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Bacteriophage T4 Head Maturation: Release of Progeny DNA from the Host Cell Membrane

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We have presented a new approach to studying bacteriophage T4 head maturation. Using a modified M-band technique, we have shown that progeny deoxyribonucleic acid (DNA) was synthesized on the host cell membrane throughout infection. This DNA was released from the membrane later in infection as the result of formation of the phage head; detachment of the DNA required the action of gene products 20, 21, 22, 23, 24, 31, 16, 17 and 49, known to be necessary for normal head formation. Gene products 2, 4, 50, 64, 65, 13 and 14, also involved in head morphogenesis were not required to detach progeny DNA from the membrane; the presence of the phage tail and tail fibers also was not required. DNA was released in the form of immature heads and initially was sensitive to deoxyribonuclease (DNase). Conversion to DNase resistance followed rapidly. The amount of phage precursors present at the time of DNA synthesis determined the time of onset and detachment rate of DNA from the M band as well as the kinetics by which the detached DNA become DNase resistant.

Bacteriophage T4 head maturation has been studied for the most part by a single type of observation; lysates of cells infected under nonpermissive conditions with conditional lethal mutants have been examined by electron microscopy. The role of each head gene has been inferred from the stage of morphological arrest in the various mutants (20, 7, 18, 6, 30, 23, 31). More recently the problem has been approached biochemically, and the proteins from lysates of mutant-infected cells have been examined by acrylamide gel electrophoresis (21, 2, 14, 15, 3, 8, 17, 19). From these observations, capsid morphogenesis can be divided into four stages—head formation, DNA packaging, and two completion steps—but these processes are poorly understood.

It has not yet been possible to study T4 head morphogenesis by in vitro assembly. Emphasis has been on the use of mutant phenotypes rather than on the search for normally occurring intermediates in head formation. This can be misleading since head-related structures which accumulate in infections with mutant phages may not represent normal stages in maturation but may be derived from them as aberrant forms.

In this report we describe a method for studying T4 maturation which potentially can be used for isolating intermediates in capsid formation from cells infected with wild-type phages. We used the magnesium-Sarkosyl crystals or M-band technique (5, 28) as a tool for fractionating the T4 infected cell. This method can be used to separate the cell membrane DNA complex from other relevant cell constituents, in this case mature phage and free DNA. This technique was previously used to examine the association of newly made and parental T4 DNA with the host cell membrane. Phage DNA was found in the M band early in infection and became detached later (4, 5). Thus it seemed likely that the detachment of DNA, a late event, was a result of phage maturation (4).

Because cells late in infection are very fragile, we modified the M-band method accordingly for the study of these late events. We found that detachment of progeny DNA from the membrane requires functional products of the genes involved in head formation and DNA packaging.

MATERIALS AND METHODS

Bacterial strains. *Escherichia coli* CR63 was the permissive host for amber mutants of T4, and *E. coli* B was used as the restrictive host. Both were obtained from E. B. Goldberg.

Bacteriophage strains. For bacteriophage strains used, see Table 1.

Media. Phage (P) broth (29) was used as the growth medium. Dilution fluid and washing fluid used in phage purification have been described (4).

Chemicals and radiochemicals. Sarkosyl NL-30 was a gift of Geigy Chemical Corporation,

TABLE 1. *Mutants of T4D*

Phage	Gene and function	Source
*ac*41	Wild-type, acridine resistant	E. Goldberg
*am*H26	*e*, lysozyme	E. Goldberg
*am*B5	*t*, "membranase"	R. Josslin (16)
*ts*A81	55, maturation	E. Goldberg
*ts*N8	21, head	E. Goldberg
*am*N135	5, tail	E. Goldberg
*am*N52	37, tail fiber	E. Goldberg
*am*N69	12, base plate	E. Goldberg
*am*B17/H11	23, head	J. King
*am*N66	16, head	J. King
*am*N56	17, head	J. King
*am*E727	49, head	J. King
*am*N50	20, head	W. Wood
*am*N76	21, head	W. Wood
*am*B270	22, head	W. Wood
*am*E58	24, head	J. King
*am*N54	31, head	W. Wood
*am*N51	2, head	W. Wood
*am*N112	4, head	W. Wood
*am*A458	50, head	W. Wood
*am*E1102	64, head	W. Wood
*am*E38	65, head	W. Wood
*am*E609	13, head	W. Wood
*am*B20	14, head	W. Wood

Ardsley, N.Y. All radiochemicals were purchased from New England Nuclear Corp. The phosphoric acid-^{32}P was carrier free in 0.02 N HCl. Thymidine-*methyl*-^{3}H and thymidine-2-^{14}C had specific activities of 6,700 mCi/mmol and 50 mCi/mmol, respectively.

Enzymes. Lysozyme, ribonuclease (RNase; bovine pancreas), and deoxyribonuclease (DNase) I (bovine pancreas) used for phage purification, and DNase I (RNase-free, electrophoretically purified), used for determining DNase sensitivity of nascent T4 DNA, were all purchased from Worthington Biochemical Corp. Pancreatin was obtained from Calbiochem.

Growth and infection of cells. A 2-ml amount of an aerated overnight culture of *E. coli* B was used to inoculate 200 ml of broth. The culture was grown with aeration at 37 C to a titer of 2×10^8 and concentrated to 5×10^8 to 1×10^9 cells per ml. Phage were added at a multiplicity of infection of 5 to 7 and absorbed for 2 min at 37 C without shaking. Infection was timed from the start of aeration. Viable cell counts were taken at 5 min after addition of phage at which time 98 to 100% of the cells were infected.

Labeling of infected cells. Unless stated otherwise, infected cells were continuously labeled with 5 to 10 μCi of thymidine-*methyl*-^{3}H per ml added between 7 and 8 min after infection at 37 C.

Modified M-band procedure. When indicated, samples were harvested and M bands were prepared by a modification of the method of Earhart (4). Unless stated in the text, 0.4-ml samples of cultures were placed directly on the top of a linear 15 to 47% sucrose gradient prelayered with 25 μliters of 1 M NaCN. A 0.1-ml amount of a 1:1 mixture of lysozyme (2 mg/ml) and a 2.7 mg/ml concentration of ethylenediaminetetraacetic acid (EDTA) were then added and mixed with the samples. Care was taken to avoid disturbing all but the uppermost portion of the gradients. The gradient tubes were allowed to stand for 30 min and then 0.05 ml of 5% Sarkosyl and 0.1 ml of 0.1 M $MgSO^4$ were added to the top and crystals were allowed to form. The layers on top of the gradients were mixed and allowed to stand undisturbed for a further 15 min. Centrifugation, fractionation, and other methods were described by Earhart et al. (5).

DNase treatment. M bands and top fractions were collected. Samples were treated with 50 μg of DNase I per ml for 2 h at 30 C. Controls indicated that newly made DNA in the M band was 96% sensitive and that purified T4 phage were resistant.

Preparation of unlabeled phage stocks. Phage from a single plaque was suspended in 10 ml of broth containing a few drops of chloroform, mixed, and allowed to stand 30 min at room temperature. Soft agar overlay plates were made by mixing about 10^4 phage with fresh *E. coli* CR63 plating bacteria. After growth, a few drops of chloroform and 5 ml of broth were added to each plate and allowed to stand for 30 min. The overlay agar was removed to centrifuge tubes, and additional broth was added to fill the tubes. Agar and bacterial debris were removed by centrifugation

at 12,000 × *g* for 5 min. The supernatant fluid was treated with RNase and DNase (10 μg/ml final concentration) for 30 min at 37 C and then about 10 mg of pancreatin was added for an additional 30 min. The lysate was filtered sequentially through membrane filters (0.80 μm and 0.45 μm pore size, Millipore Corp). Phages were sedimented by ultracentrifugation at 50,000 × *g* for 45 min, washed three times, and resuspended in washing fluid.

Preparation of ^{32}P-T4. ^{32}P-T4 phage were prepared as described by Earhart (4). After phages were purified by washing, they were sedimented to equilibrium in CsCl. The phage band was collected with a syringe and dialyzed sequentially against 4 M NaCl, 3 M NaCl, 2 M NaCl, 1 M NaCl (all in washing fluid). The last dialysis was against washing fluid without added salt.

Preparation of ^{32}P-T4 DNA. Three times phenol-extracted ^{32}P-T4 DNA was a gift of L. A. McNicol.

RESULTS

Localization of mature phage and purified phage DNA in the gradient. To determine whether mature phage or purified phage DNA were entrained in the M band, we conducted the following reconstruction experiment. *E. coli* B was infected with T4 *am*H26 (lysozyme minus) and was harvested at various times according to the procedure of Earhart (4). The cells were resuspended in buffer containing 0.025 M NaCN. Each time sample was divided into four parts which were layered on gradients and treated with lysozyme-EDTA. Two parts were mixed with ^{32}P-T4 (6×10^8 plaque-forming units) or 3.5 μg of ^{32}P-T4 DNA. The remaining two samples were mixed with labeled phage or DNA after the lysis treatment and the addition of Mg^{2+} and Sarkosyl. The results presented in Table 2 indicate that 95 to 99% of the phage and 91 to 99% of the phage DNA were found in the top fraction. Thus, neither phage nor phage DNA are entrained in the M band under our conditions.

Harvesting procedures for fragile infected cells. Earhart (4) reported that progeny DNA was synthesized on the host membrane early in infection and in the cytoplasm at later times. Since cells in the late stages of infection may be lysed during the harvesting procedure, newly made DNA in the cytoplasm may appear due to artifactual shearing of DNA from the membrane. We investigated this possibility by comparing the standard method with procedures designed to minimize lysis of cells late in infection.

At various times after infection with *am*H26 (gene e^-) *E. coli* B was pulse-labeled for 60 s with 10 μCi of tritiated thymidine per ml. Two samples were taken at each time indicated. One was treated with lysozyme on top of a gradient (see Materials and Methods). The other sample was treated by the standard M-band method (Earhart, [4]), which involves lysozyme treatment in a test tube prior to layering on the gradient. As shown in Table 3, M bands from cells treated with lysozyme in a test tube contained much less newly synthesized DNA than those from cells layered directly on the gradient. Similar results

TABLE 2. *Reconstruction experiment to determine location of mature phage and mature phage DNA in gradient*

Addition to cells[a]	Fraction	Radioactivity in each fraction (%)		
		Time after infection		
		0 min	15 min	25 min
A. ^{32}P-T4	Top	96.0	96.5	97.5
	M band	3.1	3.3	2.1
	Bottom	0.2	0.1	0.1
	Pellet	0.6	0.1	0.3
^{32}H-T4 DNA	Top	91.0	98.5	97.5
	M band	6.1	1.0	2.2
	Bottom	1.4	0.4	0.0
	Pellet	1.5	0.1	0.3
B. ^{32}P-T4	Top	98.2	98.4	98.6
	M band	1.6	1.2	1.3
	Bottom	0.0	0.4	0.0
	Pellet	0.2	0.0	0.1
^{32}P-T4 DNA	Top	96.8	95.4	97.5
	M band	2.6	4.4	2.3
	Bottom	0.0	0.0	0.2
	Pellet	0.6	0.2	0.0

[a] A, DNA and T4 present for 20 min with lysozyme-EDTA. B, DNA and T4 added to gradient after Sarkosyl and Mg^{2+}.

TABLE 3. *Location of nascent T4 DNA using two methods of sample preparation*

Time after infection (min)	Newly made DNA in M band (%)[a]	
	A	B
5	64	99
10	63	99
15	28	97
20	24	93

[a] Method A, Samples were treated by the standard procedure (4). Method B, Samples were placed directly in gradient, spheroplasted, and lysed on the gradient. Each time sample was collected after a 1-min pulse of tritiated thymidine.

were obtained using wild-type T4. We interpret this to mean that the standard procedure resulted in premature lysis of cells late in infection and that this caused shearing of nascent DNA from the M band.

We have used this improved harvesting technique to examine the localization of newly synthesized phage DNA. DNA pulse labeled early in infection was initially found in the M band (Fig. 1). Later, detachment occurred and the DNA was released into the top fraction. The same rate of detachment was observed for both wild-type and *am*H26 (gene e^-)-infected cells. Similar results were obtained for cells infected with *am*B5 (reference 16; gene t^-) at 25 C (not shown). Figure 1 shows that the detachment kinetics of

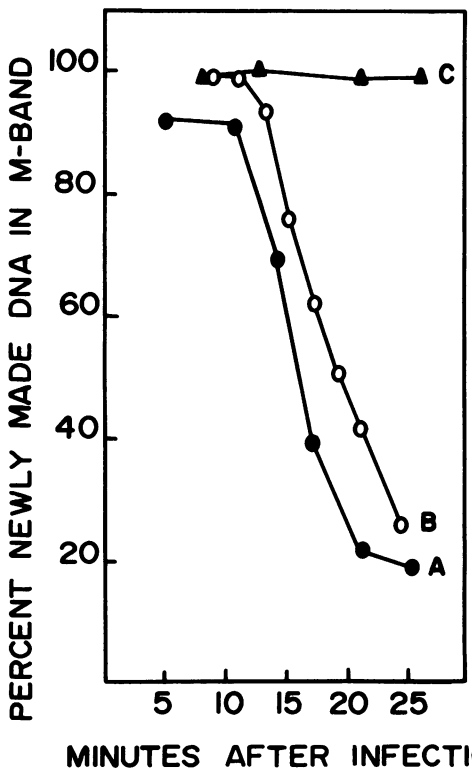

FIG. 1. *Detachment of newly made DNA from M band at 37 C. A, 4.5 min after infection of E. coli B with T4D ac 41 or T4 amH26, 15 μCi of ^{3}H-thymidine per ml was added for 45 s. Incorporation was terminated by resuspending infected cells in broth containing 500 μg of thymidine per ml (●). B, At 6 min after infection of E. coli B with T4D ac41, 0.5 μCi of thymidine 2-^{14}C per ml was added (○). C, At 4 min after infection of E. coli B with T4 tsA81 at 42 C 5 μCi of thymidine-^{3}H per ml was added (▲). Samples were taken at various times and placed on the top of gradients. The percentage of DNA in the M band was determined for each time indicated.*

pulse-chase-labeled DNA (curve A) are similar to those observed with continuously labeled progeny DNA (curve B). Therefore, in many of the experiments to be described, continuous labeling was used to avoid difficulties in washing out thymidine pools after a pulse.

Requirements for detachment of DNA from the M band. We hypothesized that detachment of DNA from the M band was the result of its encapsulation into the head structure. To test this idea, detachment was examined using various mutants of T4 defective in maturation and head formation.

Late protein synthesis was shown to be necessary for DNA detachment by the following experiment. DNA was pulse labeled during infection with *ts*A81, a maturation-defective mutant in gene 55. Under nonpermissive conditions where no late protein synthesis occurred (26), the newly made DNA did not detach from the M band (Fig. 1C).

Next we examined various mutants defective in maturation processes to determine their role in the detachment of DNA. Rates of detachment comparable with wild-type were obtained for mutants in tail formation (*am*N135, gene 5), in tail fiber formation (*am*N52, gene 37), and in base plate completion (*am*N69, gene 12). Therefore, normal morphogenesis of the tail or tail fiber was not required to detach DNA from the M band. This result was expected since the head morphogenesis pathway is independent of formation of the tail and tail fibers.

The rates of detachment of a variety of mutants defective in head morphogenesis were similarly examined. The results are summarized in Table 4. It was found that gene products necessary for head formation were required for detachment of DNA from the M band.

Genes 16, 17, and 49 are thought to be involved in DNA packaging (28) since the empty heads observed in mutant lysates appear to be formed normally (20). The products of these three genes were found to be necessary for DNA detachment from the M band. Mutants in gene 49 showed the least detachment of all the genes tested. An average of only 1% was detached.

The action of genes 2, 50, 64 (which are necessary for cleavage of head proteins at normal rates [21]), genes 13 and 14 (which are necessary to convert the filled head to a substrate for tail addition [6]), and genes 4 and 65 were not required to detach DNA from the M band. Detachment kinetics for these mutants were the same as those shown in Fig. 1 for wild-type T4.

We concluded from these results that the T4

TABLE 4. *Detachment of progeny T4 DNA from the M band after infection of E. coli with various phage mutants*

Gene	Defective function	Detachment[a]
55 *ts*A81	Maturation	−
20 *am*N50	Head formation	−
21 *am*N76, *ts*N8		− (20%)
22 *am*B270		−
23 *am*B17/H11		−
24 *am*E58		−
31 *am*N54		−
16 *am*N66	DNA packaging	−
17 *am*N56		−
49 *am*E727, *ts*C9		−
2 *am*N51	Head formation and completion	+
4 *am*N112		+
50 *am*458		+
64 *am*E1102		+
65 *am*E38		+
13 *am*E609	Head completion	+
14 *am*B20		+
5 *am*N135	Tail	+
12 *am*N69	Base plate addition	+
37 *am*N52	Tail fiber	+

[a] −, Detachment defective, usually no more than 5% of DNA is released; +, detachment functional, 70 to 90% of DNA released. See Fig. 1 for representative rate of nascent DNA detachment.

DNA is detached from the host cell membrane in the form of a phage head, requiring the action of gene products 20, 21, 22, 23, 24, 31, 16, 17, and 49, but not 2, 50, 65, 64, 4, 13 or 14.

DNase sensitivity of detached DNA. DNA in the M-band fraction is sensitive to degradation by DNase whereas DNA in the mature phage is resistant. We wished to determine the kinetics of conversion from DNase sensitivity to resistance.

Wild-type infected cells were pulse labeled with ^{3}H-thymidine at 4.5 min after infection. Incorporation of label was terminated at 5.25 min by chilling and resuspending the infected cells in phage broth containing 500 μg of thymidine per ml. Samples were taken at various times during the chase and layered directly on a chilled gradient. After centrifugation, the top and M-band fractions were treated with 50 μg of DNase per ml for 2 h at 30 C or with 50 μg of bovine serum albumin per ml as a control. Figure 2A shows that the proportion of DNA in the top fraction, which is DNase sensitive, decreased as a function of time after infection. The results presented in Fig. 2B are expressed as the ratio of DNA resistant or sensitive to DNase in each fraction to total DNA (DNA in the top and the M band before DNase treatment). It appeared from these data that DNA synthesized in the M-band fraction was released in a DNase-sensitive form onto the top fraction where it rapidly became DNase resistant.

In this experiment there appeared to be a short-lived, DNase-sensitive capsid intermediate which could have been an artifact resulting from the rupture of mature but unstable heads without tails. Therefore we repeated the experiment with a tailless mutant, *am*N135 (gene 5). The amount of DNase-resistant DNA in the top fraction was much less than that found in wild-type T4 (compare Fig. 3A and 2A). Most of the DNase-sensitive DNA probably arose from unstable free heads, since in the *am*N135 infection DNase-sensitive DNA continued to increase with time (Fig. 3B). Presumably this reflected the production of new unstable heads which were not converted to DNase-resistant phage particles. However, some DNA released in a sensitive form appeared to be a precursor for the DNase-resistant heads (Fig. 3B). It is likely then that the head is released from the M band in an uncompleted DNase-sensitive form which becomes DNase resistant by the action of head gene products, per-

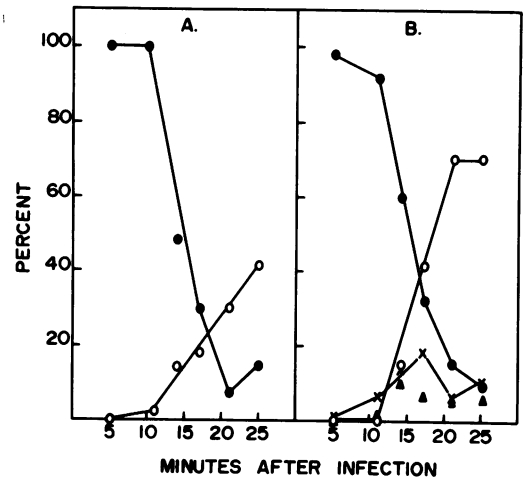

FIG. 2. *DNase sensitivity of detached DNA in a wild-type infection. At 4.5 min after infection of E. coli B with T4 ac41, 13 μCi of ^{3}H-thymidine per ml was added. Incorporation was terminated at 5.25 min by resuspending infected cells in broth containing 500 μg of thymidine per ml. Samples were placed on gradients, and M-band determinations were made at the times indicated. The M band and top fraction from each sample were divided in two, half was treated with DNase and half served as a control as described in the text. A, Percent of the DNA in the top fraction sensitive to DNase (●), and percent of the DNA in the M band resistant to DNase (○). B, DNase-sensitive DNA in M band/total DNA (●); DNase-resistant DNA in top fraction/total DNA (○), DNase-sensitive DNA in top/total DNA (×), and DNase-resistant DNA in M band/total DNA (▲).*

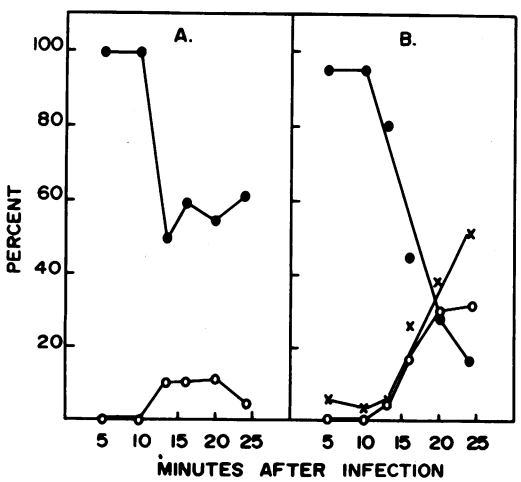

FIG. 3. *DNase sensitivity of detached DNA in an amN135 infection. ^{3}H-thymidine was present from 4.25 min to 5 min after infection of E. coli B with amN135 (gene 5$^-$). The experimental procedure was described in the legend Fig. 2. The symbols are the same as in Fig. 2.*

haps 13 and 14. Complete stabilization is lacking without the tail however (Fig. 3B).

Detachment of DNA at various times. An attempt was made to correlate the time of DNA synthesis with the time it detached from the M band. Figure 4A shows that detachment occurred at the same rate whether the DNA was pulse labeled at 6, 8, or 10 min after infection. When detachment of DNA from the M band was plotted as a function of time after pulse labeling (Fig. 4B), the results showed that, with DNA made later, the onset of DNA detachment occurred sooner than with DNA made earlier in infection. Later times were examined using *am*H26 and similar results were obtained (DNA labeled at 6, 11, and 15 min).

Later in infection, more rapid encapsulation of phage DNA may reflect the presence of larger amounts of structural phage precursors. To test this hypothesis the experiment was repeated at late times of infection using *E. coli* infected with the lysis delay mutant *am*B5. DNA was pulse-labeled for 1 min from 40, 43, and 46 min after infection at 25 C. Again, DNA synthesized later was detached sooner than DNA made earlier. The difference was less dramatic than shown in Fig. 4B. DNA made at 46 to 47 min was detached 2 min sooner after the pulse than DNA made at 40 to 41 min. Therefore, the amount of structural

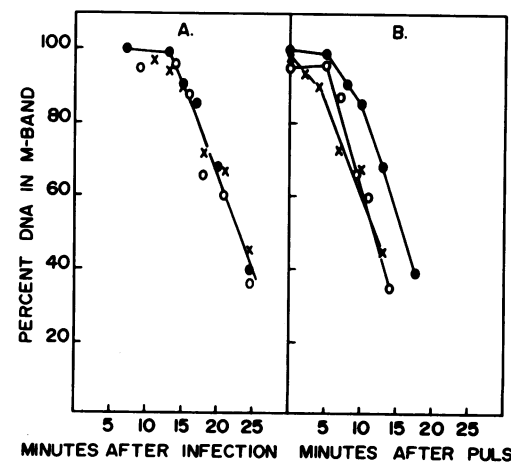

FIG. 4. *Detachment of DNA made at various times. A, E. coli B infected with T4D ac41 was labeled with 20 μCi of thymidine ^{3}H per ml from 6 min to 6.75 min (●) or 8 min to 8.75 min (○) or 10 min to 10.75 min (×). Incorporation was terminated by resuspending infected cells in broth containing 500 μg of thymidine per ml. Samples were taken and processed as described in the legend to Fig. 1. B, The data presented in Fig. 4A is replotted as percentage of DNA in the M band vs. min after the pulse, rather than time after infection.*

precursors present at the time the DNA was synthesized does affect its rate of detachment.

Figure 5 shows that DNA synthesized later and, once detached, became DNase resistant more quickly than DNA made earlier. The rate of completion of phage heads as evidenced by their increased resistance is thus also influenced by availability of structural phage precursors.

DISCUSSION

Using modifications of the M-band technique we have found that T4 DNA is synthesized on the host membrane throughout infection (Table 3). This is consistant with the results of others (1, 25, 27) who found that the replicative complex could be isolated as rapidly sedimenting material presumably due to the association of DNA with other cell components.

After synthesis, the DNA remains attached to the membrane until it is removed by encapsulation. The capsid is probably released from the membrane in a nearly complete but immature form. These conclusions are drawn from the gene product requirements for detachment (Table 4) and the DNase sensitivity of the DNA before and after detachment from the membrane (Fig. 2 and 3).

Late protein synthesis was found to be required for the release of progeny DNA from the M band. Earlier results of Frankel (9, 11) demonstrated that inhibition of protein synthesis prevented the formation of mature DNA molecules from long replicative DNA. DNA in the M band probably consists of concatemers from which DNA is released by encapsulation, since normal head morphogenesis was found to be required for detachment of the DNA from the host membrane. T4 mutant in genes 20, 21, 22, 23, 24, 31, 16, 17, or 49 released little DNA from the M band. On the other hand, products of genes necessary for completion of the assembled head, i.e., 2, 4, 50, 64, 65, 13, and 14, were not found to be necessary for detachment. These results are in agreement with those of Frankel (10) and Fujisawa and Minigawa (13) who found that gene products 20, 21, 22, 23, 24, 31, 16, 17, and 49 were necessary to cut the replicative concatemers into mature phage lengths. Thus, although Frankel et al. demonstrated in vitro that head formation is not necessary for cutting genome lengths from concatemers (12), our results and those of others indicate that in vivo normal head formation is essential for cutting genome lengths from the replicative complex.

In addition, the gene product requirements (Table 4) for detachment indicate that this DNA is associated with a nearly completed capsid.

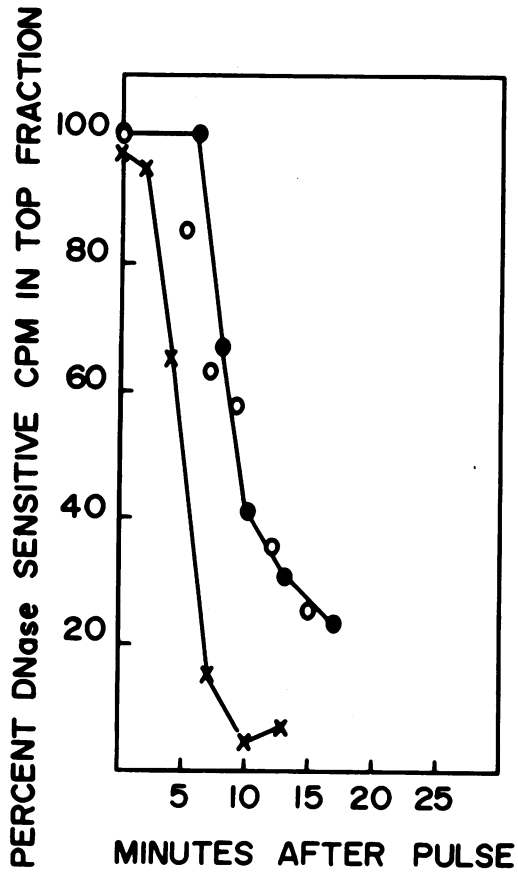

FIG. 5. *DNase sensitivity of detached DNA. Top fractions from the experiment described in Fig. 4 were divided in two, half was treated with DNase and half served as a control. The percent of each sample which was DNase sensitive is shown. The symbols are the same as in Fig. 4.*

DNA is released from the M band in a DNase-sensitive form which rapidly becomes resistant to the enzyme (Fig. 3B). Perhaps action by gene products 13 and 14, known to act in vitro to make the head a substrate for the tail (6), serves to plug the head and makes it resistant to DNase, though unstable without the tail (Fig. 4B).

The observation that DNA made later becomes detached from the M band more quickly may be explained by the increase in the pool of phage head precursors at later times (14), i.e., greater amounts of those gene products required for detachment. DNA released from the M band becomes resistant to DNase more quickly later in infection when structural precursors are more abundant. Therefore, the acquisition of DNase resistance probably reflects the amount of precursors required for completion of the capsid (P13

and P14, for example) and the amount of tail structure present in the cytoplasm (consider Fig. 2, 3, and 5).

Frankel et al. have shown that gene 49 mutant lysates are defective in a nuclease necessary to produce mature DNA lengths from replicative concatemers in vitro (12). Our results support their finding since gene 49 mutants showed the lowest detachment of DNA from the membrane, an average of only 1%, as compared to 5 to 10% for the other head mutants.

Our assay does not measure packaging directly, but measures release of the DNA from the membrane, presumably the last stage in packaging in which the DNA is cut. This process is unimpaired in gene 2, 50, and 64 mutants in which head protein is not cleaved normally (21). This indicates that cleavage of the head proteins is not essential for packaging the DNA into the phage head. Also, protein cleavage cannot be a prerequisite for gene 49 nuclease activity. However, our unpublished data indicate that at least some cleavage occurs before the DNA is released from the M band.

If the DNA were associated with a capsid structure before being released from the membrane, it would be expected that head proteins found in the M band could be released artificially by DNase treatment. Our unpublished experiments indicate that this is the case. By sodium dodecyl sulfate acrylamide gel electrophoresis it was found that the major protein in the M band late in infection is cleaved P23. It could be released from the membrane by DNase treatment prior to cell fractionation. Also, Luftig and Ganz have found that gene 49 defective heads are attached to cell debris via a DNase-sensitive complex (24).

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