JOURNAL OF VIROLOGY, June 1972, p. 1047-1056
Vol. 9, No. 6

Bacteriophage T4 Head Morphogenesis

III. Some Novel Properties of Gene 13-Defective Heads

DANIEL L. HAMILTON AND RONALD B. LUFTIG

Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina 27710

Received for publication 24 January 1972

The nature of phage precursors in gene 13-defective infected cells was studied by electron microscopy and pulse-chase isotopic labeling experiments. Our results suggest that both stable (20%) and fragile (70%) filled-head precursors accumulated in the absence of gene 13 product. Upon extraction, the fragile heads were found to lose most of their deoxyribonucleic acid and appeared unfilled with an average density of 1.34 g/cm^3 and a sedimentation coefficient of 300*S*. These unfilled heads differed from empty gene 13-defective heads which did not have any associated deoxyribonucleic acid and banded at an average density of 1.31 g/cm^3. Furthermore, it was found that a *ts*N38 (temperature-sensitive mutant in gene 13)-infected culture maintained at 41.5 C for increasing times led to a decrease in specific infectivity of 1,000*S* phagelike particles. Electron microscopy of these particles revealed that the decreased infectivity was due to an improper union of head and tails.

The discovery of large numbers of bacteriophage T4 conditional lethal mutants by Epstein et al. (3) proved to be a major factor in permitting elucidation of the T4 morphogenetic pathway. Much of the attention in previous studies on the pathway had been directed at tail (6, 7) and tail fiber assembly (15), as well as at the early steps involving capsid formation (8). One aspect which has not been extensively studied is how the head and tail are joined. This problem is of particular interest because it is through this junction that the deoxyribonucleic acid must pass to infect its host.

One of the genes, which has been implicated in preparing the head for union with the tail, is gene 13 (2). Edgar and Lielausis (1) demonstrated that isolated filled heads lacking the product of gene 13 could be complemented in vitro, which strongly suggests that they are a precursor to the action of the gene 13 product. To specify what function the product of gene 13 performs in this process, we have undertaken a detailed investigation of gene 13-defective head precursors. We have partially characterized them through the use of both pulse-chase, temperature-shift experiments with labeled amino acids and electron microscopy. Some additional experiments which bear on the mechanism of action of gene 13 product are also presented. The preceding paper in this series is Luftig and Ganz (10).

MATERIALS AND METHODS

Media and buffers. M9 minimal medium (M9) contained 3.7 g of Na_2HPO_4, 3 g of KH_2PO_4, 1 g of NH_4Cl, 6 g of NaCl, 4 g of glucose, 1.2 g of $MgSO_4$, 11 $\times$ 10^{-3} g of $CaCl_2$, and 17 $\times$ 10^{-5} g of $FeCl_3$ per 1,000 ml of distilled water. H broth and EHA top and bottom agar were prepared as described by Steinberg and Edgar (14). PO_4 buffer (BU) and dilution buffer for phage serial dilutions were prepared as described by King (6). Tenfold-diluted PO_4 buffer containing 2 $\times$ 10^{-2} M $MgSO_4$ is termed 0.1 $\times$ PO_4-Mg buffer.

T4D phage strains. The phage mutants used in this study 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 (2, 3). Amber (*am*) mutants form plaques on the permissive host *Escherichia coli* CR63 but not on the restrictive hosts B/5 or S/6/5. Temperature-sensitive (*ts*) mutants form plaques at 25 but not at 42 C. Phage stocks were prepared as described by Epstein et al. (3) by using either H broth or M9 as the growth media.

E. coli host strains. *E. coli* host strains used in this study have been previously described by Luftig, Wood, and Okinaka (11).

Reagents. Chloroform was B & A reagent grade. Sucrose for gradients was from B & A. Crystalline deoxyribonuclease I, used in preparing extracts, was obtained from Sigma Chemical Co. L-Lysine-*U*-^{14}C monohydrochloride at 312 mCi/mmole was obtained from Amersham-Searle. Thymidine (*5-methyl*-^3H) from New England Nuclear Corp. was 6.7 Ci/mmole.

General procedures for temperature-shift experiments. The procedure for temperature-shift experiments has been previously described (11). The label-

TABLE 1. *Characteristics of mutants used in this study*

Mutant	Gene	Defect	Components accumulated[a]
*ts*N38	13	Head	13 Defective heads, tails, tail fibers
*am*E609	13	Head	13 Defective heads, tails, tail fibers
*am*E727	49	Head	49 Defective heads, tails, tail fibers
*ts*C9	49	Head	49 Defective heads, tails, tail fibers
*ts*N1	34	Tail fiber	Filled heads, tails, defective tail fibers
*ts*N34	27	Tail	Filled heads, tail fibers

[a] Components listed are those that accumulate under nonpermissive conditions.

ing regime for pulse-chase temperature-shift experiments is described in Fig. 4.

Zone sedimentation through sucrose gradients. Linear sucrose gradients (9.8 to 10.0 ml; 20 to 40%, w/v) were made in 0.1 × PO_4-Mg buffer. Samples of 0.2 to 0.4 ml were layered onto gradients and centrifuged in an SW41 rotor of a Spinco model L3-50 ultracentrifuge for 40 min at 22,000 rev/min at 8 to 12 C. Fractions were collected through the bottom of the tubes by using a tube-piercing and collecting device equipped with a no. 20 gauge needle which yielded about 600 drops per gradient.

Absorbancies at 260 nm were determined on 20-drop fractions by using a Gilford 2400 spectrophotometer equipped with microcuvettes. For measuring radioactivity, 20-drop fractions were precipitated in 5% trichloroacetic acid and the precipitates were collected on a membrane filter. The filters were then dried and 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 at approximate efficiencies of 80% for ^{14}C and 30% for ^{3}H. Background was subtracted from all fractions.

Electron microscopy. Negative staining: negative-stained specimens were prepared as described by Luftig et al. (11). Specimens were examined in a Phillips EM 300 electron microscope at 80 kv. The microscope had a 200-μm condenser and 50-μm thin silver (French) objective apertures. Micrographs were taken on Kodalith LR 70-mm film.

Particle counts were made as described by King (6). Magnification was calibrated as described by Luftig (9).

Sectioning: procedures used for fixation and embedding were modified from those described by Kellenberger, Eiserling, and Boy de la Tour (5). Five milliliters of a culture sample (see above) was immediately mixed with 5 ml of 2% OsO_4 in Michaelis buffer, *p*H 6.0. Casamino Acids (Difco) were added to a final concentration of 2%, and the suspension was left overnight at room temperature. The cells were then sedimented and embedded in Epon 812.

Samples were sectioned on an MT2-B Sorvall Porter-Blum ultramicrotome by using glass knives made with a Messer Sunkay knifemaker. Sections were mounted on grids carrying carbon-coated collodion films and stained by floating on a saturated solution of uranyl acetate for 1 hr.

In vitro complementation. Infected-cell extracts were prepared for in vitro complementation according to Meezan and Wood (12). The *am* E609 extract was subjected to zone centrifugation, and the fraction of the 1,200*S* peak was incubated with an extract of *am*B17 (gene 23) for 2 hr prior to plating.

RESULTS

Recovery of gene 13 function. Previous investigations of Edgar and Lielausis (1) have implicated gene 13 as a control element in T4D head-tail union. We have undertaken a detailed study of the role of gene 13 in phage production in order to obtain additional information about this interaction. Temperature-shift experiments were performed with *ts*N38, a mutant in gene 13. Fig. 1 indicates that a culture of *ts*N38-infected cells can recover the gene 13 function in vivo when it is shifted late in infection from the restrictive (41.5 C) to the nonrestrictive temperature (25 C). The culture had been incubated at 41.5 C for 24 min before shifting; intracellular

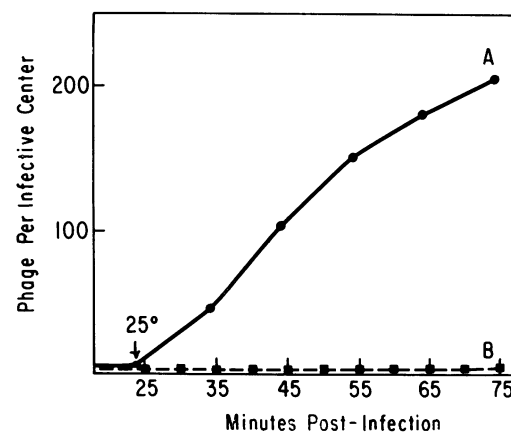

FIG. 1. *Phage production in tsN38-infected cells following a temperature shift. Bb cells at 41.5 C were infected with tsN38 as described and incubated for 24 min. One portion of the culture was then shifted to 25 C (curve A) whereas a second was left at 41.5 C (curve B). Phage titers were measured at the times shown by dilution through $CHCl_3$-saturated buffer and plating on S/6 at 30 C.*

phage were assayed at 5 or 10 min intervals for both the shifted (curve A) and unshifted culture (curve B). The shifted cells showed an immediate rise in phage production with an average burst size of 160 phage within 40 min after the shift. This is similar to the wild-type yield of 175 ± 30 phage under these conditions. The *ts*N38-infected control culture kept at 41.5 C showed a burst of only five phage per infected cell in the same time interval, indicating that the large phage yield obtained after the temperature shiftdown is due predominantly to rescue of the gene 13 function.

We also found, in agreement with Edgar and Lielausis (1), that filled heads isolated from *am*E609-infected cultures could be complemented in vitro with an extract that supplied the gene 13 product (Table 2). Thus, it would be expected that *ts*N38-infected cells also accumulate maturable filled heads under restrictive conditions. The rapid initial kinetics of phage production observed after a temperature shiftdown (Fig. 3, curve B) support this contention. In the

TABLE 2. *In vitro activity of gene 13-defective 1,200S head particles*[a]

Fraction tested	Tested against	
	Gene 13⁻ 1,200S particles	Gene 23⁻ extract
Gene 13⁻, 1,200S particles	0.8	51.6
Gene 23⁻ extract	—	0.4

[a] Titers are expressed × 10^{-9}/ml.

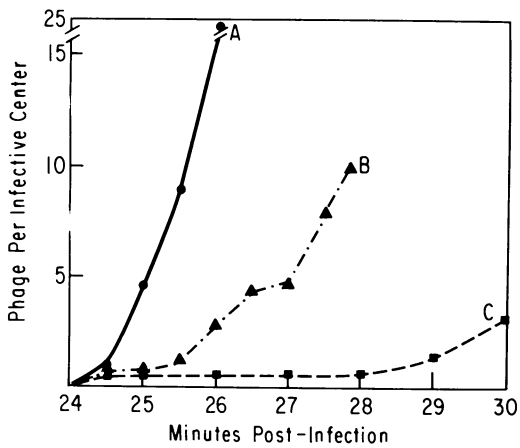

FIG. 2. *Kinetics of phage produced immediately after a temperature shift. Bb cells were infected at 41.5 C with various mutants and assayed for phage after a shift to 25 C, as described in Fig. 1. One culture was infected with tsN1 (curve A), a second with tsN38 (curve B), and a third with tsC9 (curve C).*

*ts*N38 infection, phage-producing capacity was recovered within 2 min; this result is comparable to the 1-min recovery for *ts*N1, a mutant in which only tail fibers have to be added to the phage precursors. In contrast to this rapid rescue is the 5 min recovery period for *ts*C9-infected cells which accumulate defective heads in vivo that are not filled (11).

These results, however, only yield indirect evidence as to the in vivo nature of precursors to gene 13 product. In order to obtain direct evidence sections of embedded *E. coli* Bb infected with either *am*E609, *ts*N38, *am*E727, or T4D were examined through the technique of thin-section electron microscopy (Fig. 3).

Amber, rather than temperature-sensitive, mutants were utilized predominantly in these experiments to minimize recovery of gene function during specimen preparations. The particles observed in the gene 13-defective cells appear to be almost all filled and nearly identical in appearance to those of T4D$^+$ (Fig. 3B, C). Furthermore, they are distinctly more dense than the incompletely filled heads found in the gene 49-defective cells. (Fig. 3A). These results indicate that there are a large number of intracellular filled-head precursors to gene 13 action.

Because filled heads accumulate in a *ts*N38-infected culture grown under restrictive conditions, we reasoned that an assay system to study the mode of gene 13 action could be developed through "pulse-chase" radioactive labeling, temperature-shift experiments with these cells. The schedule of additions to and removals from an *E. coli* Bb culture is illustrated in Fig. 4. Infection and superinfection with *ts*N38 at 41.5 C was followed by a 5-min pulse of ^{14}C-lysine and chase with a 1,000-fold excess of ^{12}C-lysine. The culture was then shifted to 25 C; samples removed at the indicated times were lysed, and the extracts obtained were analyzed on sucrose gradients. A typical set of sedimentation profiles is shown in Fig. 5. The distribution of label found at various times postshift was unexpected. Instead of the flow of amino acid label from the gene 13-defective filled-head (1,200*S*) peak to the phage (1,000*S*) peak, we observed that most of the counts flowed from unfilled heads (300*S*) to phage. Thus, at 0 min after the shift 80% of the counts were in unfilled heads; however, by 40 min later 60% of these counts had shifted into phage. For *ts*N49 and *am*E609, two other mutants in gene 13, we found the following results from a pulse-chase labeling experiment: (i) *ts*N49 showed a redistribution of label from unfilled and filled heads, into phage, similar to that found for *ts*N38 (Fig. 5); and (ii) *am*E609 gave the same distribution profile seen in Fig.

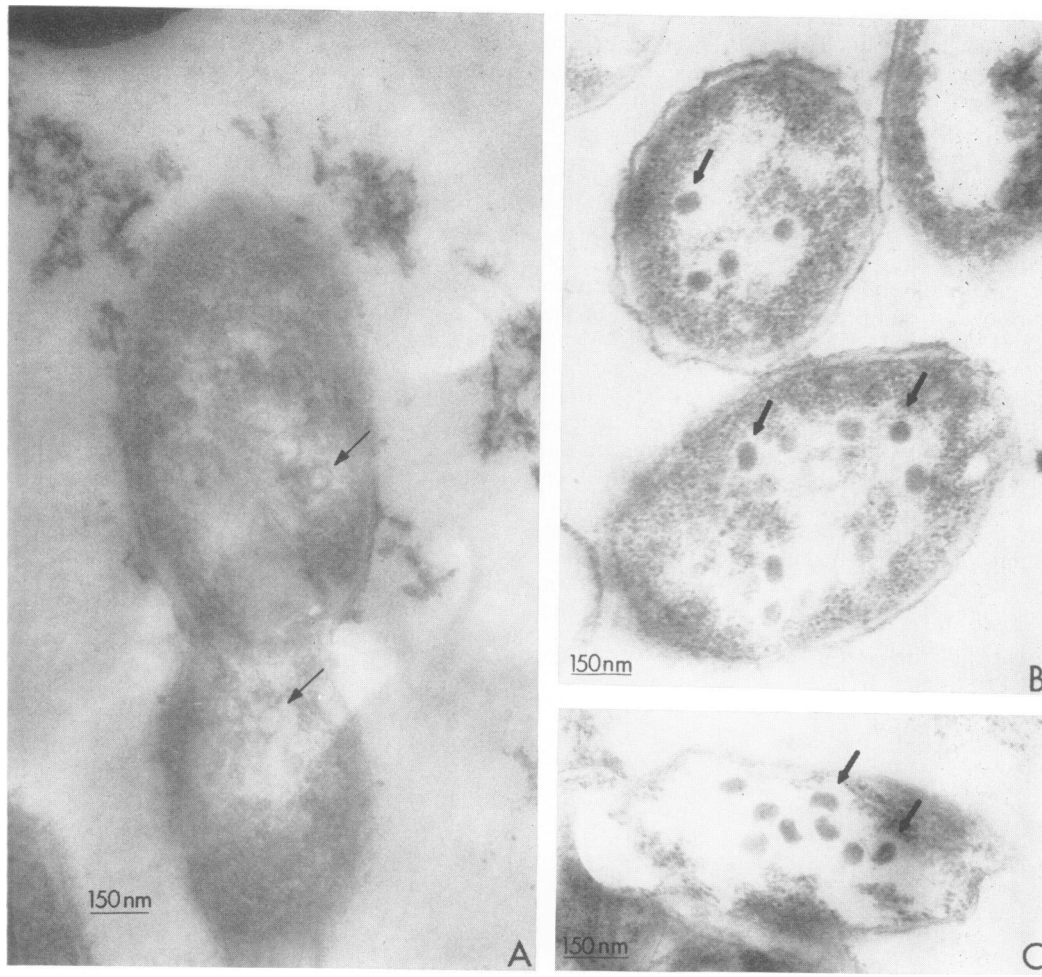

FIG. 3. *Thin sections of T4D-infected cells. In (A) E. coli was infected with amE727 (gene 49) and prepared for sectioning at 30 min postinfection as described; (B) is a wild type T4D⁺-infected cell; (C) is an infection with amE609 (gene 13). Sections through tsN38 infected cells gave the same appearance as in (C). The arrows in (A) point to structures we have referred to as unfilled heads, whereas the heavier arrows in (B) and (C) point to structures we have designated as filled heads. Magnification = × 70,000.*

5(a) at 0, 20, and 40 min shiftdown (D. Hamilton, *unpublished observations*). These results, as well as those shown in Table 2 and Fig. 3 for *am*E609, strongly indicate that the defect of gene 13 seen in *ts*N38-infected cells is gene rather than mutant specific. We also note from Fig. 5 that the sum of total counts in the 300*S* and 1,000*S* peaks is approximately additive at the various times postshift. This suggests that the isolated, gene 13-defective unfilled heads represent phage precursor particles. However, based on what has been previously discussed, we would have expected most, if not all, of the gene 13-defective head particles to be filled. This discrepancy can be explained by several alternatives. (i) All gene 13-defective structures are stable, filled heads (however, most become unfilled as a result of our isolation procedures) or (ii) there are two classes of gene 13-defective filled heads, one class being intrinsically more fragile than the other. The experiments described below were performed to ascertain which was the best alternative.

Gene 13-defective phage precursors. We initially examined extracts from gene 13-defective cultures in the electron microscope. Both *am*E609- and *ts*N38-infected cells showed filled (20%) and empty (70%) appearing head particles (Table 3). Furthermore, the production of such unfilled heads occurred regardless of whether

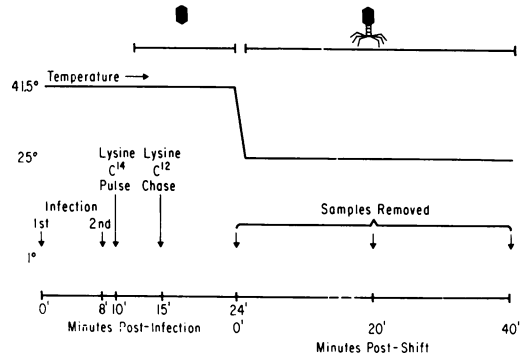

FIG. 4. *Labeling and incubation regime for pulse-chase temperature-shift experiments with tsN38. Bb cells were grown, infected, and shifted in temperature as described. ^{14}C-lysine was added at 10 min post-infection to a level of 0.2 to 0.4 μCi/ml. At 15 min postinfection a 1,000-fold excess of ^{12}C-lysine was added. After the temperature shift, samples were removed and placed on ice. Extracts were then made and analyzed.*

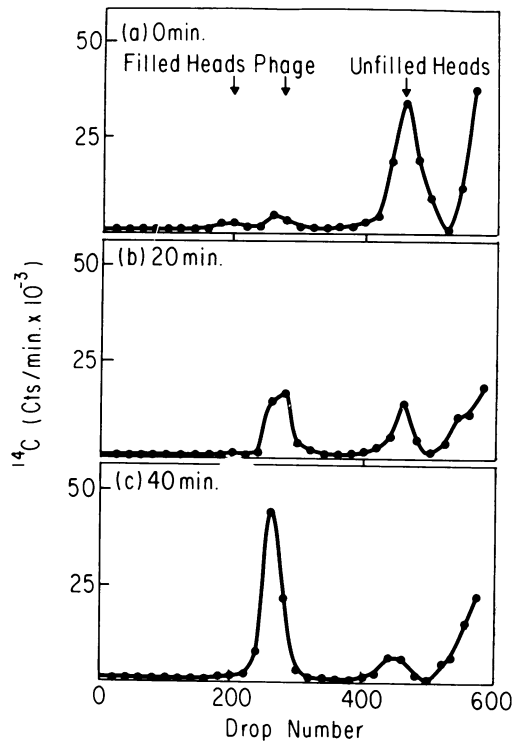

FIG. 5. *Sedimentation profile of labeled proteins in tsN38-infected cultures at various times after a shift-down to 25 C. Cells were infected and labeled according to the regime described in Fig. 3. Five-milliliter samples were removed at 0, 20, and 40 min after the shift to 25 C. Extracts were prepared and analyzed by zone sedimentation as described. The particles present in the peaks were determined by electron microscopy examination of these fractions. For tsN49, another mutant in gene 13, we found that 1, 30, and 45% of the label in the two peaks redistributed itself from unfilled and filled heads into phage at 0, 20, and 40 min postshift, respectively. The lower amount of counts now shifting into phage (30 compared to 60%) by 40 min postshift apparently reflects the overall 50% decrease in burst size seen with tsN49 as compared with tsN38 rescued cultures.*

*am*E609-infected cultures were lysed by chloroform-deoxyribonuclease, lysozyme-ethylenediaminetetraacetate (EDTA) or freeze-thaw treatment (Table 3). The appearance of both filled and unfilled gene 13-defective particles by these several methods lends support to the second alternative discussed. The unlikeliness of the first alternative was strengthened by the following two additional experiments. In the first case, we tested the stability to isolation of already filled heads, such as those present in a gene 27-defective culture (11). We infected two cultures in parallel, one with *ts*N34 (gene 27) and the other with *ts*N38 (gene 13); after 24 min at 41.5 C, labeled extracts were prepared and centrifuged on sucrose gradients as described in Fig. 3. An analysis of the amino acid label distribution over the gradient peaks showed the profile given in Table 4. It can be seen that relative to the total counts in each experiment, about five times as many counts (39%) resided in the gene 27 filled-head peak compared with the gene 13 filled heads (7%). This makes it unlikely that 300*S* gene 13$^-$ heads arose solely from degradation of *stable*, filled heads.

The second study involved a density gradient analysis of gene 13$^-$ heads obtained at various times after a pulse-chase temperature-shift experiment with *ts*N38. In the unshifted culture, two density peaks were observed (Fig. 6A): one at 1.30 to 1.32 g/cm^3 and a second, containing 95% of the total counts, at a density of 1.33 to 1.36 g/cm^3. At various times after the temperature shift, we found the heavier peak decreased in proportion to the lighter peak from an original 15:1 ratio to almost 1:1 (Table 5). This result indicates that only the heavier (average ρ = 1.34 g/cm^3) 300*S* heads are the presumptive phage precursors. The increased density of these structures seems to be caused by a small amount of associated, nuclease-resistant deoxyribonucleic acid (DNA). This was shown by modifying the labeling schedule of Fig. 4 with an additional 5-min pulse of ^{3}H-thymidine administered at 10 min postinfection. After CsCl equilibrium centrifugation, only the heavier density peak of 300*S* unfilled heads was now found to contain ^{3}H

TABLE 3. *Particle counts of head structures in gene 13⁻ extracts*

Time incubated (min)	Mutant	Unfilled heads (UH)	Filled heads (FH)	Ratio (UH:FH)
24[a]	*ts*N38	153 (70%)	37 (18%)	4.1:1
32[a]	*ts*N38	106 (67%)	25 (18%)	4.2:1
30[a]	*am*E609	160 (76%)	40 (19%)	4.0:1
30[b]	*am*E609	72	17	4.2:1
30[c]	*am*E609	149	32	4.6:1
75[d]	*am*E609		29 (17%)	

[a] $CHCl_3$ was added to saturation and the sample was vortexed.

[b] Lysozyme-EDTA was added by the method of Frankel (4).

[c] Freezing of the pellet in dry ice-ethanol was followed by immediate thawing at 30 C.

[d] Data taken from King (6).

TABLE 4. *Comparison of the amino acid label distribution in tsN34- and tsN38-infected cell extracts*[a]

Sedimentation position	Structure	*ts*N34 counts/min (%)	*ts*N38 counts/min (%)
1,200*S*	Filled head	10,500 (39)	6,500 (7)
1,000*S*	Phage	3,600 (13)	10,600 (11)
300*S*	Unfilled head	13,100 (48)	77,900 (82)

[a] Infection and labeling were carried out as described in Fig. 4.

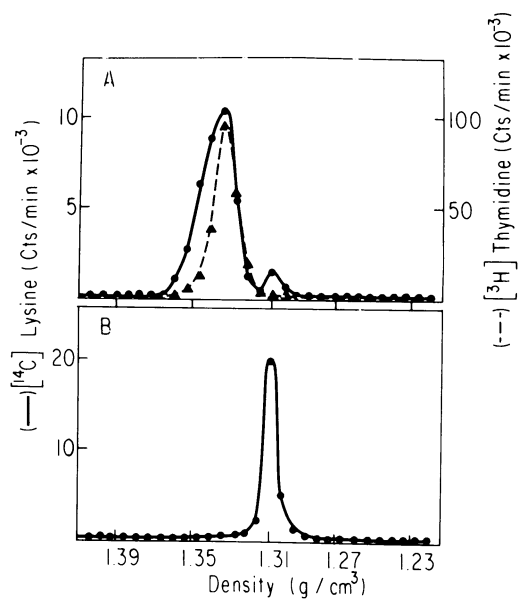

FIG. 6. *Density distribution of 300S particles previously isolated by zone sedimentation. Bb cells were infected and labeled according to the regime described in Fig. 4. At 24 min after infection a sample was removed and an extract was prepared and analyzed by zone sedimentation as described in Fig. 5. The 300S band was isolated for subsequent CsCl density analysis. Electron microscopy examination showed the band contained only unfilled heads and ghosts. The 300S band was made up to a density of 1.30 g/cm³ by addition of solid CsCl (Fisher Scientific Co.). The solution was centrifuged to equilibrium in an SW50 rotor of a Beckman model L3-50 ultracentrifuge at 36,000 rev/min for about 40 hr at 15 C. Fractions were collected directly on membrane filters and analyzed for radioactivity as described. Several fractions were collected in a tube for density determination by measurement of refractive index in a Bausch & Lomb refractometer. Distribution of ^{14}C-lysine is shown by closed circles (—●—) in (A). The triangles (---▲---) in (A) represent ^{3}H-thymidine distribution. (B) shows the distribution of ^{14}C-lysine obtained when the 1,200S zone sedimentation band of gene 13-defective filled heads was artificially disrupted prior to density analysis.*

and ^{14}C label (Fig. 6A). The 300*S* unfilled heads obtained by artificial disruption (freeze-thaw) of stable 13⁻ filled heads (Fig. 6B; average density of 1.31 g/cm³) did not contain any associated DNA by these criteria. The control of frozen-thawed, 300*S* gene 13-defective structures showed no change in labeling pattern from Fig. 6A. Hence, several lines of evidence have made highly unlikely the alternative that gene 13-defective, heavy-density unfilled heads arose from stable 13⁻ filled heads. Instead, it indicates that in addition to stable, gene 13-defective filled heads there are also present unstable, filled heads. On the basis of both the labeling (Fig. 5) experiments and electron microscope counts (Table 2) we estimate that this is in a ratio of about 20% stable and 70% unstable filled heads. We have termed the latter structures fragile, filled heads.

Mode of gene-13 action. The results presented thus far indicate that when the gene 13 function is defective a large proportion of the already packaged heads tend to lose most of their DNA when isolated. It would seem that this instability of the head structure is induced by absence of gene 13 function. In order to understand this induction of head instability, we investigated the effect of prolonged high temperature on already filled, gene 13-defective heads. If *ts*N38-infected cultures were maintained at 41.5 C for longer than 24 min a decrease in phage yield was observed (Fig. 7). The yield decreased to one-third or one-half of the expected burst size when cultures were held at 41.5 C for 32 or 40 min, respectively. This reduction is not due to a flaw in tail attachment; pulse-chase radioactive-labeling experiments with a shiftdown at 32 min

TABLE 5. *Density gradient analysis of the 300S, gene 13⁻ heads present after a temperature shift of tsN38-infected cells*[a]

Determination	Min postshift			
	0	10	20	40
Heavy density peak[b]	34,700	26,700	16,300	8,000
Ratio of counts in the heavy to light density peaks[b]	15:1	6:1	3:1	1:1

[a] Infection and labeling was carried out as described in Fig. 4. The 300S bands were analyzed as described in Fig. 6.

[b] Heavy refers to those counts between densities of 1.33 to 1.36 g/cm³; light refers to those counts between densities of 1.30 to 1.32 g/cm³.

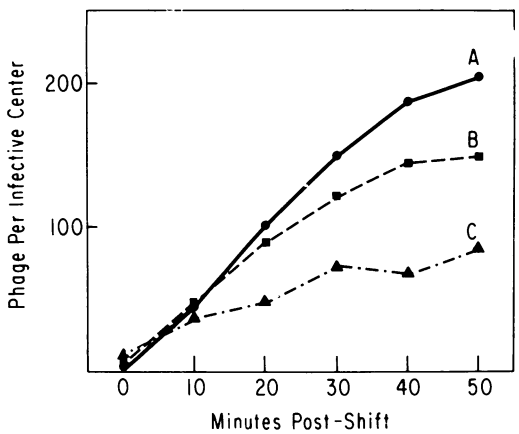

FIG. 7. *Effect of prolonged temperature on phage production of tsN38-infected cultures. Bb cells were infected as described in Fig. 1. After a shiftdown to 25 C, phage production was assayed. Cultures were shifted at 24 min (curve A), 32 min (curve B), or 40 min (curve C) after infection.*

showed that label flowed from the 300S to 1,000S peaks as efficiently as for a 24 min postinfection temperature shift (Table 6). The result shown in Fig. 7 could then be explained if some of the particles in the 1,000S peak (Table 6) were not viable phage. Thus the 1,000S peaks of *ts*N38-infected cells incubated for 24 or 32 min at 41.5 C before shiftdown were assayed for their specific infectivity. Specific infectivity is defined as the ratio of plaque-forming units to the total number of particles as measured by absorbancy (260 nm). We found (Table 7) that this ratio decreased from 1.00 to 0.60 when the culture was left for the additional 8 min at 41.5 C. This indicates that 40% of the 1,000S particles in the latter peak are aberrant phage structures. An

TABLE 6. *Redistribution of labeled protein from 300S to 1,000S at various times after a temperature shift of tsN38-infected cells*

Time of shift (min)	Min postshift	Peak[a]			
		300S	1,000S	1,200S	Total
24	0	77,900	10,600	6,500	95,000
	20	34,000	55,700	2,200	91,900
	40	16,900	71,500	1,100	89,500
32	0	37,800	7,500	2,000	47,300
	20	19,500	27,200	300	47,000
	40	7,100	38,900		46,000

[a] The ^{14}C-lysine counts were summed over the indicated peaks.

TABLE 7. *Specific infectivity of the 1,000S peak obtained after various times of incubation of a tsN38-infected culture*

Determination	Time of incubation at 41.5 C[a]	
	24 min	32 min
No. of particles (OD_{260})[b]	4×10^{10}	6.14×10^{10}
Plaque-forming units (PFU)[c]	4.4×10^{10}	3.9×10^{10}
Ratio (PFU-particles)	1.10	0.63

[a] Followed in both cases by a 40 min incubation at 25 C.

[b] Number of particles was determined by comparing the OD_{260} to a standard curve for wild type T4D⁺.

[c] Plaques were assayed on S/6 at 30 C.

electron microscope examination of the 24 and 32 min peaks was then performed to see if any structural alterations had occurred.

The 1,000S particles obtained from the 24 min shift were identical in appearance to wild-type phage (data not shown). In contrast to this observation, many of the 1,000S particles obtained from the 32 min shift did not appear normal (Fig. 8A, B). Such head structures had either disconnected, broken, or missing tails. Isolated tails observed in other areas of the grid contained tail fibers. Because fibers cannot attach to free tails (6), this suggests that the heads seen in this fraction probably had tails on them which were lost during specimen preparation. There were also many wild-type phage present. The particle counts done over several fields of view (Table 8) are consistent with the ratio of 60% viable phage being present in the 1,000S peak.

The studies thus far reported have pointed out several deficiencies that result as a consequence

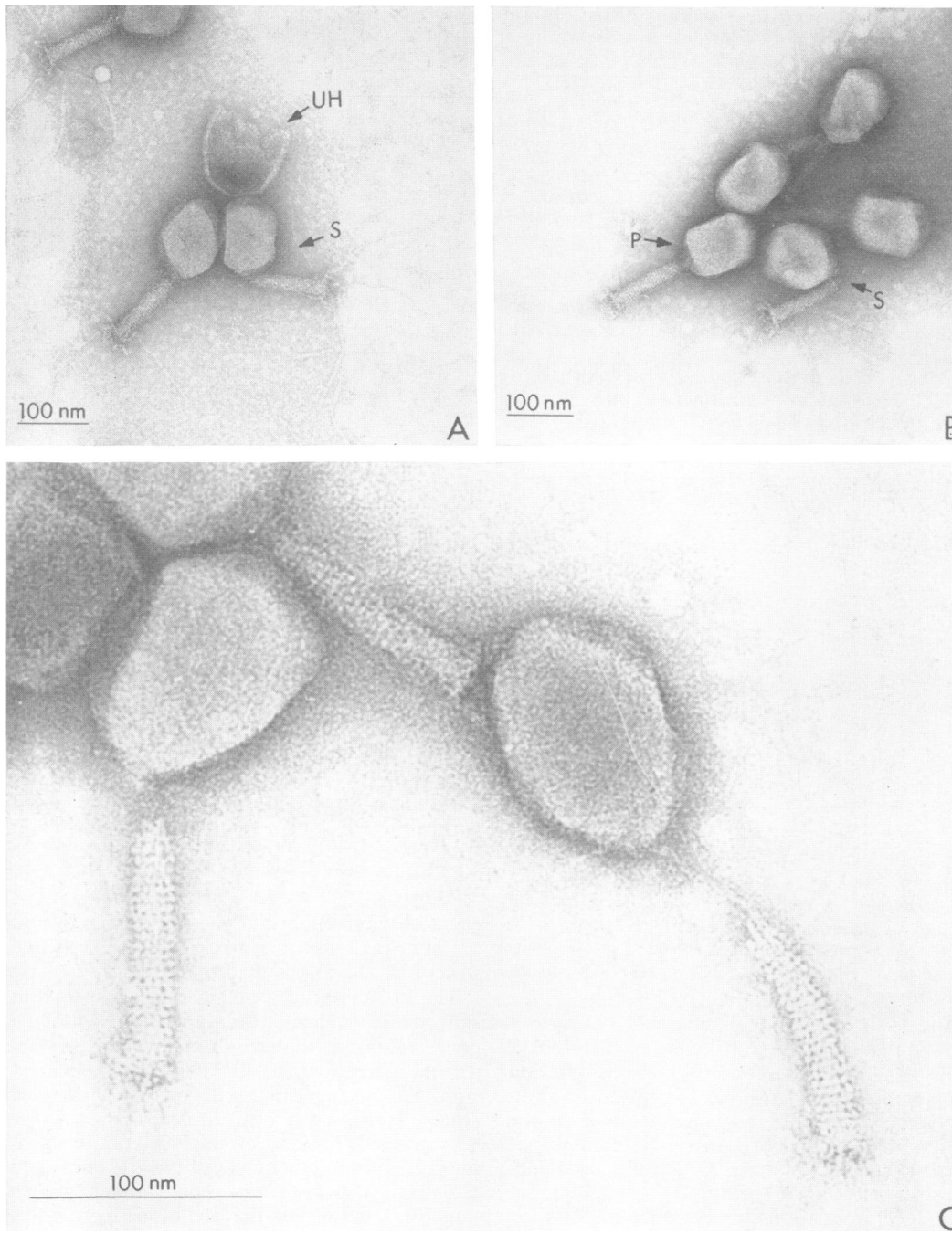

FIG. 8. *Phagelike structures isolated from tsN38-infected cells incubated for an extended time (32 min) at 41.5 C followed by a 40 min recovery period at 25 C. Sections (A) and (B) show representative particles from the 1,000S peak. Isolation and preparative techniques have been described in Table 7. The structure labeled P is a normal-appearing phage; those marked UH (unfilled head) and S (phage with disconnected tail), are counted as aberrant. The latter is the predominate type of aberrant form observed. Magnification = ×132,000. Section (C) is a highly magnified micrograph of two such aberrant, phagelike particles. Magnification = ×430,000.*

TABLE 8. *Morphology of 1,000S particles obtained after rescue of the gene 13 function*[a]

Determination	Time of incubation at 41.5 C	
	24 min	32 min
No. of particles counted	425	317
No. of normal-appearing phage[b]	412	196
Ratio (particles-phage)	.97	.62

[a] The 1,000*S* particles were prepared as described in Table 7.

