. 1a). To prevent any interference by newly synthesized phage DNA, we chose a T7 mutant phage defective in DNA synthesis (T7 amber mutation in gene 5). The degradation of the host DNA began at about 5 min after the onset of infection, continued for 10 min, and reached a plateau at 60% of the initial ³H labeling. There was no significant difference in this respect between the wild type and the *E. coli* K-12-M strain. In another experiment, the synthesis of phage DNA was measured by incorporation of [³H]thymidine, which was added simultaneously with the phage (Fig. 1b). In K-12 and B^E, the synthesis started about 5 min after infection and leveled off at 15 min p.i., the time at which lysis occurred. We also observed some incorporation of [³H]thymidine in the T7-infected K-12-M cells, but at a greatly reduced rate. The total amount of synthesized DNA reached only about 20% of that found in infected *E. coli* B^E and *E. coli* K-12 cells.

Phage-directed protein synthesis in T7-infected *E. coli* K-12-M and B^E cells was also studied. Figure 2a shows the autoradiographs of [³⁵S]methionine-labeled phage proteins separated by SDS-polyacrylamide gel electrophoresis. At the indicated times, the label was added to T7-infected cultures during 2-min pulses. We observed that phage protein synthesis in K-12-M-infected cells started normally about 2 min p.i. with the early proteins, but after 8 min p.i. the rate of synthesis decreased slightly. We compared the phage-directed protein synthesis of K-12-M cells with that of B^E cells by electrophoresis and obtained similar patterns (Fig. 2b). No protein band was missing in the case of T7-infected K-12-M cells, but the rate of protein synthesis was slightly reduced compared with that in B^E cells. Studies on the incorporation of ¹⁴C-labeled amino acids into acid-precipitable material indicated that the phage proteins synthesized in K-12-M cells reached only about 30% of the amounts in K-12 or B^E cells (data not shown). A transcriptional block in the K-12-M cells is thus not very likely, since phage-directed protein synthesis was only slightly reduced.

We conclude that, after infection of *E. coli* K-12-M by T7, host DNA is degraded, phage DNA is produced, and phage-coded proteins are synthesized. We felt that the observed reduction of synthesis rates was not sufficient to explain the maturation block, and another cause must be responsible for the abortive outcome of the phage infection: only a much more specific action could explain the nearly complete block of phage maturation.

Analysis of viral DNA accumulated in infected K-12-M cells. The T7 progeny DNA was labeled with [³H]thymidine as described in Materials and Methods. At the indicated times, samples of

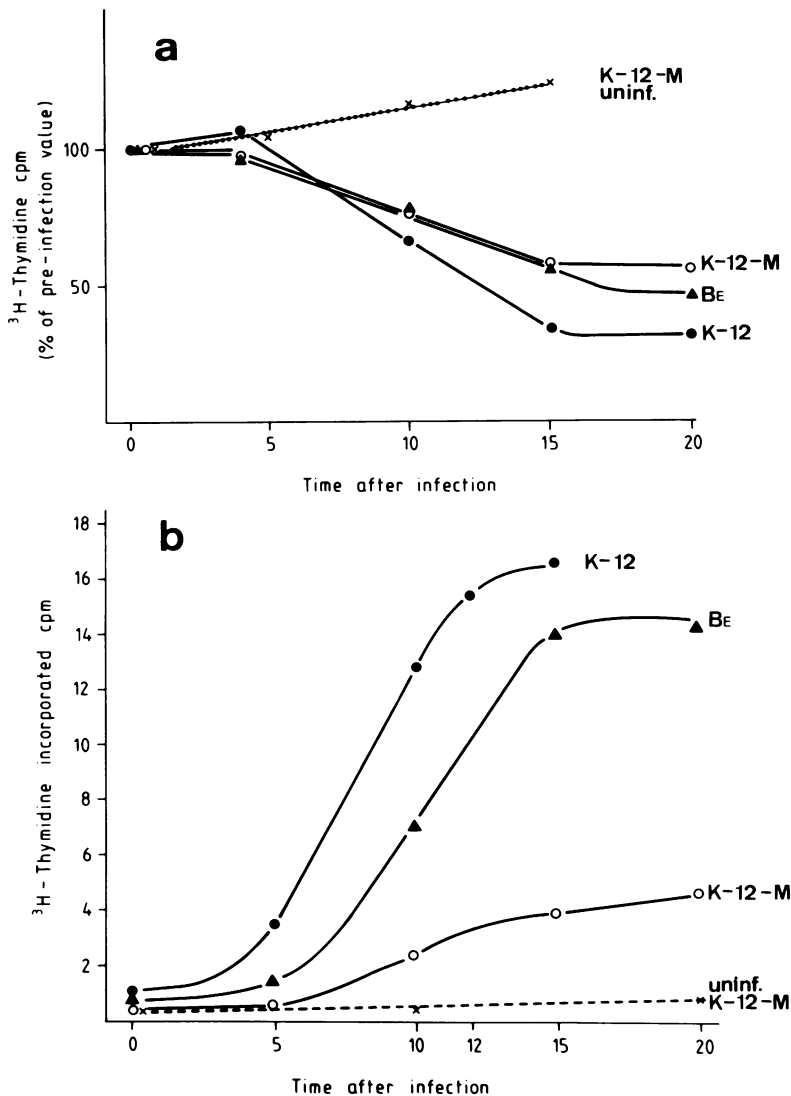


FIG. 1. T7-induced degradation of host DNA and phage-directed DNA synthesis. (a) Host DNA (*E. coli* B^E, K-12, or K-12-M) was prelabeled over 10 generations (37°C) as described in Materials and Methods. Infection (MOI = 5) was carried out with an amber mutant defective for T7 DNA polymerase (gene 5 product). The TCA-precipitable radioactivity was assayed at the indicated times. (b) Exponentially growing cultures of *E. coli* B^E, K-12, and K-12-M at a concentration of 2×10^8 cells/ml were UV irradiated to inhibit synthesis of bacterial DNA. After 2 min of aeration at 37°C, wild-type T7 phage (MOI = 5) and [³H]thymidine (1 μ Ci/ml) were added. After various times, samples were taken and assayed for acid-precipitable radioactivity. Symbols: (○) K-12-M, (▲) B^E, and (●) K-12 cells after T7 *am5* infection; (*) uninfected K-12-M cells.

the infected cultures were washed, lysed, and layered on top of 5 to 20% sucrose gradients. The sedimentation profiles of the phage DNA harvested 10 min after infection of B^E cells by T7 (Fig. 3a) showed that only part of the phage DNA cosedimented with the ¹⁴C-labeled T7 DNA used as a marker. Most of the DNA was observed as faster-sedimenting material (FS fractions), which we interpreted as a concat-

meric form of phage DNA (16). The amount of these concatemers decreased strongly at 14 min p.i. (Fig. 3a). At this time the situation was similar to what we observed in T7-infected K-12 cells (Fig. 3c).

In T7-infected K-12-M cells, the DNA sedimented in a broad peak ranging from unit-size T7 DNA to concatemeric forms when observed at 10 min p.i. (Fig. 3b). Ten minutes later, the

