Bacteriophage T4 Head Morphogenesis

II. Studies on the Maturation of Gene 49-Defective Head Intermediates

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An investigation into the metabolic requirements for maturation of gene 49-defective heads indicated that adenosine triphosphate energy and continued deoxyribonucleic acid (DNA) but not ribonucleic acid synthesis were needed. The fate of DNA present at restrictive temperatures (41.5 C) in *tsC9* (gene 49)-infected cells was also examined. After lysis of infected cells, the 12 to 32% deoxyribonuclease-resistant DNA associated with isolated gene 49-defective heads was found to be attached to a deoxyribonuclease-sensitive complex associated with the debris. Pulse-chase experiments where ³H-thymidine was used to label the DNA at 41.5 C suggested that more DNA from this pool was present in phage recovered after rescue of the gene 49 function than could be accounted for by the deoxyribonuclease-resistant portion. Further, when these experiments were repeated with an additional density shift (¹⁵N¹³C-glucose to ¹⁴N¹²C-glucose), the DNA extracted from phage rescued at 10 min after the temperature shift-down was found to be 90% conserved. These results suggest a model whereby DNA packaging into capsid precursors is separated from DNA replication and the energy from DNA synthesis provides the driving force for packaging. Pulse-chase, temperature-shift experiments with E920g (gene 66) or E920g;*tsC9* mutant-infected cells showed that gene (49, 66)-defective heads, which were isolated as small, isometric-shaped unfilled heads, were a precursor to "petite" phage. This suggests that the maturation process is independent of the size and shape of the head membrane. Similar experiments with the double mutant *tsC9;amN120* indicate that gene 49-defective heads can also be filled in the absence of tails.

Although many steps in the T4 morphogenetic pathway have been elucidated (5, 17, 18, 32, 33), the process by which phage deoxyribonucleic acid (DNA) is packaged into the capsid remains a mystery. Kellenberger, Ryter, and Séchaud (16) proposed a model whereby a condensed piece of phage-sized DNA served as a nucleation center for capsid assembly. The major evidence for this model, namely the appearance of capsidless DNA condensates in sections of infected cells, has now been withdrawn (15). Improved sectioning techniques showed that these structures were actually bounded by a head membrane (15).

Recently, head structures which contain less than a full complement of phage DNA have been isolated from amber mutants in genes 46, 47, and 49 (22, 29; Fed. Proc. 28:861, 1969). This suggests an alternative model for maturation in which the capsid can be formed without the

presence of a precleaved full complement of DNA. In this model, some of the phage DNA would be introduced into the head after the capsid has been formed. This model was supported by the following experiments with *tsC9*, a mutant in gene 49 (23): (i) mutant-infected cells grown under nonpermissive conditions accumulated head structures which did not appear filled in sections examined by electron microscopy; (ii) on the basis of "pulse-chase" isotopic labeling, temperature shift-down experiments, the heads appeared to represent an intermediate stage in capsid formation; and (iii) these gene 49-defective structures were not completely filled with DNA since they incorporated some DNA that had been labeled with ³H-thymidine after a temperature shift-down of the infected culture. The exact amount of DNA present inside these gene 49-defective heads *in vivo* has not been resolved. However, since the isolated 300S structures have 12 to 32% of a phage DNA equivalent associated with them, we can say that *in vivo* they contain at least 12% but not 100%

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DNA. An additional point relevant to the proposed model is that DNA isolated from gene 49-infected cell cultures under restrictive conditions exists in a large $>1,000S$ form (Frankel et al., *in press*). The defect in gene 49 prevents a normal conversion of this rapidly sedimenting DNA in vivo, to phage-size pieces of DNA (10). In vitro studies (Frankel et al., *in press*) suggest that this large $>1,000S$ DNA may be a normal precursor to phage, rather than an aberrant by-product.

The dual existence inside gene 49-defective cells of heads that are not completely filled and a $>1,000S$ form of DNA makes the condensate model unlikely, since it contradicts the requirement for a precleaved piece of phage-size DNA prior to capsid formation. However, the question remains open as to how DNA is packaged in vivo from the $>1,000S$ form into gene 49-defective heads to yield a completely filled head. The purpose of this communication is to examine this process in greater detail by: (i) an examination of the requirements for metabolic energy and macromolecular synthesis during packaging; (ii) a study on the association of vegetative DNA with the gene 49-defective heads; and (iii) an investigation into the role played by the capsid size and the tail in head formation. On the basis of these experiments, a tentative model is proposed to explain how a phage equivalent of DNA is ultimately packaged into partially filled capsid precursors.

MATERIALS AND METHODS

Media and buffers. M9 minimal and M9 medium with a mixture of 18 amino acids (M9A) has been described (23). Some experiments contained 3.7 g instead of 7 g of Na_2HPO_4 , with no change in results. H broth and EHA top and bottom agar were prepared by the methods of Steinberg and Edgar (30). Phosphate buffer (BU) and dilution buffer for phage serial dilutions were prepared as described by King (17). BUM was BU/10 with $2 \times 10^{-2} \text{ M}$ MgSO_4 .

Phage strains. The mutants used in this study and some of their properties are listed in Table 1. All are derivatives of T4D from the collection of R. S. Edgar and W. B. Wood and have been described previously (6, 7, 8, 14). The double mutants (*tsC9*; *amN120*), (*tsC9*; *amtA3*), and (*tsC9*; E920g) were obtained from a (1:1) mixed infection with the single mutants under permissive growth conditions. We thank W. B. Wood for constructing the latter two mutants. Phage stocks were prepared as described by Epstein et al. (8) using M9A as the growth medium. For all isotope incorporation experiments, phage stocks were purified by two or three cycles of differential centrifugation and suspended in BUM.

E. coli host strains. *Escherichia coli* Bb, obtained from S. Champe, was used for all incorporation experiments. It is restrictive for *am* mutants. Strains

TABLE 1. Characteristics of T4D⁺ mutants used in this study

Mutant	Gene	Mutant phenotype under restrictive conditions
<i>tsC9</i>	49	Nucleocapsid maturation blocked; leads to accumulation of unfilled capsid precursor structures [King (17); Luftig et al. (23)].
<i>amE727</i> <i>amN120</i>	49 27	Defect in base-plate synthesis; no tails formed; filled heads formed that can complement in vitro [Edgar and Wood (6)].
E920g	66	Up to 70% of phage formed have small, isometric capsids containing a two-thirds-size piece of DNA [Eiserling et al. (7)].
<i>tsP36</i>	43	Defect in T4-induced DNA polymerase [Riva et al. (27)].
<i>amtA3</i>	<i>t</i>	Block in lysis; presumably a defect in degradation of the cytoplasmic membrane [Josslin (14)].

B/5 and S/6/5 were also used as restrictive hosts for *am* mutants and for making stocks of *ts* mutants. *E. coli* CR63 was used for plating and making stocks of *am* mutants.

Reagents. Chloroform was Mallinckrodt analytical grade. Sucrose was from Fisher. Cesium chloride optical grade powder was from Harshaw Chemical Co. or Fisher. Crystalline deoxyribonuclease I, used in preparing extracts, was obtained from Sigma Chemical Co. L-Lysine- ^{14}C (U) monohydrochloride at 310 mCi/mmol, ^3H -thymidine (5-methyl- ^3H) at 7 Ci/mmol, and ^3H -uridine (^3H in 5 position) at 29 Ci/mmol were all obtained from Amersham-Searle. The stable isotopes $^{15}\text{NH}_4\text{Cl}$ and ^{13}C -glucose came from Isomet Corp. and Merck, Sharpe, & Dohme of Canada, Ltd., respectively. The rifampin initially used was a gift of D. McMahon, Cal Tech. The same results were found with rifampin obtained from Sigma.

Procedures for temperature-shift experiments. (i) *E. coli* Bb was grown at 41.5°C to 4×10^8 to 5×10^8 cells/ml (Petroff-Hauser count) in M9 from an original inoculum ($\frac{1}{40}$ volume) of a fresh overnight culture. Then the scheme outlined in Fig. 1 was followed for either *tsC9* alone or any of the *tsC9* double mutants. Infections and superinfections were performed with the phage at a multiplicity of about 4 phage per cell. For amino acid "pulse-chase" experiments, 0.2 μCi of ^{14}C -lysine or ^{14}C -leucine per ml was added at 15 min, followed by a 1,000-fold excess of unlabeled amino acid at 21 min. Both amino acid labels gave the same results, so ^{14}C -lysine was routinely used. On the average, 80% of the label was incorporated into 5% cold trichloroacetic acid-precipitable counts over the 6-min interval. The temperature shift-down was accomplished by placing the culture into an ice bath for 1.5 to 2 min to lower the temperature to 25°C and then transferring it to a 25°C bath. Samples (5 ml)

FIG. 1. Labeling and incubation regime for pulse-chase, temperature-shift experiments with either *tsC9* or a double mutant of *tsC9* (gene 49) and an amber defective in base-plate synthesis. *Escherichia coli* Bb cells were grown, infected, and temperature-shifted as described in the text. The flow of label from unfilled to filled heads in the double mutant or to phage in the single mutant was followed by zone centrifugation.

were removed at various times postshift, and extracts were prepared as previously described (23).

(ii) In those experiments where DNA was also labeled, the procedure given above was modified to include an additional pulse of ^3H -thymidine (10 to 20 $\mu\text{Ci}/\text{ml}$) at 5 min postinfection, followed by a 10,000-fold excess of cold thymidine 10 min later. At 0, 10, and 20 min postshift, 5-ml samples were removed for extract preparation (23). The extracts were subjected to zone centrifugation, and fractions were analyzed for ^3H and ^{14}C by scintillation counting.

(iii) For the experiments with cyanide or azide, the following modification was employed in the scheme of Fig. 1. At 24 min postinfection, the inhibitor was added to a final concentration of 4 mM or 30 mM. The infected culture was then temperature-shifted down at 25 min postinfection. Both the burst size and the flow of label from 300S unfilled heads into 1,000S phage (see below) was monitored at 10-min intervals after the temperature shift-down. No lysis of cells was observed from 0 to 30 min postshift. At the time of shift-down, 0.3 μCi of ^3H -arginine per ml was added to the infected cultures to determine the efficiency of metabolic inhibition. With 4 mM and 30 mM cyanide, as well as 30 mM azide, there was no incorporation of ^3H label into phage particles. For 4 mM azide, by 30 min postshift, label was incorporated into both phage and unfilled heads. However, only one-tenth as many counts were in the phage peak, as compared to an untreated culture.

(iv) For the shift-up experiment with *tsP36*, cells were initially infected and superinfected 8 min later with a multiplicity of infection (MOI) of 4 at 25°C. At 27 min after infection, the culture was divided into two equal parts: one part was left at 25°C, and the

other part was shifted up to 41.5°C. This was accomplished by placing the infected cells in a 60°C water bath for 15 sec and then putting them into a 41.5°C bath. At 27.5 min postinfection, 0.2 μCi of ^{14}C -lysine per ml was added to each culture. Then 5-ml samples were removed to ice at 28, 38, and 58 min postinfection, and extracts were prepared as previously described (23) and subjected to zone centrifugation.

(v) In the rifampin experiment, *E. coli* Bb cultures were untreated or prepared with ethylenediaminetetraacetic acid (EDTA) according to the method of Leive (19). The regime of Fig. 1 was then followed for the single mutant *tsC9*, with the modification that rifampin (200 $\mu\text{g}/\text{ml}$ in untreated or 25 $\mu\text{g}/\text{ml}$ in EDTA-treated cells) was added at 2 min before the shift-down from 41.5 to 25°C. Reid and Speyer (26) found that at these concentrations ribonucleic acid (RNA) synthesis was shut off within 2 min in uninfected *E. coli* K-12. Extracts were prepared at 0, 10, 30 min postshift and subjected to zone centrifugation. To determine how effective rifampin was in shutting off messenger RNA (mRNA) synthesis late in infection, we monitored uridine-5- ^3H (10 $\mu\text{Ci}/\text{ml}$ plus 0.25 μg of unlabeled uridine per ml added at the same time as rifampin) incorporated into trichloroacetic acid-precipitable material. In the EDTA-treated cells, we followed the rate of incorporation of ^3H -uridine pulses (13). For both cultures, we found that at 3 min after rifampin addition, the relative rate of ^3H -uridine incorporation was $<5\%$ that for the control without the drug.

Sucrose gradient centrifugation. Linear sucrose gradients (4.8 to 5.0 ml; 10 to 30%, w/v) were made in BUM. Samples (0.2 to 0.3 ml) of infected cell extracts were layered onto the gradient prior to

centrifugation in an SW50 rotor for 25 to 40 min at 22,000 rev/min with temperature at 8 to 12 C. Fractions were collected through the bottom of polyallomer or cellulose nitrate tubes using a tube-piercing and collecting device equipped with a no. 20 gauge needle, which yielded about 300 drops per gradient. The gradient allowed separation of 300S unfilled head structures from 1,000S or 1,200S filled heads. This was confirmed by electron microscopy of the gradient fractions.

For counting radioactivity, 10- or 15-drop fractions were generally collected directly onto Whatman GF/A glass fiber filters. In some duplicate experiments, fractions were collected into test tubes which contained 2 ml of 5% cold trichloroacetic acid. The acid-insoluble material was then collected on a 0.45- μ m membrane filter (Millipore). For both methods, the dried filters were placed in glass vials containing 6 ml of scintillation fluid (20 ml of Liquifluor, New England Nuclear Corp., to 1 pint of toluene) and counted in a Beckman liquid scintillation counter. Since the two methods gave identical results except for the top 15% of the gradient, the first and simpler of the two was routinely used.

Density gradient centrifugation. (i) A sample of 0.2 ml was added to 2.8 ml of CsCl (H_2O). The refractive index (n_D) of the solution was adjusted to give a density of 1.30 g/cc for gene 49-defective head samples and 1.70 g/cc for DNA preparations. Then 2 ml of paraffin oil was layered above the solution to prevent collapse of the tube during centrifugation. The tubes were placed in a Spinco SW50 rotor and centrifuged at 35,000 rev/min for about 40 hr at 15 to 20 C. Fractions were collected through the bottom of the tube using a Buchler tube-piercing device which yielded about 250 drops per gradient. Five- or ten-drop fractions were collected directly onto GF/A filters for liquid scintillation counting, with every fifth fraction placed in a test tube for n_D determination. Densities were then calculated from the n_D values.

(ii) The details for the DNA density shift experiment with *tsC9*-infected cells are as follows. A culture of Bb was infected and pulsed with ^{14}C -lysine and 3H -thymidine as described above, except that the cells were grown and infected in M9 medium with $^{15}NH_4Cl$ and ^{13}C -glucose (1 mg/ml) replacing the usual $^{14}NH_4Cl$ and ^{12}C -glucose. At 23 min postinfection the cells were collected on a membrane filter (Millipore, 0.45 μ m pore size), washed with two culture volumes of cold $^{14}N^{12}C$ medium, and then resuspended in one volume of the same at 25 C. Aeration was resumed, and 10 min later a sample was removed. An extract was prepared and centrifuged as described above. The phage peak was pooled and dialyzed against BUM. DNA was extracted by a modification of the Guthrie and Sinheimer (12) method. A mixture of phage (0.8 ml), 1% bovine serum albumin (0.2 ml), and 1 ml of BUM saturated phenol were gently rolled together in a conical centrifuge tube at room temperature. The two phases were separated by centrifugation for 15 min in a desk-top International (1,500 rev/min). The aqueous phase was removed with a broken-off Pasteur pipet. It was then dialyzed for a day against three changes of BUM. We found that 90%

of the 3H -thymidine label was recovered. DNA prepared by this procedure had an absorbancy at 260 nm/absorbancy at 280 nm ratio of 1.7; and absorbancy at 260 nm/absorbancy at 230 nm ratio of 2.0. Then 0.5 ml of this DNA was mixed with CsCl and water to a final density of 1.7 g/cc. Equilibrium gradient centrifugation was performed as described above (i). Fractions were assayed for 3H by liquid scintillation counting, and the peak counts were plotted (see lower graph, Fig. 3b). We found over 90% of the label was recovered. As a control to check that the heavy label was washed out, we performed the density shift as described above with *tsC9*-infected cells. 3H -thymidine was then added at 2 min after the cells were removed from the filter and placed at 25 C. Total cellular DNA was extracted by the sodium dodecyl sulfate (SDS)-lysozyme procedure of Frankel (9). We found at 2 min after the shift-down, 63% of the label was in light-light DNA, and, at 5 min later, over 80% of the label was in this position. This indicates that the filter and wash procedure was successful in replacing the heavy-density pool of nucleotides with a light-density pool.

As a control to determine the density positions of totally light or heavy DNA, DNA was extracted from T4+ phage grown in $^{15}NH_4Cl$, ^{13}C -glucose (1 mg/ml) modified M9 or in M9 medium, where pulses of ^{14}C -lysine and 3H -thymidine were added as previously described. These DNA species were centrifuged to equilibrium under identical conditions as above. The fractions of each tube were assayed for 3H by scintillation counting, and the peaks were plotted (see Fig. 3a). They mark the position of heavy-heavy T4 DNA and light-light DNA as isolated under our conditions. The physical manipulations used in this experiment were especially designed to maintain the integrity of the T4 DNA molecule. On the average over several experiments we found that the sedimentation coefficient for DNA extracted by this method was 57S, suggesting that the DNA is mostly intact (9).

Electron microscopy. The procedures for negative staining of viral components have been described (23). Specimens were examined in a Siemens Elmiskop I electron microscope at 80 kv. Particle counts were made as described by King (17). Magnification was calibrated by the method of Luftig (21).

RESULTS

Requirements for nucleocapsid maturation. It has been previously shown that *tsC9* (gene 49) mutant-infected cultures accumulate defective heads, which are not completely filled with DNA and act as intermediates in head maturation (22, 23). As an initial step toward ultimately determining how phage DNA is assembled into the T4 capsid, we have performed the following experiments utilizing defective head structures.

(i) **Requirement for metabolic energy.** Since temperature-shift experiments with *tsC9* can serve as a convenient probe for analyzing head maturation, we have examined the effect of

various metabolic inhibitors on this process. Potassium cyanide and sodium azide are potent uncouplers of oxidative phosphorylation (20) and can be used to assay whether cellular energy metabolism is required for certain steps of a coliphage infection (1). However, a difficulty with using cyanide in the T4 system is that it causes premature lysis when added late in infection (4). We overcame this difficulty by constructing the double mutant *amtA3;tsC9*. It is blocked in the *t* gene and lysis is prevented, even in the presence of cyanide (14). A temperature shift experiment as outlined in Fig. 1 was then performed with *tsC9;amtA3*, where cyanide or azide (4 mM, 30 mM) was added before the shift-down. We asked whether label initially located in gene 49-defective, unfilled heads could be redistributed to phage at various times after a temperature shift-down. The results (Table 2) show that with inhibitor at 30 mM both the transfer of radioactive label to phage and the burst size are reduced. On the average, 13% of the total peak counts at 30 min are in phage, compared to the control with no inhibitor added, where 73% of the label is in phage. This suggests that adenosine triphosphate (ATP) energy is needed for DNA to be packaged. The residual 13% of label in phage and the small burst size (viz., 15% of control) present at 30 min postshift may be a consequence of the ATP energy still remaining in the cell at the time of uncoupler addition. It is also possible, however, that cyanide and azide are effecting this decrease by interacting with the gene 49 product. Isolation of the gene 49 product is required to answer this directly.

Since packaging can occur in the presence of either chloramphenicol or puromycin (200 µg/ml) (23), the suggested metabolic energy requirement

is evidently not associated with "de novo" protein synthesis. To determine just how this energy is utilized, we assayed head filling under conditions where DNA or RNA synthesis was blocked.

(ii) **Gene 43 function is required.** The mutant *tsP36* (gene 43) grown under restrictive conditions prevents the appearance of T4 DNA polymerase activity as assayed by incorporation of radioactively labeled thymidine (Table 3, and reference 29). To study whether the energy needed for packaging was used in DNA synthesis, we infected cells at 25°C, and 28 min later shifted the culture up to 41.5°C. ¹⁴C-lysine was added at the time of this shift-up, samples were removed 0 to 30 min later, and extracts were assayed as before. From Table 3, we observed that 90% of the label incorporated into the 300S peak at 10 min postshift was still present 20 min later, whereas in the unshifted control 85% of the label had been converted to phage by that time.

We interpret our results as indicating that DNA synthesis is required for the packaging of DNA into unfilled heads. However, there are at least two alternative possibilities. (i) The packaging may be primarily correlated with the maintenance of RNA rather than DNA synthesis. This premise is based upon the recent finding of Riva et al. (7) that normal late mRNA synthesis is not sustained after a shift-up of cells infected with *tsP36*. (ii) The failure to fill the head membranes could be due to the presence of a residual nucleic acid activity in the nonfunctional polymerase, which degrades intracellular phage DNA. Nossal (25) has shown this for *amb22* (gene 43)-infected cell extracts.

We have eliminated the first possibility in section (iii) below, and we believe that the second alternative is unlikely for two reasons. First, Cascino et al. (2) have shown that labeled DNA present in *tsP36*-infected cells is not degraded at the nonpermissive temperature, indicating the absence of a T4 deoxyribonuclease. Secondly, there is no a priori reason to expect a *ts* mutation in T4 which leads to formation of an altered complete polypeptide chain to have the same activity as an amber fragment of a chain, even though the *am* and *ts* mutants map in the same cistron. In fact, Nossal (25) has shown the nuclease is only present in one of several *am* mutants of gene 43.

(iii) **RNA synthesis is not required.** We added rifampin, an inhibitor of RNA synthesis in vivo (13, 26) to *tsC9*-infected cells at the time of their shift-down (Fig. 1). Then we followed the flow of label from 300S structures into phage at various times after the temperature shift (Table 4).

TABLE 2. Effect of uncouplers of oxidative phosphorylation on recovery of phage from (*amtA3;tsC9*)-infected cells^a

Compound added	Concn	Relative burst size at 30 min postshift (%)	Per cent label in phage peak at 30 min postshift
		100	73
Cyanide	4 mM	34 (9-53)	33 (17-44)
Cyanide	30 mM	4 (2-7)	7 (2-12)
Azide	4 mM	62 (47-68)	43 (40-50)
Azide	30 mM	27 (14-41)	19 (10-33)

^a An untreated *tsC9*-infected culture served as a control. Each percentage represents the average over four experiments, with the range of values indicated by the parenthesis.

^b The burst sizes are relative to the range of 160 to 260 per infective center, found for a *tsC9*-infected culture.

TABLE 3. *Redistribution of label from 300S unfilled heads into phage after DNA synthesis is blocked^a*

Temperature conditions	¹⁴ C-Lysine counts/min at various times after infection ^b					
	28 Min		38 Min		58 Min	
	300S	1,000S	300S	1,000S	300S	1,000S
Shift from 25 to 41.5 C at 27 min post-infection						
Expt 1	3,400	0 (0%)	13,300	200 (1.5%)	12,300	1,200 (9%)
Expt 2	300	0 (0%)	3,600	300 (8%)	2,900	600 (16%)
No shift; cells kept at 25 C through experiment						
Expt 1	2,700	0 (0%)	11,100	3,300 (23%)	2,700	14,000 (84%)
Expt 2	300	0 (0%)	4,000	1,500 (27%)	3,300	11,200 (77%)

^a Incorporation of labeled thymidine into acid-precipitable counts immediately after the shift-up was found to be between 3 and 5% that of a culture where the polymerase function was not shut off. This rapid shut-off by *tsP36* had previously been reported (27).

^b The counts tabulated above are the sum found in the 300S or 1,000S peak fractions from the 10 to 30% sucrose gradient. The percentages in parenthesis are those for the 1,000S counts relative to the sum.

TABLE 4. *Effect of rifampin on the flow of label from 300S gene 49-defective heads into phage^a*

Min postshift	¹⁴ C-Lysine counts/min ^b	
	300S	1,000S
0	40,400	1,500 (4%)
10	27,300	14,000 (28%)
30	18,600	21,700 (63%)

^a The counts above are for a non-EDTA-treated *tsC9*-infected culture where 200 µg of rifampin per ml was added 2 min before the temperature shift-down. A control experiment without added rifampin showed comparable values of 6, 36, and 74% for the 1,000S peak at 0, 10, and 30 min after the shift.

^b Values in parenthesis are those for the 1,000S counts relative to the sum and represent the average over three experiments.

There was no significant difference in label distribution compared to an untreated culture, which indicates that packaging can proceed in the absence of RNA synthesis.

(iv) **DNA replication need not occur at the site of packaging.** The above result that the gene 43 function is required led us to ask whether or not the DNA needed to be immediately replicated at the site of the capsid precursor in order for it to be packaged. If the latter assumption were true, then we would expect the following two corollaries to hold for the phage produced soon after a temperature shift-down of a *tsC9*-infected culture. (i) Part of its DNA should come from the

vegetative DNA pool present at 41.5 C. (ii) The two DNA strands should each come from different pools, viz., DNA present at 41.5 C and newly made at 25 C.

The first corollary was tested by labeling the pool of DNA present at 41.5 C with a pulse of ³H-thymidine and preparing extracts from samples removed at 0 to 20 min postshift. Figure 2 shows that DNA present at the restrictive temperature is incorporated into phage formed after the rescue of the gene 49 function. Further, from Table 5 we note that the total amount of DNA precursor label incorporated into phage by 20 min is much greater than can be accounted for by the 60% loss of counts from the unfilled head peak. It suggests that the DNA present in these phage is not solely attributable to the 12 to 32% DNA in gene 49-defective heads and is at least partly derived from the vegetative DNA pool at 41.5 C. The second corollary was tested by a "pulse-chase" density-shift experiment. Cells were grown in ¹⁵N¹³C-glucose (heavy) medium, infected with *tsC9*, and pulsed with both ³H-thymidine and ¹⁴C-lysine as previously described. Then at the time of the temperature shift-down, the cells were filtered, washed, and resuspended in fresh ¹⁴N¹²C-glucose (light) medium at 25 C. Phage recovered 10 min after the shift were purified by zone centrifugation, and the DNA was gently extracted by phenol treatment. If DNA replication were required for packaging, then most of this DNA would be hybrid in density, containing one heavy and one light DNA strand. However, when the DNA was centrifuged

FIG. 2. Rescue of ^3H -thymidine-labeled phage at 0 and 20 min after recovery of the gene 49 function, assayed by zone centrifugation. As a control we note the expected flow of about 50% of ^{14}C -lysine counts from the 300S to the 1,000S peak at 20 min postshift. See Table 5 for experimental details.

TABLE 5. Incorporation of labeled DNA precursors into phage rescued after recovery of the gene 49 function^a

Min postshift	^3H -Thymidine counts/min ^b	
	Unfilled heads (300S)	Phage ^c (1,000S)
0	7,500	2,900
10	3,100	13,300
20	2,300	14,600

^a *E. coli* Bb was infected with *tsC9* as described in the text.

^b ^3H -thymidine counts above are the sum of the peak fractions from 10 to 30% sucrose gradients. On the average over three identical experiments, by 20 min postshift we found that 60% of the label had disappeared from unfilled heads. Even if all of these lost counts were incorporated into phage, it could only account for about 30% of the total ^3H -thymidine counts located in phage at 20 min after the shift.

to equilibrium, we found (Fig. 3) that 90% of the counts were in a heavy position and less than 10% were hybrid. A control confirmed that our filtering procedure had removed the pool of heavy nucleotides. We conclude that DNA repli-

cation does not directly accompany packaging and that most of the double-stranded DNA present in *tsC9*-infected cells at high temperature is conserved during the packaging process.

Effect of deoxyribonuclease on extracts prepared from gene 49-infected cultures. In all the assays reported in this paper, 80 μg of deoxyribonuclease per ml had been added to extracts made from *tsC9*-infected cells. If, however, the extracts were prepared without deoxyribonuclease, only 15% of the expected 300S counts were liberated into the supernatant fluid (Table 6). The remainder pelleted with the chloroform-treated cell debris. If deoxyribonuclease was now added to the pellet, the residual 300S head counts were recovered. These debris-attached structures were almost all (95%) found to band in CsCl at the increased density (about $\rho = 1.34$) position characteristic of nucleoprotein-containing heads (23). As can be seen by Fig. 4, 300S heads of this density, rather than those that band at 1.30 g/cc, represent maturable capsid precursors. This follows from the observation that at 0 to 50 min postshift (41.5 C $\rightarrow$ 25 C) of *tsC9*-infected cells, the higher density peak exhibited a fivefold decrease in counts, while the other peak remained constant (Fig. 4). Hence, our results indicate that maturable gene 49-defective heads are attached to cell debris through

FIG. 3. Fate of heavy-density labeled DNA after recovery of the gene 49 function in light density medium. See text for experimental details.

TABLE 6. Effect of deoxyribonuclease on the release of 300S, gene 49-defective heads from extracts of *tsC9*-infected cells^a

Fraction	¹⁴ C-Lysine counts/min in 300S peak ^b	Per cent of counts in CsCl broad density peak ^c
No deoxyribonuclease added to extract		
Unfilled heads in supernatant	60,500	23
Unfilled heads recovered from pellet re-suspended in deoxyribonuclease medium	119,300	94
Total	179,700	
Deoxyribonuclease added to extract		
Unfilled heads in supernatant	200,900	85

^a The labeling scheme outlined in Fig. 1 was followed. At 23 min postinfection, 5-ml samples were removed, and extracts were prepared with or without deoxyribonuclease and centrifuged on sucrose gradients.

^b The counts were normalized with regard to volume and represent the total counts present in each preparative step listed.

^c The pooled unfilled heads centrifuged on CsCl gradients gave a broad nucleoprotein ($\rho = 1.335$ g/cc) and sharp protein ($\rho = 1.3$ g/cc) peak (Fig. 4). The percentage represents counts of the broad peak, relative to the sum of the two peaks.

a deoxyribonuclease-sensitive connection. It should be noted that deoxyribonuclease is not leading to removal of DNA from already filled heads, since we have previously shown that an *amN120* infection yielded >80% filled heads (23) and a *tsC9;amN120* infection produced >50% filled heads (see Table 8), where infected cell extracts were treated in the same manner with deoxyribonuclease. A head-filling model that takes into account these and the other results described above will be presented below.

Influence of head shape on capsid formation. Mosig (24) discovered that T4 populations include a small fraction of particles which contain DNA of two-thirds the normal molecular length. The capsids of these unusual "petite" particles are isometric (80 by 80 nm) and enclose roughly two-thirds of the volume of prolate-shaped (80 by 110 nm) wild-type heads. Particles such as these are also produced at a frequency of up to 70% in gene 66 mutant (E920g)-infected cells

(7). This mutant and the double mutant (E920g;*tsC9*) were utilized in "pulse-chase" experiments to probe the nature of "petite phage" capsid precursors. Initially, temperature-shift experiments as outlined in Fig. 1 were performed. At 0 to 30 min postshift, samples were removed, and extracts were prepared and centrifuged on a sucrose gradient. Each fraction from the gradient was assayed by both electron microscopy and

FIG. 4. CsCl equilibrium centrifugation of 300S, gene 49-defective heads isolated from *tsC9*-infected cells at 0, 30, and 50 min after a temperature shift-down (41.5 $\rightarrow$ 25 $^{\circ}$ C). See text for details. Note that the ordinate scales differ for each time point. Two density peaks are observed; the one at $\rho = 1.34$ g/cc is not artifactually created by incomplete leakage of DNA from a completely filled head, since both phage and filled heads artificially disrupted by freeze-thawing band exclusively at the protein position (D. Hamilton and R. Luftig, unpublished observations).

FIG. 5. (A) and (B): *Escherichia coli* Bb was infected with (tsC9;amN120) according to the scheme of Fig. 1. Extracts were prepared and centrifuged on 10 to 30% sucrose gradients. (A) is a field from a peak (300S) fraction of the 0-min postshift extract; (B) is from a peak (1,200S) of the 30-min postshift extract. (C) and (D): *E. coli* Bb was infected with (tsC9;E920g), and extracts were subjected to zone sedimentation. (C) is from a peak (300S) fraction of the 0-min postshift extract; (D) is from a peak (1,000S) of the 30-min postshift extract. Arrows in (C) and (D) point to petite unfilled heads and petite phage, respectively. All grids were stained with 2% uranyl acetate, pH 4.2. Magnification is $\times 120,000$.

isotope counting. In both mutants at 0 min postshift, the gradient fractions from the 300S label peak were found to contain a mixture of isometric and prolate-shaped unfilled heads (Fig. 5C). The smaller, isometric, unfilled heads could be clearly distinguished on the micrographs, since they had the same dimension (80 nm) in two mutually perpendicular directions. The prolate, unfilled heads, in contrast, were clearly nonisometric. To insure that these heads were not an artifact of our preparative procedure, we determined the ratio of small heads to total heads for identical fractions from an E920g and a *tsC9* gradient. The values 53% and 6%, respectively, were obtained over several fields. This indicates that at least 85% of the small, isometric unfilled heads seen in E920g-infected cells do not represent end-on views of prolate-shaped heads. At 30 min postshift, the 1,000S peak showed a mixture of isometric and prolate-shaped phage particles (Fig. 5D). We then constructed calibration curves for the per cent of isometric/total particles over the 300S and 1,000S peaks which were superimposed upon the radioactivity profiles from the "pulse-chase" experiments (Table 7). This enabled us to calculate the amount of label present in both petite, unfilled heads and phage.

For petite, gene 49-defective unfilled heads, we calculated that at 0, 10, and 30 min postshift, about 5, 35, and 65% of the amino acid label had flowed into small-headed phage (Table 7). This is similar to results found with *tsC9* for prolate-shaped heads. For E920g alone, we found at these times on the average over several experiments that 60, 70, and 90% of the counts flowed into small-headed phage, which suggests that when the gene 49 function had been present throughout infection, then unfilled heads did not accumulate but were rapidly filled. This was confirmed by administration of a 1-min pulse late in infection; the results shown in Table 8 indicate that, within 7 min postchase, over 70% of the label was converted to small-headed phage. We conclude that the packaging of DNA is independent of the process which determines the size and shape of the head membrane.

Role of tails in capsid maturation. There is evidence that T4 tails can affect the conformation of the head structure as well as its stability (3, 6). Recently, it was found (11) that heads isolated under nonpermissive conditions from *amB255*, a base-plate-defective mutant in gene 10, were quite fragile, suggesting that complete tails were needed for proper head maturation. Although the separate pathways for assembly of heads and tails are well established (33), the possibility that gene 49 function was sensitive to the pres-

TABLE 7. *Redistribution of labeled protein from small, isometric unfilled heads (about 300S) to petite phage (about 1,000S) following temperature shift of E920g or E920g;*tsC9*-infected cells^a*

Min post-shift	¹⁴ C-Lysine counts incorporated			
	E920g; <i>tsC9</i>		E920g	
	1,000S	300S	1,000S	300S
0	900 (5%)	16,300	10,700 (71%)	4,300
10	6,000 (35%)	11,100	9,000 (74%)	3,100
30	9,600 (63%)	5,600	13,100 (89%)	1,600

^a Each fraction in the 300S and 1,000S peaks represented a mixture of small, isometric (petite) and normal, prolate-shaped heads. The per cent of petite particles for each fraction was calibrated by electron microscopy, e.g., the five peak fractions of 300S, unfilled heads had 15, 29, 42, 55, and 68% petite structures, respectively. Then the per cent of radioactive counts in such small-headed structures, for each fraction, was obtained by using the per cent value for the number of structures multiplied by the factor, 0.87. This latter value is the ratio of protein molecular weights in the (small/large) heads and was calculated from the increased fraction of protein, viz., +0.062, found when going from wild type to petite phage (7).

TABLE 8. *Effect of a short radioactive pulse on the redistribution of label from petite unfilled heads into petite phage^a*

Min after chase	¹⁴ C-Lysine counts incorporated ^b	
	Petite phage (1,000S)	Petite unfilled heads (300S)
0	(15%) 600	5,700
2	(29%) 1,000	3,400
4	(44%) 2,600	3,100
7	(71%) 3,000	3,200
12	(86%)	

^a Infection scheme outlined in Fig. 1 was modified by administration of a 1-min pulse of ¹⁴C-lysine at 15 min after infection, followed by the chase with ¹²C-lysine. The chase was effective within 1 min, as assayed by incorporation of acid-precipitable counts.

^b Values in parenthesis represent the average over at least three experiments.

ence of tails had not been investigated (23). Thus, the double mutant *tsC9;amN120* was constructed and a temperature-shift experiment as outlined in Fig. 1 was performed. Initially, extracts prepared from cells removed at 0 and 30 min postshift were layered on sucrose gradients and centrifuged under conditions which

separated filled from unfilled heads. Electron microscope examination of fractions from the 0-min extract showed most of the isolated particles were empty-appearing head structures (Fig. 5A) which sedimented at about 300S, whereas the 30-min extract had a peak of filled heads (Fig. 5B) sedimenting at 1,200S. This suggests that head maturation can proceed in the absence of tails. The recovery of filled heads at 0 to 50 min postshift was quantitated by tabulation of the ^{14}C -lysine counts which occurred under the two major peaks of the gradient. As can be seen from Table 9, the sum of counts in the two peaks was roughly the same over the time interval studied, consistent with a precursor-product relationship for the isolated 300S and 1,200S head structures. Further, by 50 min, over 50% of the label was converted to filled heads, which is consistent with the earlier electron microscope observation that gene 49-defective head structures can be packaged in the absence of tails. This process is not gene-specific, since double mutants of *tsC9* with genes 6, 7, 10, or 29 also exhibited a similar redistribution of label into filled heads (Luftig and Ganz, *unpublished observations*).

DISCUSSION

The results we have presented extend as well as substantiate our previous suggestion (23) that the gene 49 product has a key function in T4 head maturation. This role of gene 49 can be viewed in two different aspects as described below.

Role of gene 49 in cleaving a "head-full" of DNA. We have shown that T4 head maturation in gene 49-defective cells can occur in the absence of tails and is independent of the size and shape

of the capsid. This is consistent with the result that the gene 49 protein is a structural component of the capsid (33). Since Frankel et al. (*in press*; also, Fed. Proc. 30:1263, 1971) have also found that gene 49-defective extracts are deficient in a nuclease activity, it is tempting to speculate, as they have, that an *in vivo* role of the gene 49 product may be to sit at the base of the capsid (33) and cleave the DNA when a capsid-full is obtained. A suggestion similar to this had been proposed on genetic grounds (31). This suggestion is further supported by the finding in (*tsC9*;E920g)-infected cells, that small, isometric-shaped, unfilled heads are apparently precursors to the small-headed, "petite" phage which contain two-thirds the normal DNA complement (7). However, the result (Frankel et al., *in press*; Fed. Proc. 30:1263, 1971) that the >1,000S DNA of gene 49 mutant-infected cells can be cleaved *in vitro* into normal phage-size pieces of DNA appears to contradict the need for a full capsid before cleavage can occur. It is probable though that this *in vitro* process is not directly analogous to the *in vivo* situation, since phage-size pieces of DNA are not found free in the cytoplasm of T4-infected cells (9). The *in vitro* cleavage may possibly be due to a built-in tertiary structure in the >1,000S DNA, imparted to it through association with the gene 49-defective heads. This may make it more susceptible to a specific nuclease activated by the gene 49 product. This model would then predict that DNA isolated from (*tsC9*;E920g)-infected cells should be cleaved *in vitro* into both normal and two-thirds size pieces of T4 DNA. The preliminary studies of Fujisawa and Minigawa (10) indicate that such a cleavage does occur *in vivo* for E920g-infected cells.

Role of gene 49 in packaging DNA into capsid intermediates. One possible mechanism for head formation is that phage DNA is packaged with head protein by a total self-assembly process. This model is probably not valid, since ATP energy is required for head maturation (Table 2). Further, the temperature-shift studies with *tsP36*-infected cells suggest that this metabolic energy is correlated with DNA synthesis as the driving force behind the packaging process. This led us initially to test a simple model for head formation, where DNA replication was directly coupled to filling of the heads. It was ruled out, however, on the basis of a DNA-labeling density-shift experiment where both strands of DNA labeled at 41.5 C in *tsC9*-infected cells were found to be conserved in phage rescued at 10 min after a temperature shift-down.

The tentative model that we wish to propose

TABLE 9. Flow of label from gene 49-defective heads into filled heads for the double mutant (*amN120*;-*tsC9*)^a

Min postshift	^{14}C -Lysine counts incorporated	
	Unfilled heads (300S)	Filled heads (1,200S)
0	48,000	0 (0%)
10	33,000	4,900 (13%)
20	29,300	13,400 (31%)
30	19,700	17,100 (46%)
50	15,800	18,500 (54%)

^a In all of the tables presented in this paper, the peak counts have the gradient background subtracted. The percentages in parenthesis are those of filled head counts relative to the total in the two peaks.

here for DNA packaging utilizes the above results as well as the finding that the 12 to 32% deoxyribonuclease-resistant pieces present in the isolated 300S gene 49-defective heads are most likely associated in vivo with the large >1,000S DNA form. In this model, we propose that the vegetative DNA pool has two components: one, attached to the membrane, is actively synthesizing DNA [similar to the ϕ X-174 system (28)], undergoes recombination, and may be the competent DNA suggested by Riva et al. (27) which synthesizes late mRNA; the other component is attached through the deoxyribonuclease-resistant pieces to the incompletely filled capsid precursors. As DNA synthesis proceeds, it causes DNA, which now is in a noncompetent (27) form, to be pushed into the heads. We have not mentioned how the gene 49-defective heads themselves are assembled. One possibility is that they are formed by polymerization of T4 proteins around the 12 to 32% pieces of deoxyribonuclease-resistant T4 DNA. These pieces may have a special tertiary structure which permits such a polymerization. We are presently in the process of isolating and investigating the structure of these DNA fragments.

Another alternative for how the head may be formed derives from the recent experiments of Laemmli (Abst. 1971 Cold Spring Harbor Phage Meet.). He has proposed that initially a capsid intermediate is formed (prohead I) that consists mainly of uncleaved gene 23 protein, is almost completely filled with DNA, and sediments at 900S. The head protein is then cleaved to yield a structure (prohead II) whose DNA content is unstable upon isolation. As the final step in maturation, prohead II is thought to be converted into a mature head with a stable DNA content. Both Laemmli's model and the one we have proposed agree in that they postulate that some phage DNA is needed for initiation of capsid assembly and that gene 49 is required for conversion of a capsid intermediate into a mature head. The alternatives apparently differ in the amount of DNA assumed to be present in the initial capsid precursor. Our data indicate that there is at least 12%, but less than 100%. Hence, it appears that our models agree in the initial and terminal steps of head maturation, but further investigations are needed to ascertain what is occurring between these steps. The detailing of requirements for maturation provided in this paper should serve as a basis for attempting to resolve this dilemma through in vitro experimentation.