[b] See Fig. 8 for a description of normal and aberrant phage particles.

of the absence of gene 13 function. A clue to what this function is may be seen in the high-resolution electron micrograph of Fig. 8C. Here the tail has disconnected from the head, leaving a rather tenuous connecting strand between these structures. The particular nature of this connection, as well as a biochemical characterization of the gene 13 product, is being undertaken to further characterize the union between T4D$^+$ head and tail.

DISCUSSION

Pulse-chase, temperature-shift experiments suggested that 300*S*, gene 13-defective unfilled heads isolated from infected cultures served as phage precursors. However, sections through these cells show that all head structures appear filled in vivo. On this basis, the above gene 13-defective head precursors have been termed fragile, filled heads. The unfilled heads presumably occur as a result of leakage of DNA from these fragile, filled heads. However, about 12 to 32% of the total DNA remains associated with these 300*S* structures. This is in contrast to the stable, gene 13-defective filled heads where breakage of heads releases all of the DNA. In vitro complementation experiments have shown that these are also phage precursors. Thus, there are two classes of precursor particles to gene 13 action, i.e., filled heads and fragile, filled heads. Their presence may be explained by the idea that the absence of gene 13 product results in an accumulation of precursors blocked at several steps in T4 head maturation. Whether such an action is related to a structural function of gene 13 which must occur in order to remove the block or is due to an enzymatic role cannot be assessed by these results. Snustad (13) reported that the gene 13 product was stoichiometric in behavior, indicating a possible structural role for gene 13, but his results were subject to the provision that polar effects were not involved. Gene 13 *am* mutants, however, do exert a polar effect on gene 14 (1), and the stoichiometric behavior shown by the gene 13 product might be due instead to gene 14 product.

It has also been shown that if *ts*N38-infected cultures are maintained at 41.5 C for extended periods of time, they produce a structural defect in many 1,000*S* particles recovered after a shift to permissive temperature. This defect is observed as an unstable head-tail union. Our results do not indicate whether this aberrant byproduct results from a direct failure of gene 13 product to carry out its function or is due to some other function which acts abnormally due to lack of gene 13 product. Presently, investigations are in progress to isolate the gene 13 product so that we can directly determine its role in head-tail union.

ACKNOWLEDGMENT

We thank C. Ganz for her excellent technical assistance. We are extremely grateful to R. Webster and D. Hall for their critical reading of the manuscript. The research was supported by Public Health Service grants 1-RO1-CA-11976 from the National Cancer Institute and predoctoral traineeship 2 TO1-GM01019-09A1 from the National Institute of General Medical Sciences and National Science Foundation grant GB-8077 to D.L.H.

LITERATURE CITED

1. Edgar, R. S., and I. Lielausis. 1968. Some steps in the assembly of bacteriophage T4. J. Mol. Biol. 32:263–276.
2. 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.
3. Epstein, R. H., A. Bolle, C. M. Steinberg, E. Kellenberger, E. Boy de la Tour, R. Chevalley, R. S. Edgar, M. Sussman, G. A. Denhardt, and A. Lielausis. 1963. Physiological studies of conditional lethal mutants of bacteriophage T4D. Cold Spring Harbor Symp. Quant. Biol. 28:375–394.
4. Frankel, F. R. 1966. Studies on the nature of replicating DNA in T4 infected *Escherichia coli*. J. Mol. Biol. 18:127–143.
5. Kellenberger, E., F. A. Eiserling, and E. Boy de la Tour. 1968. Studies on the morphopoiesis of the head of phage T-even. III. The cores of head-related structures. J. Ultrastruct. Res. 23:335–360.
6. King, J. 1968. Assembly of the tail of bacteriophage T4. J. Mol. Biol. 32:231–262.
7. King, J. 1971. Bacteriophage T4 tail assembly: four steps in core formation. J. Mol. Biol. 58:693–709.
8. Laemmli, U. K., F. Beguin, G. Gujer-Kellenberger. 1970. A factor preventing the major head protein of bacteriophage T4 from random aggregation. J. Mol. Biol. 47:69–85.
9. Luftig, R. B. 1968. Further studies on the dimensions of viral and protein structures using the catalase crystal internal marker technique. J. Ultrastruct. Res. 23:178–181.
10. 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.
11. 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.
12. Meezan, E., and W. B. Wood. 1971. The sequence of gene

product interaction in bacteriophage T4 tail core assembly. J. Mol. Biol. 58:685–692.
13. Snustad, D. P. 1968. Dominance interactions in *Escherichia coli* cells mixedly infected with bacteriophage T4D wild-type and amber mutants and their possible implications as to type of gene-product function: catalytic vs. stoichiometric. Virology 35:550–563.
14. Steinberg, C. M., and R. S. Edgar. 1962. A critical test of a current theory of genetic recombination in bacteriophage. Genetics 47:182–208.
15. 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.