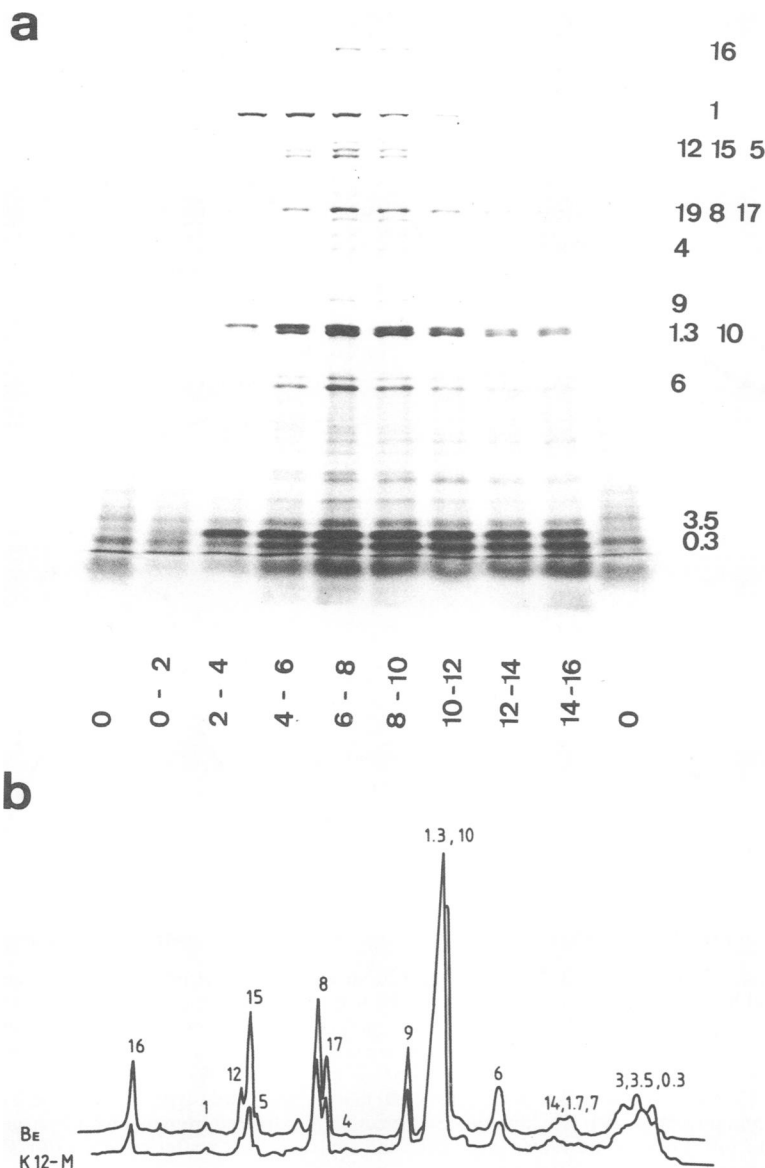


FIG. 2. SDS-polyacrylamide gel electrophoresis. (a) Time course of synthesis of phage-coded proteins after infection of K-12-M. The numbers of the abscissa indicate the duration of the [35 S]methionine pulse, and the numbers of the ordinate refer to corresponding T7 genes (for details, see reference 12). (b) Banding pattern of phage-encoded proteins synthesized between 8 and 10 min p.i. in K-12-M cells (lower line) and in B^E cells (upper line).

sedimentation profile indicated no increase of the unit-sized DNA (Fig. 3b). We conclude that the faster DNA (i.e., the concatemeric forms) was converted into unit-sized DNA in infected B^E and K-12 cells, but not in *E. coli* K-12-M cells. Here, the concatemeric forms were apparently accumulated without further processing. The FS fractions appeared to be heterogeneous, confirming the observation that concatemeric

forms of very different lengths were present (30). This finding was further investigated with the pulse-chase experiments described below. From our data we cannot decide if the observed small amount of unit-sized DNA was derived from concatemers or whether it had never entered such a form. We also observed only DNA concatemers in *E. coli* B^E cells infected by a mutant in gene 10 (defective for the main capsid protein,

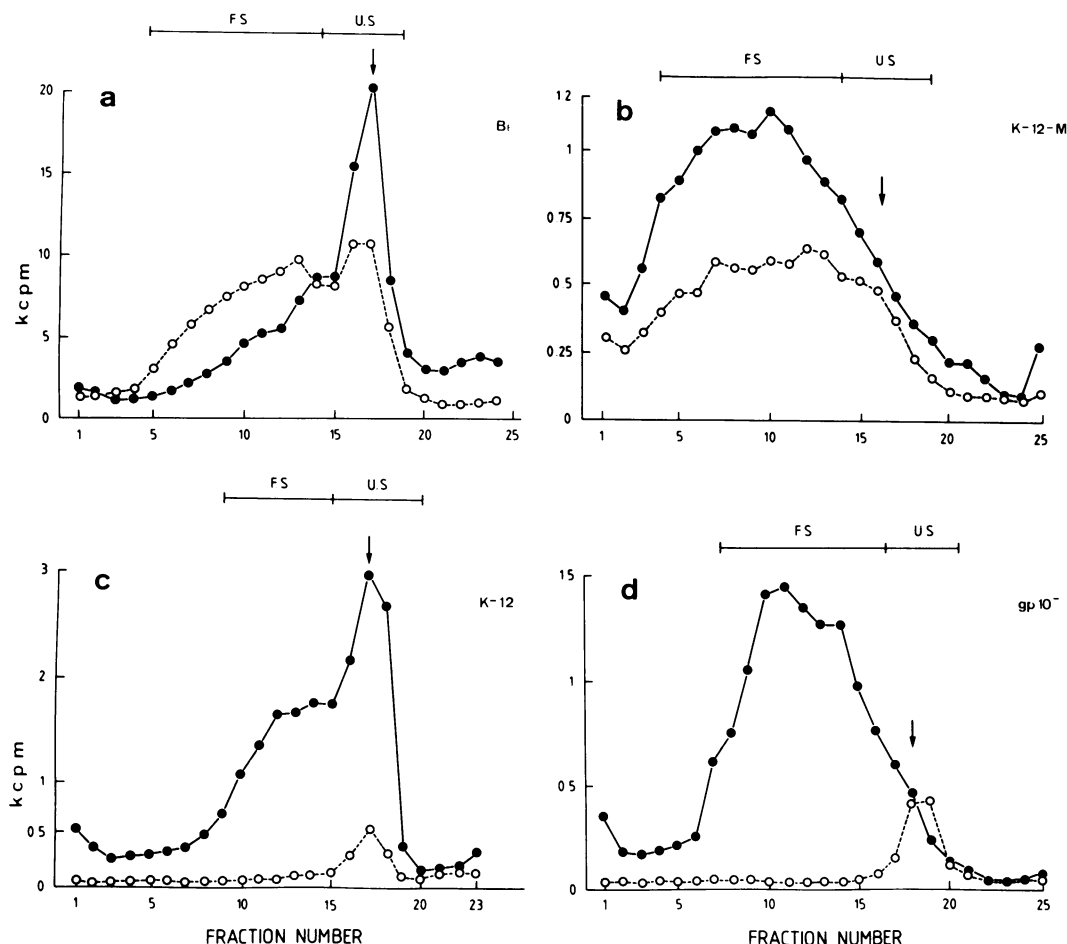


FIG. 3. Analysis of phage-directed DNA on neutral sucrose gradients. ^3H -labeled T7 DNA synthesized in *E. coli* B^E , K-12, and K-12-M cells was analyzed on 5 to 20% neutral sucrose gradients. US (unit size) indicates the fractions with [^{14}C]thymidine-labeled T7 DNA used as a marker. The peak fraction is indicated by an arrow (for actual data, see panels c and d). The DNA that sedimented faster than unit-sized DNA is indicated by FS. (a) T7 wild-type DNA synthesized in B^E cells and analyzed 10 (○) and 14 (●) min p.i. (b) T7 wild-type DNA synthesized in K-12-M cells and analyzed 10 (○) and 20 (●) min p.i. (c) T7 wild-type DNA synthesized in K-12 cells and analyzed 14 min p.i. (●—●). [^{14}C]thymidine-labeled DNA extracted from mature T7 phage was used as a marker (○). (d) T7 *am10* DNA synthesized in B^E cells and analyzed 14 min after infection (●). [^{14}C]thymidine-labeled DNA extracted from T7 $^+$ phage was used as a marker (○).

gp10 [35]) (Fig. 3d). We conclude that the presence of preheads may be correlated with DNA maturation.

To correlate the steps of the DNA maturation pathway with the different forms of DNA, vegetative T7 DNA was studied by pulse-chase experiments as described in Materials and Methods. [^3H]thymidine (0.2 $\mu\text{Ci/ml}$) was added to a T7-infected *E. coli* B^E or K-12-M culture 6 min p.i. at 37°C. Two minutes later the label was chased by adding 2 mg of cold thymidine per ml. At different times after the chase, the viral DNA was extracted and analyzed on neutral 5 to 20%

sucrose gradients (Fig. 4). One minute after the chase (Fig. 4a), the phage DNA in *E. coli* B^E was recovered mainly in the concatemeric form (fraction I); a smaller part comigrated with ^{14}C -labeled T7 marker DNA (fraction II). Four minutes after the chase (Fig. 4a), both forms were present, but the sedimentation profile clearly showed that most of the T7 DNA was now in unit-length size. In the normal host, the concatemeric form was thus nearly completely matured into unit-sized DNA within 3 min. The concatemeric fraction (I) may have been heterogeneous, with two peaks (fractions Ia and Ib) indicated by

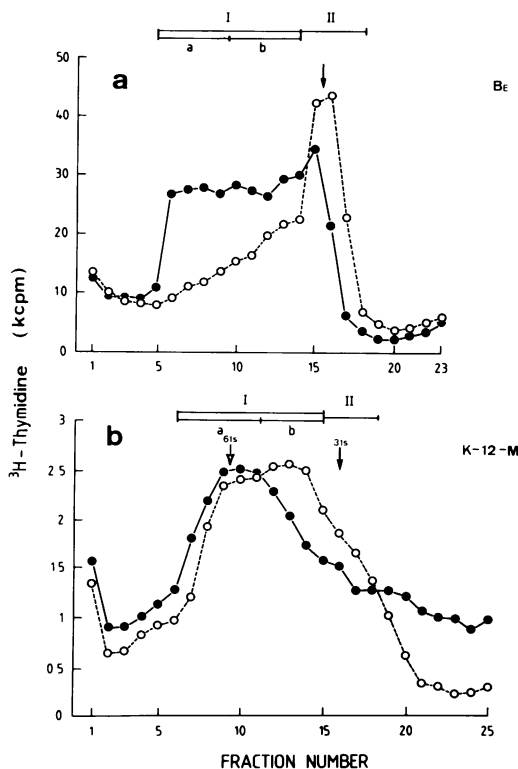


FIG. 4. Maturation of phage DNA studied by pulse chase. ^3H thymidine incorporation into phage DNA was performed as follows. Six minutes after infection, $0.2 \mu\text{Ci}$ of ^3H thymidine per ml was added to the culture and 2 min later chased with 2 mg of nonradioactive thymidine per ml. Then $500\text{-}\mu\text{l}$ samples were taken 9 min and 12 min p.i. and submitted to sucrose gradient centrifugation as in Fig. 3. A T7-infected culture of B^E (a) and an infected culture of K-12-M (b) were analyzed after 1 (●) and 4 (○) min of chase. The ^{14}C thymidine-labeled phage DNA extracted from T4 (open arrow) and T7 (closed arrow) was used as a marker.

the cosedimentation of ^{14}C -labeled T7 and T4 DNA, which we used as markers (Fig. 4b). The sedimentation constant of T7 unit-sized DNA has been determined to be 31S, and that of T4 DNA has been determined to be 61S (4). The sedimentation constants of the material present in the peaks of fractions Ia and Ib of Fig. 4b were therefore 65S and 45S, assuming an ideal linearity of the gradient between fractions 6 and 20. This corresponds approximately to tetrameric and dimeric forms.

In T7-infected K-12-M cells, phage DNA accumulated in concatemeric forms (Fig. 4b). Almost no unit-sized DNA was recovered, even 4 min after the chase. We conclude that phage DNA in T7-infected K-12-M cells remained in concatemeric forms, very similar to the case of

the $gp10^-$ mutant of T7 which is unable to assemble intracellular preheads (Fig. 3d). The concatemeric fractions of T7 DNA synthesized in the K-12-M cells appeared to be heterogeneous, as found with the normal host B^E (Fig. 4a). The profiles indicate a shift from the 65S to the 45S fraction after a 4-min chase (Fig. 4b). These observations strongly suggest that the newly synthesized phage DNA can mature only through tetrameric into dimeric concatemers. To determine whether DNA maturation is blocked at a step of nicking the concatemers at unit-size intervals, we analyzed extracted DNA on alkaline 5 to 20% sucrose gradients. Figure 5a shows the results with T7-infected B^E cells harvested 1

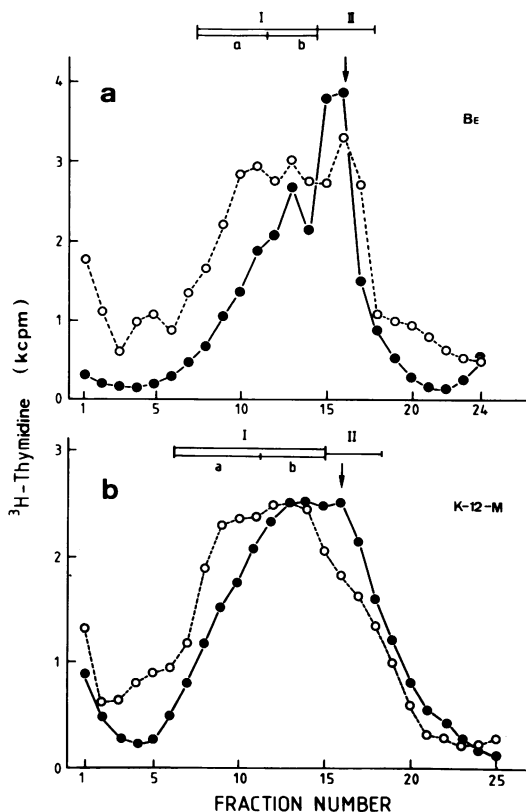


FIG. 5. DNA sedimentation pattern in alkaline sucrose gradients. The pronase-treated samples (see Materials and Methods) were incubated together with ^{14}C thymidine-labeled T7 DNA, used as a marker, in a 0.3 M NaOH and 0.7 M NaCl buffer for 2 h. Then $200 \mu\text{l}$ of each sample was put on top of a 5 to 20% alkaline sucrose gradient. Centrifugation and treatment of the samples were as described in Materials and Methods. T7 DNA was analyzed after 1 min of chase in B^E cells (a) and after 4 min of chase in K-12-M cells (b) (●). The open symbols refer to corresponding profiles obtained under neutral conditions; the arrows indicate fractions containing ^{14}C thymidine-labeled T7 DNA.

min after the chase. The phage-directed DNA was found in concatemeric forms (fraction I) and in a major peak of unit-length DNA (fraction II). Fraction I was composed mainly of fast-sedimenting molecules (fraction Ib), but some very fast sedimenting molecules were also present (fraction Ia). By comparing the two profiles in Fig. 5a, we noted that, after alkaline treatment, part of the radioactivity of fraction I was shifted to fraction II; this demonstrates that some of the concatemeric DNA was present in a nicked state. However, the majority of concatemers had no nicks. The phage DNA from T7-infected K-12-M cells was also analyzed after alkaline treatment (Fig. 5b). One part of the DNA sedimented as concatemers; another part sedimented as unit-sized DNA. Alkaline treatment induced a shift from the concatemers to unit-sized phage DNA. This demonstrates that some of the accumulated concatemers had nicks at distances of about one T7 genome length.

Intracellular morphology of T7-infected *E. coli* K-12-M cells. Thin sections of *E. coli* B^E cells infected by T7 wild type at 10 min p.i. are shown in Fig. 6a. The first newly assembled preheads seemed to be preferentially located at the periphery of a nucleoid-like DNA pool. They became filled with DNA in a very compact form, which then appeared as darkly staining particles ("black particles"). Freshly assembled preheads continued to appear and to mature into black particles (Fig. 6b).

A different situation was shown by T7-infected K-12-M cells (Fig. 7a). As in the above case, the intracellular preheads appeared at the periphery of the area of DNA (nucleoid and phage-DNA pool). No black particles appeared after continued incubation. Apparently no packaging occurred, despite the presence of newly synthesized T7 DNA. We will call the accumulated head-related structures M-preheads (Fig. 7b). These M-preheads contained a small electron-scattering core and were similar to the preheads during early stages of T7 infection (Fig. 6). It has to be emphasized that electron microscopy reveals morphological similarities but is obviously not able to distinguish true preheads—which are maturable—from prehead-like, but abortive, structures. We can also not exclude that these particles originally contained packaged DNA which was released artificially during the processing for embedding procedures. To test if the M-preheads were true precursors, we performed an *in vitro* packaging test, described below.

Isolation and *in vitro* packaging of preheads accumulated in T7-infected K-12-M cells. The arrest of virus maturation in T7-infected K-12-M cells could be the consequences of the following causes: (i) the accumulated preheads are abortive structures, i.e., are unable to become filled

with DNA; (ii) the vegetative phage DNA is incompetent for this process; or (iii) the packaging process itself is affected. To test the first hypothesis, we submitted the M-preheads to *in vitro* packaging according to Miyazaki et al. (26).

To exclude particles other than preheads which occur in minor amounts that are not detectable by electron microscopy, we isolated the M-preheads on a sucrose gradient. The corresponding peak contained preheads of various aspects (Fig. 7c). A small amount (about 1%) of the particles had a roundish outline and thick shells and contained excentrically placed cores (Fig. 7d). These M-preheads I had the same morphology as the preheads accumulated after infection of mutants in gene 5, 8, 15, and 16 of the normal host (29). The M-preheads II represented the bulk of the isolated preheads; they had thin shells, were often collapsed, and also contained a core (Fig. 7e). We were not able to separate M-preheads I and II by sedimentation. In the following experiments, the mixture of these two types of particles had to be used. Preheads II were not produced in the normal host after infection with gene 5 mutants (data not shown).

In Table 1 we give the results of our *in vitro* packaging experiment. Both *gp5*[−] and M-preheads were competent to become packaged. The most delicate step of the procedure was found to be the quantitative lysis of the cell suspension. *In vitro* maturation of the "*gp5*[−] preheads" has already been demonstrated by several authors (17, 25). In our experiments the isolated M-preheads were maturable into infectious phages with an efficiency (resulting PFU/input preheads) of 1%. This *in vitro* maturation efficiency was about the same as with *gp5*[−] preheads. We do not think that the difference of a factor of 2 is significant. The fact that the efficiency of *in vitro* packaging was the same for preheads of these two origins argues strongly against the first hypothesis, that M-preheads are abortive structures.

In addition to the M-preheads I and II, we also discovered some particles which appeared to be partially filled (Fig. 7c). We investigated whether they might represent intermediates of the DNA-packaging process or whether they were breakdown products of already packaged particles.

Isolation of putative DNA-packaging intermediates. To isolate putative DNA-packaging intermediates, we followed the isolation procedure already described (27). T7-infected *E. coli* B^E and K-12-M cells were labeled with ¹⁴C-amino acids and [³H]thymidine at 5 min p.i. At the indicated times the cells were harvested and lysed, and the extracts were analyzed on a

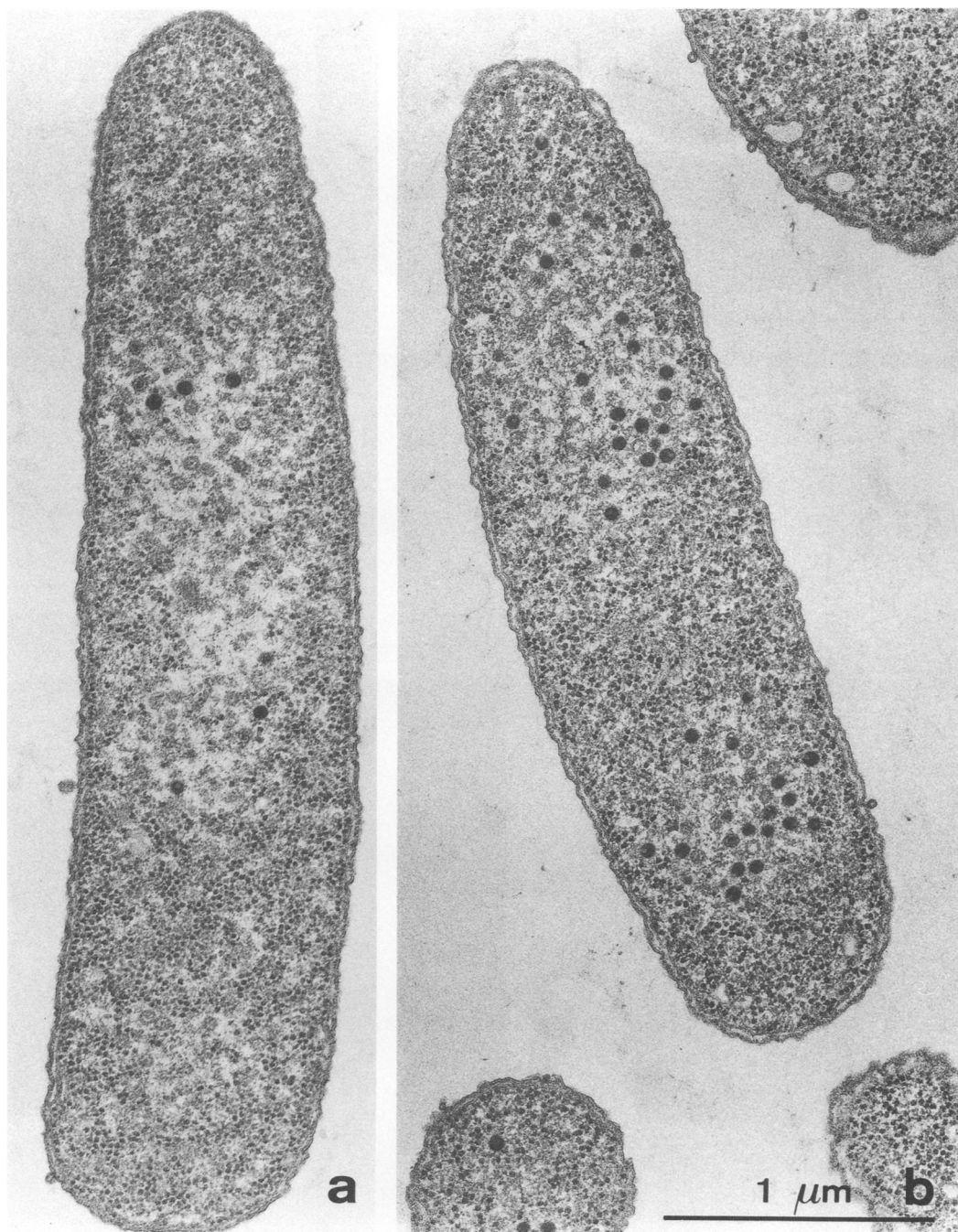


FIG. 6. Thin-sectioned B^E cells after T7 infection. T7-infected *E. coli* B^E cells were processed for thin sectioning as described in Material and Methods. (a) 10 min after infection at 37°C; (b) 14 min after infection at 37°C.

sucrose gradient in heavy water (for details, see Materials and Methods). Figure 8a shows the analysis of radioactivity in the gradient of a T7-infected B^E culture 9 min p.i. The fractions

obtained were analyzed by electron microscopy. Phage particles sedimented at fraction 16; pre-heads I and II sedimented at fraction 35. Fraction 30 contained some putative packaging inter-

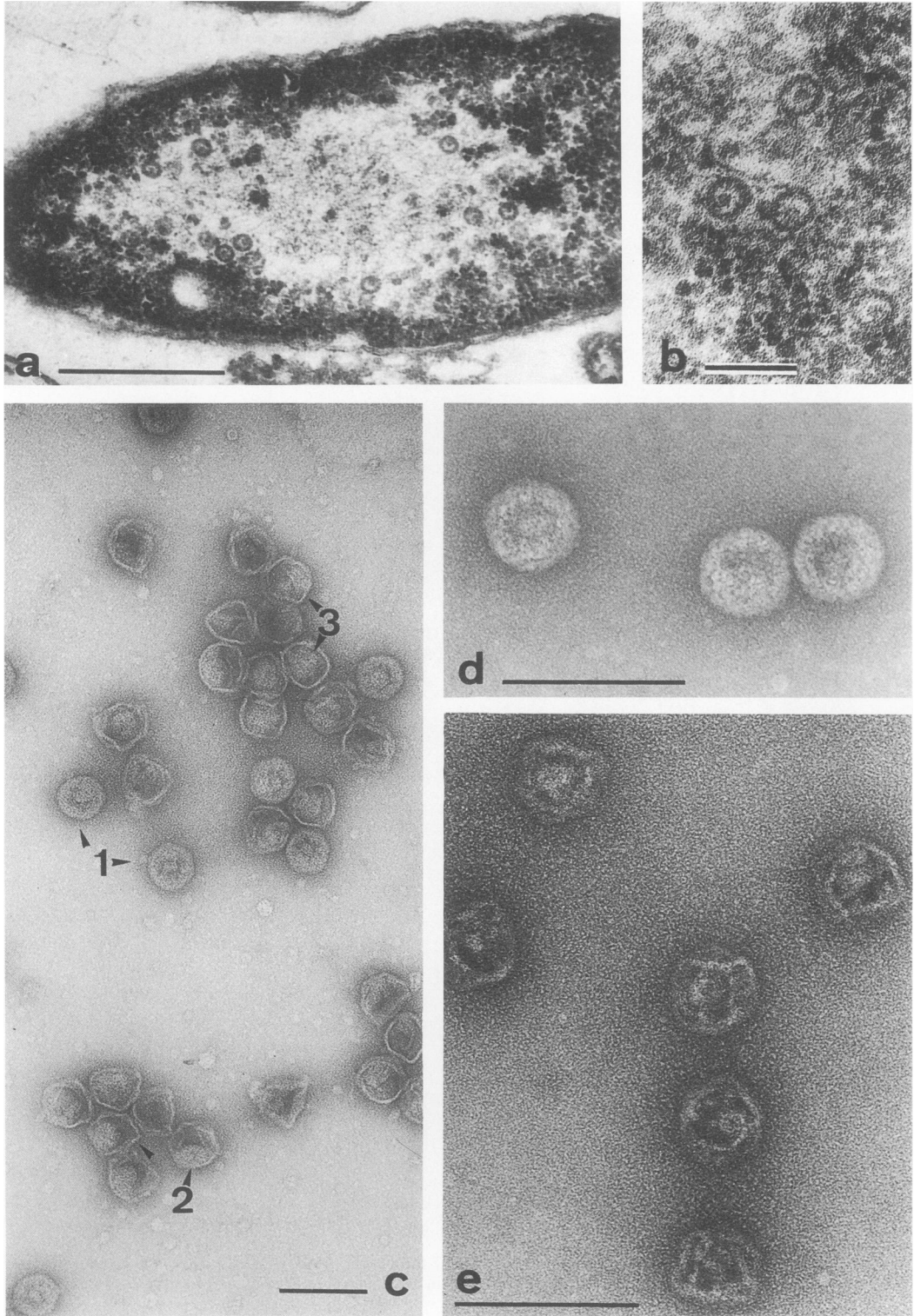


FIG. 7. Electron micrographs of preheads accumulated in infected K-12-M cells. (a) Thin-sectioned K-12-M cells 14 min after T7 infection. The bar represents 500 nm. (b) Thin-sectioned M-preheads. (c) Isolated prehead fraction of a sucrose gradient of a lysate of T7-infected K-12-M cells. We distinguish "immature" prehead I (1), "mature" prehead II (2), and suspected packaging intermediates (3). The samples were dialyzed against the isolation buffer and negatively stained with 1% uranyl acetate. (d) Isolated prehead I from infected K-12-M cells negatively stained with 1% uranyl acetate. (e) Isolated prehead II from infected K-12-M cells negatively stained with 1% uranyl acetate. The bars in panels b through e represent 100 nm.

TABLE 1. In vitro packaging of isolated preheads

Group	In vitro components	PFU $\times 10^6$ /ml
Controls	<i>gp10</i> ⁻ extract	2.5
	<i>gp5</i> ⁻ preheads	0.001
	M preheads	0.05
Experiments	<i>gp10</i> ⁻ extract + <i>gp5</i> ⁻ preheads	170
	<i>gp10</i> ⁻ extract + M preheads	75

mediates but also preheads II. The upper fractions contained DNA aggregates, free DNA, and cell components, but no capsid-like structures.

In the corresponding experiment with a T7-infected K-12-M culture the cells were harvested 14 min after infection and assayed for radioactivity (Fig. 8b). It is striking that almost no labeled material was found in fraction 16, confirming that very few phage particles were produced. By electron microscopy of material from fractions 31 to 34, we detected some particles with an apparently incomplete DNA content (Fig. 9). These particles were always accompanied by random coils of DNA. For the sake of simplicity, we will call these particles packaging intermediates, although we can obviously not rule out that the observed particles had simply lost some of their DNA during isolation or specimen preparation for electron microscopy.

A common feature of all of these particles was that they were apparently attached to DNA in some way. We thus prepared the fractions of interest by the method of Dubochet et al. (5), in current use for observing DNA and DNA-protein complexes by electron microscopy, as described in Materials and Methods (Table 2).

In fractions 31 and 34 we observed a large amount of capsid-DNA complexes, but not in the upper fractions (Table 2). Stretches of DNA were found to emerge from single capsids; in other cases up to four capsids were aligned with DNA to form a linear stretch (Fig. 10c). We measured the total length of DNA extruding from the associated particles and found that it was longer than T7 unit length (15.5 to 36.2 μ m). The stretches of DNA between associated particles did not belong to distinct length classes, as judged from the 12 molecules measured on electron micrographs (data not shown). In most cases observed, two DNA filaments protruded from one particle (Fig. 10a,b). This can be interpreted as a single filament entering and leaving a particle and forming a loop inside or as ends of two individual DNA molecules entering the same particle. To distinguish between these two possibilities, we analyzed the DNA length of the complexes on sedimentation gradients after di-

gesting the capsids by pronase as described below.

Analysis of the DNA of possible packaging intermediates. The fractions of interest of B^E and K-12-M extracts (Fig. 8) were treated with pronase and analyzed on 5 to 20% neutral and alkaline sucrose gradients. Figure 11 shows results obtained for fractions 30, 31, and 34. In fraction 30 of the B^E extract, we observed, besides T7 unit-length DNA, some DNA in its concatemeric form (Fig. 11a). Practically no unit length DNA was observed in T7-infected K-12-M cells. The DNA of fractions 31 and 34 sedimented very fast and was therefore in its concatemeric form (Fig. 11b, c). Alkaline treatment of these concatemers showed that they all had nicks at a distance corresponding to about one T7 genome length (Fig. 11d). With regard to the other fractions of the K-12-M extract, we observed mainly unnicked concatemers in fraction 45 and some T7 unit-length DNA, but mainly pieces of smaller sizes in fraction 50 (data not shown).

DISCUSSION

Multiplication of bacteriophages depends on many host functions. A mutation of a gene coding for one of these functions may prevent the production of phage progeny, but not, at least under laboratory conditions, measurably affect growth of the uninfected bacterium. It is therefore conceivable that such a mutant is only detectable because of an incompatibility regarding virus host interactions. Many such host mutants affecting the growth of several coliphages have been characterized: mutations in the *groE* gene of *E. coli* B affect the head assembly of T4 and lambda (9, 15); those in *groN* prevent the correct interaction of the lambda gene *N* product with the host RNA-polymerase and block the transcription of the early genes (8). In the case of phage T7, the bacterial thioredoxin has been found to be an obligatory component of the T7 DNA polymerase (24); in another mutant, *E. coli* Y49, the T7 head assembly seems to be abnormal (40). Besides host-dependent DNA restriction, various functions interfere with early or late events of phage multiplication. Accordingly, they might affect transcription, translation, or replication of phage DNA, or they might block DNA maturation, head assembly, or DNA packaging.

Our attempt was to elucidate the mechanisms of the T7 abortion factor, *groM*. In a first step, we investigated the early events of T7 phage multiplication. Host DNA degradation as well as phage-directed DNA and protein synthesis indicated that none of these processes was qualitatively changed. In comparison with the normal T7-infected K-12 strain, the rate of phage DNA

