On the Internal Structure of Bacteriophage Lambda

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ABSTRACT The structure of bacteriophage lambda has been studied by electron microscopy of negatively stained particles. The phage particles will eject their DNA if they are heated or dialyzed against a chelating agent. The ghost particles, so formed, have a channel running down their tails. Since the channel is not visible in normal particles, the channel may be filled with part of the DNA molecule. