Function of Gene 49 of Bacteriophage T4
I. Isolation and Biochemical Characterization of Very Fast-Sedimenting DNA

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Very fast-sedimenting DNA was isolated from cells after infection with gene 49 defective phage T4. This DNA appeared membrane bound throughout the time after infection and could be isolated either in the membrane-bound form (M-DNA) or free of membrane (released DNA) depending on the lysis procedure. Released DNA formed complexes of marked stability with sedimentation velocities between 1,400S and 2,100S. These complexes did not seem to contain material other than DNA. This was concluded from the results of RNA, protein, and membrane labeling experiments and density analysis. In addition, these complexes were resistant against treatment with n-butanol, phenol, chloroform-methanol, sodium dodecyl sulfate, Sarkosyl, Pronase, RNase, or lysozyme. The observation that more than 90% of the purified very fast-sedimenting DNA is retracted by magnesium-Sarkosyl crystals (M-band) suggests that the M-band technique may not be sufficient as a test for DNA-membrane attachment.

Assembly of the head of phage T4 involves the action of some 18 genes (4). In many cases a defect in one of these genes results in the accumulation of visible aberrant head structures (e.g., gene 31 = lumps [14], gene 20 = polyheads, or gene 21 = tau particles [15]).

At least three of the genes in the head pathway (genes 16, 17, 49) seem to be involved in the correct packaging of the DNA into the heads, as has been concluded from the accumulation of partially or totally empty heads after infection with mutants in these genes (13, 16-18).

All mutants in genes of the head pathway, which act prior to the filling with DNA, also accumulate fast-sedimenting DNA. After infection with mutants in genes 16 or 17, this DNA sediments with approximately 200S and consists of concatenated molecules held together in large complexes (6, 8). These complexes seem to contain the DNA of a whole burst (10).

Mutants in gene 49 also produce fast-sedimenting DNA complexes, but these show s values considerably higher than 1,000S. In the following we refer to this DNA as very fast-sedimenting (VFS) DNA. It has already been shown that VFS-DNA can be converted into 200S DNA in vitro (7), suggesting a relationship between the two types of complexes. This conversion is dependent on an active gene 49 product (P49), suggesting a nucleolytic function, although other activities are not excluded since we do not have a better understanding of the DNA structure. For this reason we initiated an investigation of the VFS-DNA in gene 49-infected cells. The results presented in this and the following paper (11) indicate that the VFS-DNA can be obtained free from detectable protein, membrane, or RNA without changing its characteristic rapid sedimentation behavior. This and other findings suggest that the gene 49-defective DNA is probably generated by some strong DNA-DNA interaction leading to structures of marked complexity (11).

MATERIALS AND METHODS

Chemicals and reagents. Pancreatic DNase and pancreatic RNase were purchased from Worthington Biochemicals Corp. Lysozyme (Muramidase) and Pronase were purchased from Sigma Chemical Corp. Pronase was dissolved in 10⁻² M Tris, pH 8.0, 0.2 M EDTA and self-digested at 37 C for 120 min before use. Thymine, L-tryptophan, CsCl (p.A.), CsCO₃ (suprapure), and trichloroacetic acid were from Merck. 2'-d-adenosine was purchased from Boehringer and Brij 58 (polyoxyethylene cetyl ether) was from Atlas Chemical Industries. Sarkosyl (NL87, sodium lauryl sarcosinate) was purchased from Geigy, sodium dodecyl natrium sulfate from Schuchard, and Tris from Sigma Chemical Corp.

Oleic-[9, 10-¹H] (N) acid (specific activity, 1 to 3 Ci/mmol) and [6-¹H] thymidine (specific activity, 20 to 30 Ci/mmol) were purchased from New England Nuclear Corp., and [2-¹H] thymidine (specific activity, 50 mCi/mmol) was obtained from Amersham Buchler.

Media and reagents. (i) NB medium. NB medium was prepared from 8 g of nutrient broth (Difco) and 5
g of NaCl per 1,000 ml of water. For solidification 15 g of agar per 1,000 ml was added. Soft agar contained 6 g of agar per 1,000 ml.

(ii) M9 medium. M9 medium contained, per 1,000 ml of water: Na2HPO4·12H2O, 15 g; KH2PO4, 3 g; and NH4Cl, 1 g. After sterilization, 1 ml of 1 M MgSO4, 1 ml of 0.1 M CaCl2, 5 ml of 10⁻³ M FeCl3, and 50 ml of 10% glucose were added per 1,000 ml of medium.

(iii) DNA buffer. DNA buffer contained 0.02 M Tris (pH 8.0), 0.02 M NaCl, and 10⁻³ M EDTA.

(iv) T2-T4 buffer. T2-T4 buffer was prepared from 4 g of NaCl, 5 g of K2SO4, 1.5 g of KH2PO4, and 5 g of Na2HPO4 per 1,000 ml of water. After sterilization, 0.1 ml of 1 M MgSO4, 0.1 ml of 0.1 M CaCl2, and 1% gelatin were added from sterile solutions.

(v) TMK buffer. TMK buffer consisted of 0.001 M Tris (pH 7.0), 0.01 M magnesium acetate, and 0.1 M KCl.

(vi) Lysis mix. Lysis mix contained 2 mg of lysozyme per ml, 0.05 M EDTA, and 0.1 M KCN.

Bacteria and phage. Escherichia coli B23 was used as nonpermissive and E. coli CR63 as permissive host for T4 amber mutants. T4 am-E727 (gene 49), T4 tsC9 (gene 49), and T4 amB17 (gene 23) were obtained from W. B. Wood. Each strain was purified by single-plaque isolation and stored in high-titer stocks over a drop of chloroform at 4°C.

Growth of phage. An overnight culture of E. coli B23 (for growth of wild-type phage) or CR63 (for growth of amber or temperature-sensitive phage) was diluted 50-fold into fresh NB medium and incubated at 37°C. At an optical density at 650 nm = 0.4 (approximately 4 × 10⁸ cells/ml) the cells were infected with a multiplicity of infection of 0.1 and shifted to 28°C in the case of temperature-sensitive mutants. Lysis occurred after 2 to 3 h and was completed by the addition of chloroform. Cell debris and unlysed cells were removed by low-speed centrifugation in a Sorvall centrifuge at 10,000 × g.

Concentration of the phage was achieved by a 30-min high-speed centrifugation at 75,000 × g in a Beckman ultracentrifuge. The pellets containing the phage were gently redissolved in a small volume of T2-T4 buffer. Single-plaque lysates were prepared following the same protocol, except that a single plaque was isolated from a fresh NB plate and added to the culture at optical density at 650 nm = 0.1. Phage radioactively labeled in their DNA content were prepared as described earlier (12).

Sucrose gradient analysis. Sucrose gradients (10 to 30%) were prepared by using a Buchler mixing device. The sucrose solution contained 10⁻³ M EDTA, 10⁻³ M Tris (pH 8.0), and 1 M NaCl. The gradients were prepared approximately 30 min before use by mixing 2.4 ml of each concentration layer over a cushion of 0.2 ml of CsCl (1.8 g/ml). Samples of 0.3 to 0.5 ml were layered on top of the gradients. All centrifugations were done in a Beckman ultracentrifuge at 4°C. After centrifugation the gradients were collected by inserting a disposable micropipette connected to an LKB Perpex pump. Twenty-seven or 18 fractions per gradient were collected. Alkaline sucrose gradients contained 0.3 M NaOH, 0.7 M NaCl, and 10⁻³ M EDTA.

Preparation of phage-infected cells. An overnight culture of E. coli B23 was diluted 50-fold into fresh M9 glucose medium. At optical density at 650 nm = 0.4, 10 ml of the culture was withdrawn and centrifuged at 10,000 × g for 10 min at room temperature. The pelleted cells were resuspended in 1 ml of T2-T4 buffer and incubated for 15 min at 37°C for starvation. After the addition of 20 µg of I-tryptophan per ml, five phage per cell were added. After an additional 5 min at 37°C, the phage-infected cells were diluted 50-fold into fresh M9 glucose medium. This is considered to be time 0 and the start of the experiment. The number of infected cells was determined by plating appropriate dilutions on NB agar plates just before and 10 min after infection. Infections were always better than 98% of the total cell number. For labeling the DNA after phage infection 200 µg of 2′¬-d-adenosine and 1 µCi of [¹⁴C]thymine or 10 µCi of [³²P]thymidine per ml were added to the medium. A chase was performed by adding 2 mg of unlabeled thymine per ml.

Lysis procedure. One volume of phage-infected cells was mixed with 1 volume of ice-cold lysis mix in an Erlenmeyer flask. The sample was incubated in ice for 15 min before 1% (final concentration) Brij 58 was added (9). After gentle mixing the cells were kept on ice for at least 10 more min before further analysis.

M-band analysis. The M-band procedure followed the method described by Earhart et al. (3).

Density gradients. CsCl block gradients were prepared by layering 1.5 ml of each of three densities (1.8, 1.5, and 1.3 g/ml) of CsCl solution on top of each other in a 5-ml Beckman polyallomer centrifuge tube. Centrifugations were done for 5 h at 4°C at 37,000 rpm in a Beckman ultracentrifuge using the SW50.1 rotor. After fractionation of the gradients the refractive index of the samples was determined with a Zeiss refractometer at room temperature. Self-generating CsCl density gradients were obtained by centrifuging CsCl solutions of homogeneous density of 1.7 g/ml under the described conditions.

Determination of trichloroacetic acid-insoluble radioactivity. After the addition of 200 µg of bovine serum albumin, samples of 0.1 to 0.3 ml were put into 10 ml of 5% ice-cold trichloroacetic acid. Fifteen minutes later the trichloroacetic acid-insoluble material was collected on glass-fiber filters (Whatman GF/C), and after drying the radioactivity was determined in aciilnation fluid containing 4 g of Omnifluor (New England Nuclear) per 1 liter of toluene.

RESULTS

Isolation of VFS-DNA from T4 amE727 (gene 49)-infected cells. Phage infection and labeling of the DNA were done as described above. At 30 min after infection the cells were lysed by the lysozyme-Brij 58 technique and 0.3- to 0.5-ml aliquots of the lysates were placed on top of preformed CsCl block gradients. After centrifugation the distribution of the label in the gradient was determined by fractionation.
and measuring trichloroacetic acid-insoluble material in each fraction. Figure 1 shows the results of a typical experiment. The majority of the label appears in or close to the position where phenol-extracted authentic T4 DNA bands (position I). A second peak with variable amounts of DNA is visible near the top of the gradient (position II). The radioactive material from both peaks proved to be sensitive to DNase I (degradation of the material was more than 95% complete after 30-min exposure to 100 μg of the nuclease at 37°C). This was taken as an indication for the specificity of the label and successful lysis, excluding the possibility of trapped DNA inside unlysed cells, which would also band in position II. This method of isolating the DNA from T4-infected cells, which has independently been used by others (7, 19), proved to be more reliable than the frequently used sedimentation technique through sucrose gradients. A separation of membrane-bound and released DNA (see next section) was achieved in this step.

The result shown in Fig. 1 was also obtained by applying our isolation procedure to tsC9 (gene 49)-infected cells grown at non-permissive temperature. The DNA of the amE727 or the tsC9 mutant isolated from position I of the CsCl block gradient showed very similar characteristics in respect to sedimentation behavior (see below) and resistance against the attack of the single-strand-specific DNase S1 (11). Because of these similarities we refer to the DNA of either of these mutants as gene 49-defective DNA.

Double-label experiments with [3H]oleic acid and [14C]thymidine. The finding that a portion of the newly synthesized phage DNA bands with a low density of 1.4298 g/ml suggests attachment of other material such as protein and/or membrane. To investigate this question, cells were prelabeled with [3H]oleic acid and the newly synthesized T4 DNA was pulse labeled from 4 to 10 min after infection with [14C]thymidine. By using the same protocol as in the preceding section, cells were lysed at different times after infection and their DNA was analyzed on CsCl block gradients. The result is shown in Fig. 2a, b, and c. The two labels are found at three different positions. Pure DNA bands close to a density of 1.7 g/ml (comparable to position I in Fig. 1), whereas pure membrane is found at a density of 1.29 g/ml. A third peak of 1.40 g/ml (comparable to position II in Fig. 1) contains both labels and reflects the amount of membrane-bound DNA of each lysate.

The distribution of the labels between the three positions depends on the time of lysis. At 15 min after infection approximately 80% of both labels band with an intermediate density, whereas at 30 and 45 min the amount is reduced to approximately 45 and 20%, respectively. This change, however, could be artificially caused by mechanical shearing during the lysis procedure, and we therefore tried to lyse the cells directly on top of the CsCl gradients, using an adapted version of the protocol described by Siegel and Schaechter (20). The result of a typical experiment is shown in Fig. 2d, e, and f. One can see that most of the DNA now remains permanently bound to the membrane. Even at 45 min after infection only 15% of the DNA is removed from the hybrid density position. Degradation of the DNA by pancreatic DNase in each fraction was again taken as an indication for successful cell lysis.

According to the positions on the density gradients we would like to distinguish "released DNA" in position I from "membrane-bound DNA" ("M-DNA") in position II.

Sedimentation behavior of released DNA and M-DNA. The intracellular gene 49-defective DNA has been reported to show an unusually high sedimentation velocity (7). Is that still true for the DNA isolated by our method?

To answer this question isolated released DNA was quickly dialyzed against DNA buffer (3 times, 20 min), and its s value was determined on 10 to 30% sucrose gradients. The ma-

![Graph](http://jvi.asm.org/download/plain-text/894_kemper_and_janz/jvi_2017_894_kemper_and_janz_f1.png)

**Fig. 1. Isolation of gene 49-defective VFS-DNA.** E. coli B23 was infected with T4 amE727 (49). The phage DNA was continuously labeled with [3H]thymidine from 5 to 30 min after infection. At 30 min after infection, the cells were lysed and their DNA was analyzed on preformed CsCl block gradients.
Fig. 2. Double labeling of gene 49-defective VFS-DNA and cell membrane. The membrane of E. coli was labeled by growing the cells in M9-glucose medium containing 100 µCi of [14C]oleic acid. At OD$_{660}$ = 0.4 the cells were collected by centrifugation, washed twice with T2-T4 buffer, and infected with T4 amE727 (49-). The newly synthesized DNA was pulse labeled with [14C]thymidine from 4 to 7 min after infection. At the indicated times aliquots were removed from the culture and lysed by the lysozyme-Brij 58 technique (9) before they were placed on top of a preformed CsCl, block gradient (Fig. 2a, b, and c). To reduce the danger of shearing, parallel samples were directly lysed on the gradients (Fig. 2d, e, and f). Infected cells (0.2 ml) were gently stirred into an equal volume of lysis mix prelayered on the CsCl block gradients at 0°C. Fifteen minutes later 40 µl of 10% Brij 58 was added and carefully mixed with the sample. Incubation at 0°C was continued for an additional 10 to 15 min. All centrifugations were carried out at 37,000 rpm in a Beckman SW50.1 rotor at 4°C for 5 h. I and II indicate the positions, which are comparable to those in Fig. 1. Symbols: (●) [14C]oleic acid; (○) [14C]thymidine; (▲) density (g/ml).

Jority of the material sediments with approximately 1,700S (estimated for the peak fraction by the Hershey-Burgi equation) (Fig. 3). When samples from different positions of the peak were run on the same type of gradients they were found to sediment according to their former positions, with estimated $S$ values of 1,400, 1,800, and 2,100S, respectively. This behavior demonstrates a marked stability of the released DNA complexes.

When M-DNA (prepared by the method described for Fig. 2a-c) was taken for the same type of analysis, most of the material sedimented to the cushion at the bottom of the tube (Fig. 4). After phenol extraction the DNA was completely free of membrane label, and approximately 60% of the recovered material sedimented in a peak around 1,600S, showing the characteristics of released VFS-DNA.

This indicates that at least 60% of the DNA of position II indeed consists of VFS-DNA that may be bound to membrane.

Density analysis of released DNA. As was demonstrated in Fig. 1, the gene 49-defective DNA of position I does not band with homogeneous density after a 5-h run in a CsCl block.
lyzed in a self-generating CsCl density gradient over a 48-h run. As a control, one fraction of the DNA was treated with n-butanol before the run. The result in Fig. 5 proves possibility (ii) to be the correct explanation. The n-butanol-treated, gene 49-defective DNA shows the same density as the T4 marker DNA, whereas the majority of the untreated DNA bands with slightly lower densities.

The question of whether the membrane contaminant (which was removed here by n-butanol treatment) or any other non-DNA substance is necessary for the structural integrity of the VFS-DNA complexes now seems important.

**Further analysis of VFS-DNA.** Experiments are summarized that were performed to search for the presence of a small amount of non-DNA substances nondetectable by our density gradient analysis. For this purpose the sedimentation behavior of VFS-DNA was tested on neutral 10 to 30% sucrose gradients after treatment of the DNA with several chemicals or enzymes. The analysis is based on the assumption that an essential change in the complex composition would also cause a change in sedimentation behavior.

Treatment of the DNA at high temperature with sodium dodecyl natrium sulfate (1% so-

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**Fig. 3.** Analysis of released gene 49-defective VFS-DNA on neutral sucrose gradients. Cells of E. coli B23 were infected with T4 amE727 (49-), and 30 min later their DNA was subjected to fractionation on CsCl block gradients. A sample of released DNA (see text) from position I of the CsCl gradient of Fig. 1 was dialyzed against DNA buffer, and its density was analyzed on sucrose gradients. Samples were collected from the bottom of the tube, and their DNA content was determined by trichloroacetic acid precipitation. Samples 10, 12, and 15 of the gradient shown in Fig. 3a were dialyzed against DNA buffer and recentrifuged on 10 to 30% sucrose gradients under identical conditions. (a) VFS-DNA from position I of the CsCl gradient shown in Fig. 1; (b-d) Recentrifugations of samples 10, 12, and 15 shown in Fig. 3a.

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**Fig. 4.** Analysis of gene 49-defective M-DNA on neutral sucrose gradients. A fraction of [3H]thymidine-labeled DNA bound to [14C]oleic acid-labeled cell membrane (M-DNA) from position II of a CsCl gradient (cf. Fig. 2b) was dialyzed against DNA buffer and sedimented through a neutral 10 to 30% sucrose gradient. (a) Untreated M-DNA; (b) M-DNA phenolized prior to centrifugation. Symbols: (○) [3H]DNA label; (●) [14C]membrane label.
DIEM dodecyl natrium sulfate, 15 min, 65 C) or Sarkosyl (1% Sarkosyl, 120 min, 60 C) did not significantly alter the s value of the material. Extraction with phenol (equal volumes, 3 x 15 min, 4 C, neutralized and water-saturated phenol), n-butanol (equal volumes, 3 x 10 min, room temperature), or chloroform-methanol (equal volumes, 3 x 10 min, room temperature, chloroform-methanol = 1:2) had no effect. Treatment with RNase (60 min, room temperature, 20 &g/ml DNase-free), Pronase (120 min, 37 C, 200 &g/ml self-digested Pronase), or lysozyme (30 min, 30 C, 0.5 mg of lysozyme per ml in 10-2 M Tris, pH 8.2, 0.05 &g EDTA, pH 8.2) also had no influence on the s value of VFS-DNA.

These results make it very unlikely that the DNA complexes are stabilized by protein, RNA, membrane, or cell wall components.

M-band analysis of VFS-DNA. The M-band technique has been used successfully by several authors to show association of DNA with membrane material (2, 3, 21, 22). The magnesium-Sarkosyl crystals formed during the procedure are able to bind membrane but no DNA, except the DNA itself is bound to membrane.

When we used the M-band technique to test released VFS-DNA and M-DNA, both DNAs could be quantitatively recovered from the M-band (Fig. 6a and d). Controls show that (i) T4 DNA purified from phage does not bind in the M-band (Fig. 6c), and (ii) in the absence of M-band the VFS-DNA sediments much farther through the gradient, excluding the possibility of accidental co-sedimentation of magnesium-sarkosyl crystals and VFS-DNA (Fig. 6b).

Whereas the binding of the M-DNA in the M-band was expected, the quantitative binding of the released DNA was rather surprising, because from the density analysis only a portion of the DNA seemed to be contaminated with membrane (preceding sections and Fig. 5). If, however, the M-band technique is able to indicate very small amounts of membrane, which cannot be detected by density shift, the result would suggest that the released DNA is not free of membrane. We therefore tried to remove the hypothetical contaminant by different extraction procedures. However, as summarized in Table 1, extensive washing with n-butanol or phenol did not alter the M-band binding capacity of the DNA. As was demonstrated before (Fig. 4 and 5), these treatments were sufficient to remove oleic acid label from isolated M-DNA.

Fig. 5. Density analysis of gene 49-defective released VFS-DNA. Fraction no. 7 from the gradient shown in Fig. 1 was taken for equilibrium centrifugation in CsCl either directly or after extraction with n-butanol. The DNA was mixed with 3 ml of CsCl (1.70 g/ml), and after the addition of marker DNA (obtained by extensive phenolization of T4 phage) it was spun for 48 h at 4 C in a Beckman SW50.1 rotor. Symbols: (O) [4C]marker DNA (T4); (●) [3H]VFS-DNA; (△) density (%/cm²).

Fig. 6. M-band analysis. Gene 49-defective DNA was isolated as "released DNA" or membrane-bound "M-DNA" from CsCl gradients. After short dialysis against TMK buffer, an aliquot of the DNA was placed on top of a 5-ml 15 to 47% neutral sucrose gradient in TMK buffer. Mg2+-Sarkosyl crystals were generated and centrifuged as by Earhart et al. (3). (a) [3H]labeled "released DNA" from CsCl position I in Fig. 1 with M-band formation. (b) Control: [3H]labeled "released DNA" from CsCl position I in Fig. 1 without M-band formation. (c) Control: [3H]T4 DNA purified by phenolization from phage T4 with M-band formation. (d) Membrane-bound DNA, position II in Fig. 1 with M-band formation. Symbols in (c) and (d): (O) [3H]membrane label (oleic acid); (●) [3H]DNA label (thymidine).
and to cause the membrane-contaminated "released DNA" to bind with the density of pure T4 DNA. The existence of substances in the DNA complexes that might be resistant against organic solvents or specific enzymes (preceding section) but able to interact with the M-band components seems very unlikely. Instead, we would like to suggest that the potency of the DNA to bind in the M-band is caused by some unknown structural property of the DNA itself. The idea is consistent with the observation that the VFS-DNA cannot be found in the M-band after destroying the integrity of the complex. Limited pretreatment of the released VFS-DNA with pancreatic DNase or heat prevents the VFS-DNA from binding in the M-band (Table 1, c and d), whereas RNase did not affect the binding capacity (Table 1, e).

**DISCUSSION**

The DNA of gently lysed T4-infected cells was isolated by using preformed CsCl block gradients. Under these conditions the DNA was separated into two main fractions of different densities. The application of oleic acid as a specific membrane label led to the distinction between "released DNA" (density close to 1.70 g/ml) and membrane bound "M-DNA" (density of 1.4 g/ml). The reliable distinction between the two forms of DNA makes the method useful for analyses of intracellular DNA.

By using cells infected with gene 49-defective phage, we observed that the distribution of the DNA between the two positions varied depending on the time of lysis. With proceeding infection the fraction of released DNA increased while the amount of M-DNA decreased accordingly. It turned out, however, that this effect was due to shear forces generated during the isolation of the DNA-membrane complexes, indicating an increased lability of the binding between the two components late during infection. If shear forces were reduced to a minimum by lysing the cells directly on the gradients, as suggested by Siegel and Schaechter (20), more than 80% of the DNA remained bound to the membrane even as late as 45 min after infection. These findings support the results of others, who, by different techniques, demonstrated the attachment of DNA to the membrane as a prerequisite for T4 DNA replication (evidence reviewed by Siegel and Schaechter [21]) and maturation. Under wild-type conditions the DNA is not released from the membrane except via head filling (19, 20). Therefore packaging-defective mutants accumulate their DNA in membrane-bound structures, as was shown here for a gene 49-defective mutant. Essentially the same result was obtained with 200S DNA producing head mutants in gene 23 (amB17) or gene 24 (amB26) (data not shown).

If the membrane content of the isolated gene 49-defective DNA is removed by treatment with organic solvents, the DNA still shows an unusually high sedimentation velocity. All attempts to demonstrate a factor(s) (protein, membrane, or RNA) that might be responsible for the integrity of the complexes have failed so far. Protein, membrane, or RNA labels are incorporated into the complexes but can quantitatively be removed by an appropriate degradation procedure without changing the characteristic high s value (protein and RNA label experiments not shown). These observations are in contrast to the results with the released folded chromosome of *E. coli*, which after the exposure to RNase is immediately unfolded, suggesting a stabilizing RNA core (23).

The observation that the purified VFS-DNA binds in the M-band was surprising. Since the magnesium-Sarkosyl crystals are hydrophobic, DNA, in general is not expected to be included in the M-band, and the result has been taken as an indication for DNA-membrane attachment (3, 22). The involvement of membrane, however, was excluded by our experiments in which the VFS-DNA was extensively extracted with organic solvents without eliminating its M-band binding capacity.

It was also observed that single-stranded DNA has some tendency to bind to the magnesium-Sarkosyl crystals (M. Schaechter, per-

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**Table 1. M-band analysis of tritium-labeled released VFS-DNA from position I of CsCl gradients after treatment with modifying reagents**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>M-band</th>
<th>Counts/min</th>
<th>% Total recovered counts/min</th>
<th>Top (counts/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Phenol</td>
<td>1,050</td>
<td>76.0</td>
<td>328</td>
<td></td>
</tr>
<tr>
<td>(b) n-Butanol</td>
<td>5,500</td>
<td>82.0</td>
<td>1,243</td>
<td></td>
</tr>
<tr>
<td>(c) Heat</td>
<td>800</td>
<td>33.0</td>
<td>1,600</td>
<td></td>
</tr>
<tr>
<td>(d) DNase</td>
<td>65</td>
<td>1.7</td>
<td>3,800^a</td>
<td></td>
</tr>
<tr>
<td>(e) RNase</td>
<td>4,100</td>
<td>95.0</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>(f) Untreated</td>
<td>3,900</td>
<td>96.0</td>
<td>174</td>
<td></td>
</tr>
</tbody>
</table>

^a The DNA was dialyzed and treated in parallel portions with (a) phenol (equal volumes, 3 times, 15 min at 4 °C), of phenol which was neutralized and water saturated before use by several extractions with Tris buffer at pH 8.0, (b) n-butanol (equal volumes, 3 times, 10 min at 4 °C), (c) heat (10 min, 100 °C, followed by rapid chilling in ice), (d) DNase (100 µg/ml, 15 min at 30 °C), (e) RNase (DNase free, 60 min at 25 °C), and (f) untreated DNA. After the treatments the DNA was mixed with Sarkosyl and MgCl2 and centrifuged according to the method of Siegel and Schaechter (21). Fifteen fractions of a 5-ml gradient were collected, and the label bound in the M-band and of the rest of the gradient was determined by trichloroacetic acid precipitation.
personal communication). The binding of VFS-DNA in the M-band could then be taken as an indication for the existence of single-stranded portions in the DNA complexes. This possibility, however, was excluded by two lines of evidence. (i) Under our experimental conditions heat-denatured T4 DNA does not associate with the M-band, and (ii) gene 49-defective VFS-DNA does not contain extensive single-stranded regions (11).

We therefore would like to suggest that it is the organization of the DNA molecules themselves that, in a still unknown fashion, causes interaction with the M-band. As a consequence, the complex should be destroyed only by treatments acting on DNA but not by others that affect non-DNA substances. This is, in fact, the case, as shown in Table 1 and in the last paragraph of Results.

It should be noted here that purified 200S DNA (isolated from cells infected with gene 23- or gene 24-defective phage) was also found to bind to the M-band. These results shed some doubt on the assumption that the M-band binding capacity of DNA is a reliable indication for membrane attachment if applied to very big DNA molecules.

With the exception of the sedimentation behavior, the analyzed properties of the gene 49-defective DNA were not significantly different from the properties of the 200S DNA so far (5–7; this paper). Both DNAs can be isolated free of additional substances, and the difference between their sedimentation velocities could be either due to the amount of DNA per complex or to some still unknown structural properties.

As discussed in the following paper (11), the amount of DNA does not seem to differ between the two types of complexed DNA, and we suggest that the gene 49-defective DNA is generated by direct DNA-DNA interaction leading to the highly compact very fast sedimentation structure.

Such interaction could be achieved by incomplete recombinational events, leading to linkages between individual DNA molecules (1). A number of such "uncleaved" recombinant structures could then lead to a tight network of great stability. P49 would be responsible for clearing the intramolecular links that otherwise block the process of head filling.

LITERATURE CITED