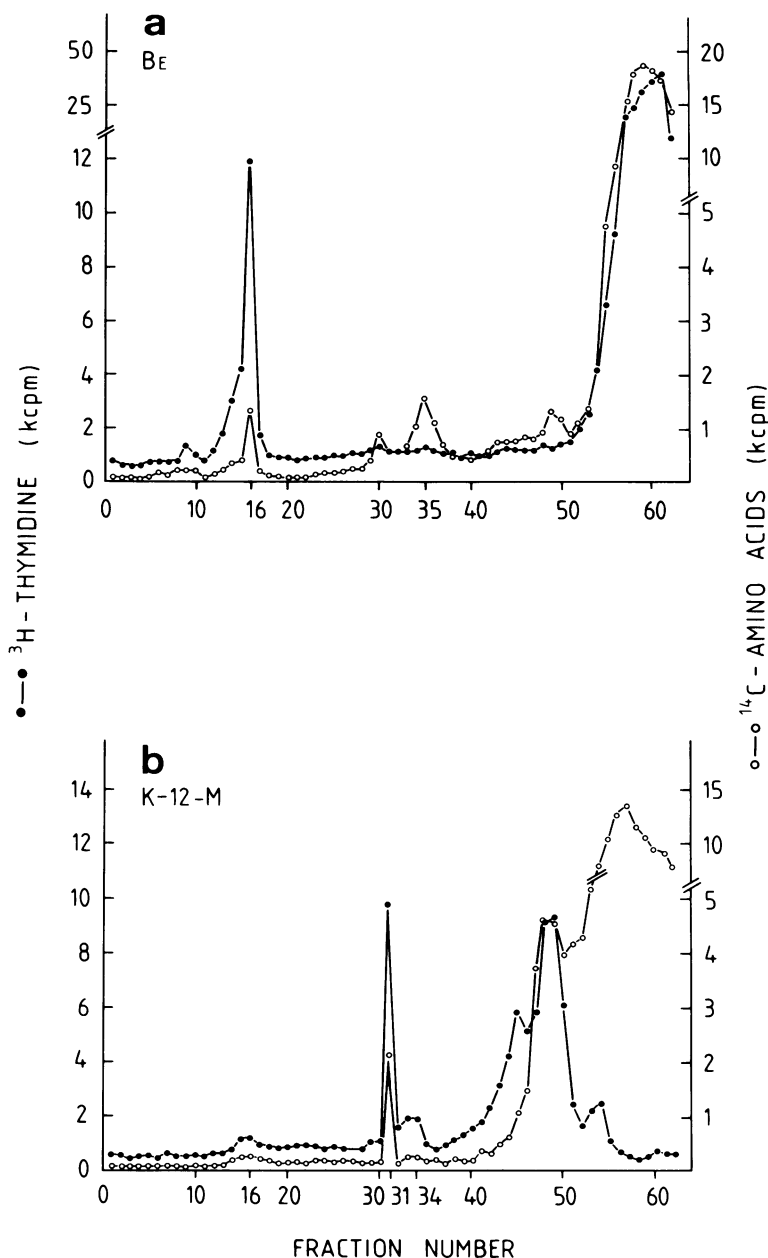


FIG. 8. Fractionation of extracts from T7-infected B^E (a) and K-12-M (b) cells after centrifugation through discontinuous sucrose gradients in heavy water. The cultures (4×10^8 cells/ml) were infected at an MOI of five phage per cell and 4 min later doubly labeled with ^{14}C -amino acids (\circ) and ^3H thymidine (\bullet). B^E cells were harvested at 9 min p.i. (a), and K-12-M cells were harvested at 14 min p.i. (b). The extracts were analyzed on discontinuous sucrose gradients in heavy water as described in Materials and Methods (27), and the resulting 0.6-ml fractions were assayed for radioactivity. Centrifugation was from right to left.

and protein synthesis was reduced to 20 to 30% in T7-infected K-12-M cells. We do not think that this reduction can explain the blocked multiplication, since only 0.5 phage was produced per cell.

We then analyzed the maturation of phage-directed DNA. The intracellular maturation of T7 DNA apparently starts with the replication of T7 unit-sized molecules, as proposed by Watson (38). These molecules are then connected to

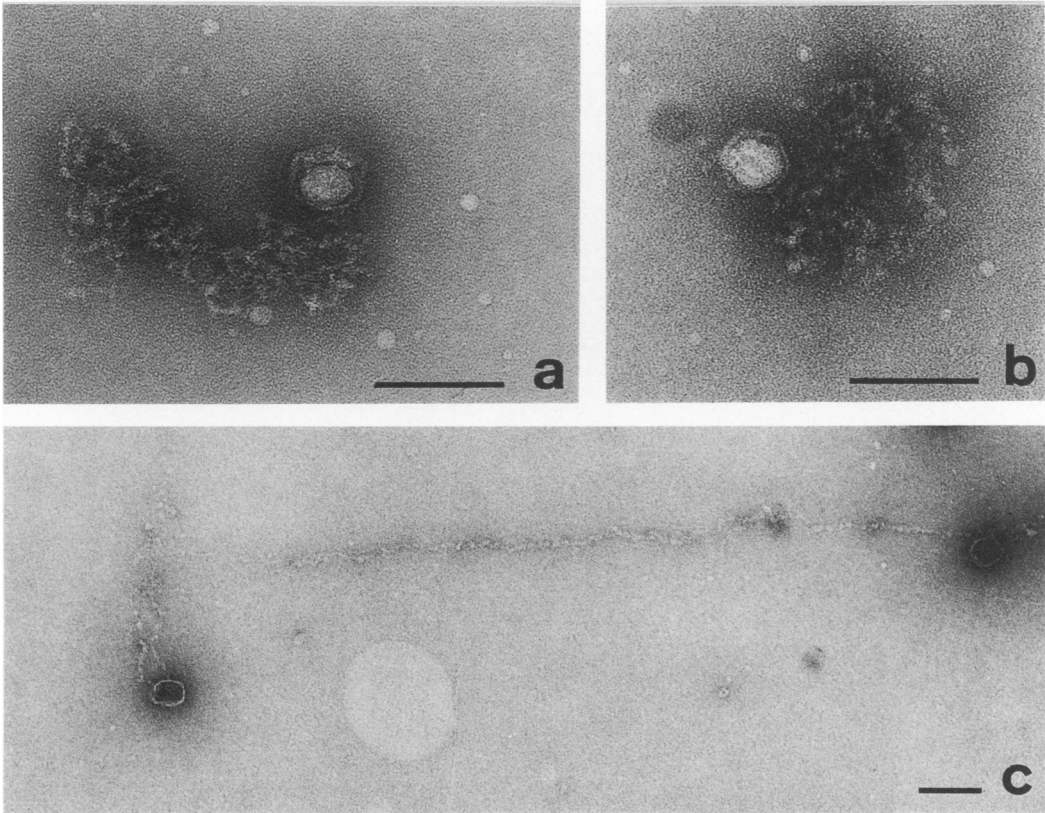


FIG. 9. T7 packaging intermediates isolated from K-12-M cells. The fractions obtained after centrifugation on a discontinuous sucrose gradient (Fig. 8) were dialyzed against 20 mM Tris-hydrochloride, pH 7.9, containing 200 mM NaCl and 1 mM EDTA, fixed by 2% glutaraldehyde for 10 min, and negatively stained with 1% uranyl acetate. Particles from fractions 31 (a and b) and 34 (c) in which the T7 DNA sedimented in its concatemeric form (Fig. 11). The bars represent 100 nm.

form concatemers of various lengths (30). Shortly before or during the packaging process, the concatemers are cut again to unit length by the products of the T7 genes 3, 6, and 19 (14, 33).

The sucrose gradient sedimentation profiles of extracts of T7-infected K-12-M cells showed that the newly synthesized phage DNA accumulated in a fast-sedimenting form (Fig. 3b and 4b). The heterogeneity of the profile of this fast-

sedimenting DNA showed that the concatemers had various lengths. Alkaline treatment of the DNA (Fig. 5) showed that nicking of the concatemers was not prevented, as the DNA was in part nicked at intervals of about unit-size length.

Infection of the normal host by a T7 mutant defective for head assembly (gene 10) led also to an accumulation of concatemeric forms (Fig. 3d). The maturation of T7 DNA from its concat-

TABLE 2. Content of fractions treated as described in the legend of Fig. 9 and 10 and analyzed by electron microscopy

Infected host	Fraction	Observed structures
B ^E	16	Phages
	30	DNA capsid complexes, prehead II, free DNA
	35	Preheads I and II
	50	Ribosomes and protein aggregates, no capsids
K-12-M	16	Few phages
	31, 34	DNA capsid complexes, free DNA of distinct length classes
	45	Ribosomes, DNA of variable length, no capsids
	50	Vesicles, protein aggregates, few capsids
	54	DNA pieces shorter than T7 unit length, no capsids