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LITERATURE CITED

1. Barnhart, B. J., and C. T. Gregg. 1967. Effects of uncouplers of oxidative phosphorylation on the infection of *Escherichia coli* K12 by phage- λ DNA. *Virology* 32:687-691.
2. Cascino, A., S. Riva, and E. P. Geiduschek. 1971. Host DNA synthesis after infection of *Escherichia coli* with mutants of bacteriophage T4. *Virology* 46:437-452.
3. Cummings, D. J., V. A. Chapman, and S. S. De Long. 1969. The sedimentation and conformational variance among T-even bacteriophages. *Virology* 37:94-108.
4. Doermann, A. H. 1952. The intracellular growth of bacteriophages. I. Liberation of intracellular bacteriophage T4 by premature lysis with another phage or with cyanide. *J. Gen. Physiol.* 35:645-656.
5. Edgar, R., and I. Lieulausis. 1968. Some steps in the assembly of bacteriophage T4. *J. Mol. Biol.* 32:263-276.
6. Edgar, R. S., and W. B. Wood. 1966. Morphogenesis of bacteriophage T4 in extracts of mutant-infected cells. *Proc. Nat. Acad. Sci. U.S.A.* 55:498-505.
7. 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.
8. Epstein, R. H., A. Bolle, S. M. Steinberg, E. Kellenberger, E. Boy de la Tour, R. Chevalley, R. S. Edgar, M. Susman, G. H. Denhardt, and A. Lielausis. 1963. Physiological studies of conditional lethal mutants of bacteriophage T4D. *Cold Spring Harbor Symp. Quant. Biol.* 28:375-394.
9. Frankel, F. R. 1966. Studies on the nature of DNA in T4 infected *Escherichia coli*. *J. Mol. Biol.* 18:127-143.
10. Fujisawa, H., and T. Minigawa. 1971. Genetic control of the DNA maturation in the process of phage morphogenesis. *Virology* 45:289-291.
11. Granboulan, P. H., J. Séchaud, and E. Kellenberger. 1971. On the fragility of phage T4-related particles. *Virology* 46:407-425.
12. Guthrie, G. D., and R. L. Sinsheimer. 1963. Observations on the infection of bacterial protoplasts with the deoxyribonucleic acid of bacteriophage ϕ X-174. *Biochim. Biophys. Acta* 72:290-297.
13. Haselkorn, R., M. Vogel, and R. D. Brown. 1969. Conservation of the rifamycin sensitivity of transcription during T4 development. *Nature (London)* 221:836-838.
14. Josslin, R. 1971. Physiological studies on the *t* gene defect in T4-infected *Escherichia coli*. *Virology* 44:101-107.
15. Kellenberger, E., F. A. Eiserling, and E. Boy de la Tour. 1968. Studies on the morphogenesis of the head of phage T. III. The cores of head related structures. *J. Ultrastruct. Res.* 21:335-360.
16. Kellenberger, E., J. Séchaud, and A. Ryter. 1959. Electron microscope studies on phage multiplication. IV. The establishment of the DNA pool of vegetative phage and the maturation of phage particles. *Virology* 8:478-498.
17. King, J. 1968. Assembly of the tail of bacteriophage T4. *J. Mol. Biol.* 32:231-262.
18. Laemmli, U. K., E. Mölbert, M. Showe, and E. Kellenberger. 1970. Form-determining function of the genes required for the assembly of the head of bacteriophage T4. *J. Mol. Biol.* 49:99-113.
19. Leive, L. 1965. RNA degradation and the assembly of ribosomes in actinomycin-treated *Escherichia coli*. *J. Mol. Biol.* 13:862-865.

20. Loomis, W. F., and F. Lipmann. 1948. Reversible inhibition of the coupling between phosphorylation and oxidation. *J. Biol. Chem.* 173:807-808.
21. Luftig, R. B. 1968. Further studies on the dimensions of viral and protein structures using the catalase crystal internal marker technique. *J. Ultrastruc. Res.* 23:178-181.
22. Luftig, R. B., and W. B. Wood. 1969. An intermediate in T4 phage head formation. *Biophys. J.* 9:A-259.
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. Mosig, G. 1963. Diversity among T4 phage particles. *Carnegie Inst. Wash. Year B.*, p. 586-589.
25. Nossal, N. 1969. A T4 bacteriophage mutant which lacks deoxyribonucleic acid polymerase but retains the polymerase associated nuclease. *J. Biol. Chem.* 244:218-222.
26. Reid, P., and Speyer, J. 1970. Rifampicin inhibition of ribonucleic acid and protein synthesis in normal and ethylenediaminetetraacetic acid-treated *Escherichia coli*. *J. Bacteriol.* 104:376-389.
27. Riva, S., A. Cascino, and E. P. Geiduschek. 1970. Coupling of late transcription to viral replication in bacteriophage T4 development. *J. Mol. Biol.* 54:85-102.
28. Salivar, W. O., and R. L. Sinsheimer. 1969. Intracellular location and number of replicating parental DNA molecules of bacteriophages lambda and ϕ X-174. *J. Mol. Biol.* 41:39-65.
29. Shalit, C., and S. Kahana. 1970. Conversion of T4 gene 46 mutant deoxyribonucleic acid into nonviable bacteriophage particles. *J. Virol.* 6:353-362.
30. Steinberg, C. M., and R. S. Edgar. 1962. A critical test of a current theory of genetic recombination in bacteriophage. *Genetics* 47:182-208.
31. Streisinger, G., J. Emrich, and M. M. Stahl. 1967. Chromosome structure in phage T4. III. Terminal redundancy and length determination. *Proc. Nat. Acad. Sci. U.S.A.* 57:292-295.
32. Ward, S., R. B. Luftig, J. H. Wilson, H. Eddleman, H. Lyle, and W. B. Wood. 1970. Assembly of bacteriophage T4 tail fibers. II. Isolation and characterization of tail fiber precursors. *J. Mol. Biol.* 54:15-31.
33. Wood, W. B., R. S. Edgar, J. King, I. Lielausis, and M. Henninger. 1968. Bacteriophage assembly. *Fed. Proc.* 27: 1160-1166.