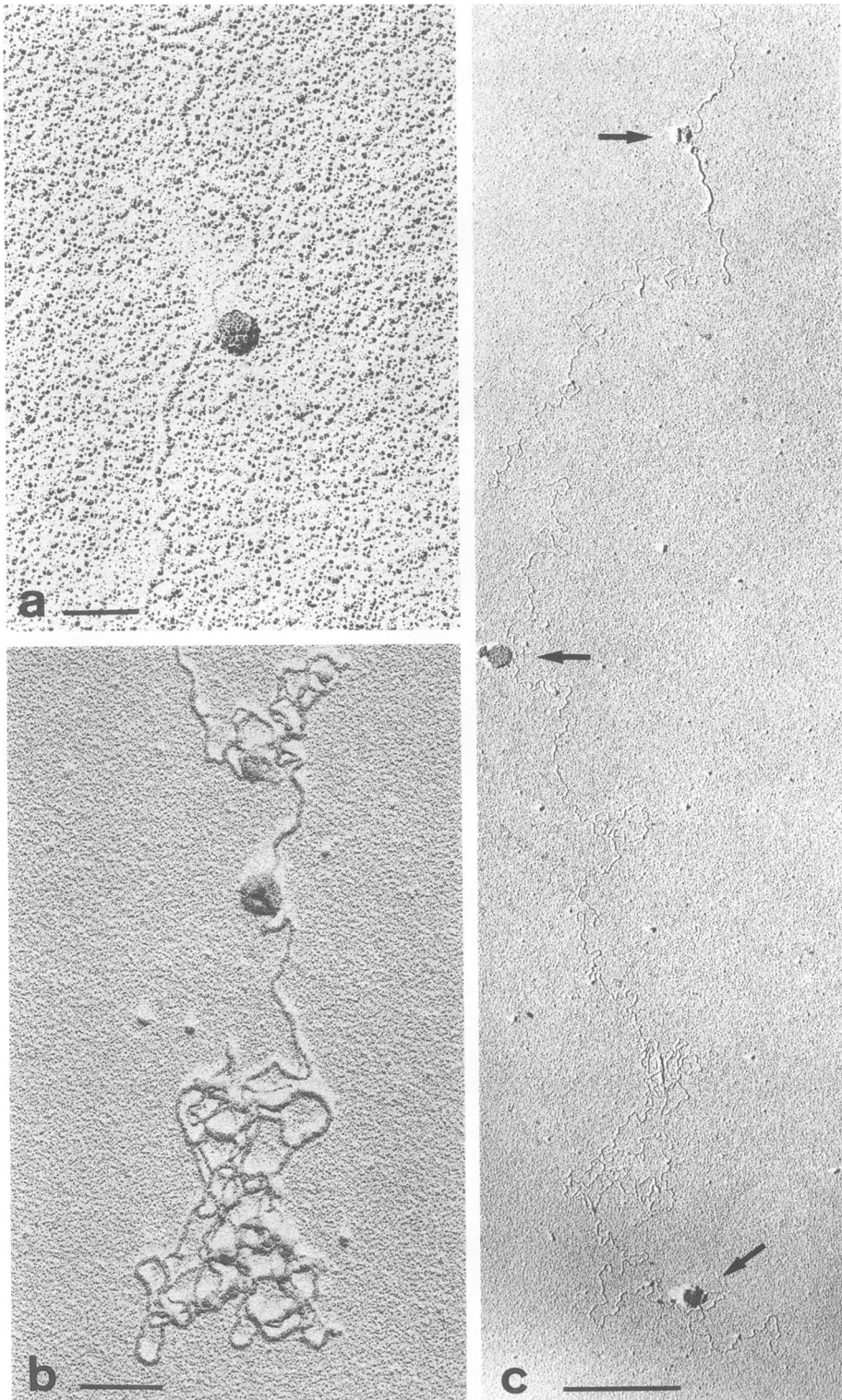


FIG. 10. T7 packaging intermediate isolated from K-12-M and B^E cells. The samples were treated as described in the legend to Fig. 9 and then further processed according to the method of Dubochet et al. (5). DNA-capsid complex from fractions 31 (a) and 34 (b) of K-12-M and from fraction 35 (c) of B^E cells. The bars represent 100 nm (a and b) and 500 nm (c). The capsid diameter is 63 nm for (a) and 61 nm for (b). The lengths of the DNA molecules between the capsids indicated by the arrows in (c) are 5.6 μ m for the upper part and 8.5 μ m for the lower.

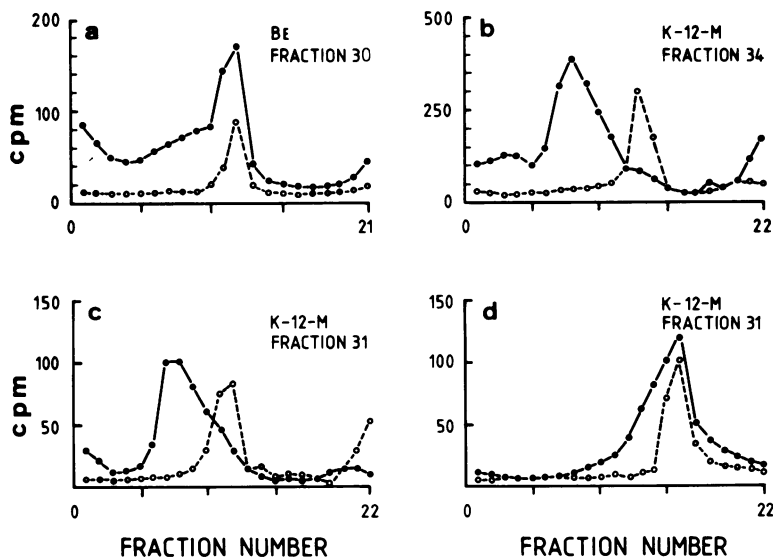


FIG. 11. DNA sedimentation patterns of the fractions containing packaging intermediates in neutral (a to c) and alkaline (d) sucrose gradients. Equal portions of the pooled fractions shown in Fig. 8 were pronase treated and layered on 5 to 20% linear sucrose gradients. T7 DNA B^E extracts (9 min p.i.) in fraction 35 (a); T7 DNA of K-12-M extracts (14 min p.i.) in fractions 34 (b) and 31 (c, d). [^{14}C]thymidine-labeled T7 DNA was used as a marker (\circ).

emic form into its unit length obviously needs the presence of preheads. The state of the phage DNA accumulated in the K-12-M cells points, therefore, to a possible block in the prehead assembly or a block in the DNA-packaging process. In thin sections of infected K-12-M cells, we detected preheads. They appeared at the periphery of a nucleoid-like structure, which presumably contained vegetative T7 DNA (since host DNA is already degraded at that time after infection). Black particles were not found (Fig. 7). This does not exclude the hypothesis that the M-preheads might have contained DNA and were artifactually emptied by the fixation procedure. Such an induced loss of already packaged DNA has been observed for the T4 *gp49*⁻ particles (10, 20, 22, 23) and for some mutants of phage P22 (21).

Consequently, two possibilities still remain for explaining more precisely the T7 maturation block: either DNA or the preheads are incompetent for packaging, or the packaging process itself is blocked. To decide between these possibilities, we compared normal and M-preheads by *in vitro* packaging experiments. In both cases, the same order of magnitude of the accumulated particles was maturable (Table 1). Thus, the preheads which accumulated in K-12-M and those of the usual host were functionally indistinguishable. We conclude that *groM* does not affect assembly of preheads but interferes with their DNA packaging. The isolation of phage-

related particles from the T7-infected K-12-M cells revealed at least three morphologically distinguishable particles (Fig. 7c). The prevalent type of particles were M-preheads II, which contained a core and a thin shell (Fig. 7e). According to the usual nomenclature, these particles represent mature preheads (7). Besides these, we found M-preheads I, which also had a core but a much thicker shell (Fig. 7d). The M-preheads I were morphologically identical to those preheads which accumulate during DNA replication-defective T7 infections (29). Maturation of preheads I to preheads II might be coupled to an interaction with DNA (7). This is further corroborated by the observation that no preheads of type II were found in the lysate of a T7 mutant defective in DNA synthesis (gene 5). The appearance of prehead II might thus be induced by phage DNA.

The following observations support the hypothesis that K-12-M preheads undergo a first-step interaction with phage DNA. Among the phage-related particles isolated from the K-12-M cells, we observed some which appeared to be half filled with DNA (Fig. 7c). From work with T4 phage, we have become aware of experimental difficulties in demonstrating true packaging intermediates. *In vivo*-formed DNA-capsid (prehead II) complexes of T7 were isolated by Serwer and Watson (31, 32). It could not be proven whether these complexes represented true packaging intermediates or whether they

were breakdown products of fragile particles which contained a complete complement of DNA. In our case of K-12-M, it is more relevant to ask whether packaging was initiated at all. Our analysis by means of length measurements and determination of sedimentation behavior of DNA attached to preheads showed DNA exclusively in concatemeric states (Fig. 11). This suggests that these structures are true packaging intermediates. From these results, we tentatively conclude that binding of preheads to phage DNA in its concatemeric form is the first step of the packaging process (and not the cutting into unit length). Similar structures have been described in other systems, for example, for phage λ (41) and for adenovirus type 5 (27). A possible pathway proposed by Serwer and Watson (32) is in accordance with the structures we observed.

The rather small amount of such DNA-capsid complexes in extracts of the normal T7 infection (Fig. 8a) suggests that either the time needed for packaging and cutting of the packaged DNA is very short or that such intermediate structures are not stable under the extraction conditions we used. According to this, we propose that the block of T7 maturation in the K-12-M cells is a late step in the DNA packaging reaction, i.e., the completion or the stabilization of the package of DNA or the final cutting of the DNA (terminase). Three observations favor this hypothesis: the presence of DNA-capsid complexes found by electron microscopy, the maturation of preheads I into preheads II, and the accumulation of concatemeric T7 DNA.

If the terminal cutting of the DNA is blocked (terminase), our results could be explained as follows. The fragility of the packaging intermediate allows us to observe mainly breakdown products. Second, if the DNA-packaging process itself is slowed down, only a few preheads become incompletely filled with DNA and will accumulate. Several explanations can be proposed for such a specific block of the DNA-packaging process. A host-coded enzyme necessary for the DNA-packaging reaction might be defective or missing. Another possibility is a lowered intracellular ATP level at this stage, since it has been shown that ATP is necessary for the DNA-packaging process (17). Furthermore, abnormal internal conditions in the host might inhibit a stable condensation of DNA. It is also known that T7 in vitro packaging systems show an absolute requirement for Mg^{2+} ions (17). Changed ionic conditions could have an effect on the condensation of phage DNA, e.g., by affecting its folding. As a consequence, the DNA, although in functional form, could not enter the prehead correctly. In a forthcoming paper (Kuhn et al., in preparation) we will show a lower level of intracellular K, Mg, and poly-

amines in T7-infected K-12-M compared with K-12 and B^E. By compensating for the leakage by Mg^{2+} ions, we were able to show that the multiplication block can be overcome. This fact strongly suggests that the decreased internal level of K and Mg concentration is the common cause of the morphogenetic block, the reduced syntheses, and lysis inhibition. Cumulative effects of several separately contributing host factors are therefore at least no longer to be considered as a first priority.

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LITERATURE CITED

- Adams, M. A. 1959. The bacteriophages. Wiley Interscience, New York.
- Arber, W., and D. Dussoix. 1962. Host specificity of DNA produced by *Escherichia coli*. I. Host controlled modification of bacteriophage λ . J. Mol. Biol. 5:18-36.
- Center, M. S. 1975. Role of gene 2 in bacteriophage T7 DNA synthesis. J. Virol. 16:94-100.
- Clark, R. W., G. H. Wever, and J. S. Wiberg. 1980. High-molecular-weight DNA and the sedimentation coefficient: a new perspective based on T7 bacteriophage and two novel forms of T4 bacteriophage. J. Virol. 33:438-448.
- Dubochet, J., M. Ducommun, M. Zollinger, and E. Kellenberger. 1971. A new preparation method for dark field electron microscopy of biomacromolecules. J. Ultrastruct. Res. 35:147-167.
- Duckworth, D. H., J. Glenn, and D. J. McCorquodale. 1981. Inhibition of bacteriophage replication by extra-chromosomal genetic elements. Microbiol. Rev. 45:52-71.
- Earnshaw, W. C., and S. R. Casjens. 1980. DNA packaging by the double-stranded bacteriophages. Cell 21:319-331.
- Georgopoulos, C. P. 1971. Bacterial mutants in which the gene N function of bacteriophage lambda is blocked have an altered RNA polymerase. Proc. Natl. Acad. Sci. U.S.A. 68:2977-2981.
- Georgopoulos, C. P., R. W. Hendrix, S. R. Casjens, and A. D. Kaiser. 1973. Host participation in bacteriophage lambda head assembly. J. Mol. Biol. 76:45-60.
- Hamilton, D. L., and R. B. Luftig. 1976. Bacteriophage T4 head morphogenesis. VII. Terminal stages of head maturation. J. Virol. 17:550-567.
- Hausmann, R. 1973. Host-dependent gene expression of bacteriophages T3 and T7, p. 263-276. In B. A. Hamkalo and J. Papaconstatinou, Molecular cytogenetics. Plenum Publishing Corp., New York.
- Hausmann, R. 1976. Bacteriophage T7 genetics. Curr. Top. Microbiol. Immunol. 75:77-110.
- Hausmann, R., and B. Gomez. 1967. Amber mutants of bacteriophage T3 and T7 defective in phage-directed deoxyribonucleic acid synthesis. J. Virol. 1:779-792.
- Hausmann, R., and K. LaRue. 1969. Variations in sedimentation patterns among deoxyribonucleic acids synthesized after infection of *Escherichia coli* by different amber mutants of bacteriophage T7. J. Virol. 3:278-281.
- Hendrix, R. W., and L. Tsui. 1978. Role of the host in virus assembly: cloning of the *Escherichia coli* *groE* gene and identification of its protein product. Proc. Natl. Acad. Sci. U.S.A. 75:136-139.

16. Kelly, T. J., Jr., and C. A. Thomas, Jr. 1969. An intermediate in the replication of bacteriophage T7 DNA molecules. *J. Mol. Biol.* 44:459-475.
17. Kerr, C., and P. D. Sadowski. 1974. Packaging and maturation of DNA of bacteriophage T7 *in vitro*. *Proc. Natl. Acad. Sci. U.S.A.* 71:3545-3549.
18. Korsten, K. H., C. Tomkiewicz, and R. Hausmann. 1979. The strategy of infection as a criterion for phylogenetic relationship of non-coli phages morphologically similar to phage T7. *J. Gen. Virol.* 43:57-73.
19. Krüger, D. H., and C. Schröder. 1981. Bacteriophage T3 and bacteriophage T7 virus-host cell interactions. *Microbiol. Rev.* 45:9-51.
20. Lämmli, U. K., N. Teaff, and J. D'Ambrosia. 1974. Maturation of the head of bacteriophage T4. III. DNA packaging into preformed heads. *J. Mol. Biol.* 88:749-765.
21. Lenk, E., S. Casjens, J. Weeks, and J. King. 1975. Intracellular visualization of precursor capsids in phage P22 mutant infected cells. *Virology* 68:182-199.
22. Luftig, R. B., and C. Ganz. 1972. Bacteriophage T4 head morphogenesis II. Studies on the maturation of gene 49-defective head intermediates. *J. Virol.* 9:377-389.
23. Luftig, R. B., W. B. Wood, and R. Okinaka. 1971. Bacteriophage T4 head morphogenesis. On the nature of gene 49-defective heads and their role as intermediates. *J. Mol. Biol.* 57:555-573.
24. Mark, D. F., and C. C. Richardson. 1976. *Escherichia coli* thioredoxin: a subunit of bacteriophage T7 DNA polymerase. *Proc. Natl. Acad. Sci. U.S.A.* 73:780-784.
25. Masker, W. E., N. B. Kuemmerle, and D. P. Allison. 1978. *In vitro* packaging of bacteriophage T7 DNA synthesized *in vitro*. *J. Virol.* 27:149-163.
26. Miyazaki, J. I., H. Fujisawa, and T. Minagawa. 1979. Biological activity of purified bacteriophage T3 prohead and prohead-like structures as precursors for *in vitro* head assembly. *Virology* 91:283-290.
27. Moncany, M. L. J., B. Revet, and M. Girard. 1980. Characterization of a new adenovirus type 5 assembly intermediate. *J. Gen. Virol.* 50:33-47.
28. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell. Biol.* 17:208-225.
29. Roeder, S., and P. D. Sadowski. 1977. Bacteriophage T7 morphogenesis. Phage-related particles in cells infected with wild-type and mutant T7 phage. *Virology* 76:263-285.
30. Schlegel, R. A., and C. A. Thomas, Jr. 1972. Some special features of intracellular bacteriophage T7 concatemers. *J. Mol. Biol.* 68:319-345.
31. Serwer, P. 1974. Complexes between bacteriophage T7 capsids and T7 DNA. *Virology* 59.
32. Serwer, P., and R. H. Watson. 1981. Capsid-DNA complexes in the DNA packaging pathway of bacteriophage T7: characterization of the capsids bound to monomeric and concatemeric DNA. *Virology* 108:164-176.
33. Strätling, W., E. Krause, and R. Knippers. 1973. Fast sedimenting deoxyribonucleic acid in bacteriophage T7-infected cells. *Virology* 51:109-119.
34. Studier, F. W. 1973. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. *J. Mol. Biol.* 79:237-248.
35. Studier, F. W. 1976. Bacteriophage T7. *Science* 176:367-376.
36. Studier, F. W. 1979. Relationships among different strains of T7 and among T7-related bacteriophages. *Virology* 95:70-84.
37. Van Driel, R., F. Traub, and M. K. Showe. 1980. Probable localization of the bacteriophage T4 prehead proteinase zymogen in the center of the prehead core. *J. Virol.* 36:220-223.
38. Watson, J. D. 1972. Origin of concatemeric DNA. *Nature (London) New Biol.* 239:197-201.
39. Wunderli, H., J. van den Broek, and E. Kellenberger. 1977. Studies related to the head maturation pathway of bacteriophages T4 and T2. I. Morphology and kinetics of intracellular particles produced by mutants in the maturation genes. *J. Supramol. Struct.* 7:135-161.
40. Yamada, Y., J. Silnutzer, and D. Nakada. 1979. Accumulation of bacteriophage T7 head-related particles in an *Escherichia coli* mutant. *J. Virol.* 31:209-219.
41. Yamagishi, H., and M. Okamoto. 1978. Visualization of the intracellular development of bacteriophage λ , with special reference to DNA packaging. *Proc. Natl. Acad. Sci. U.S.A.* 75:3206-3210.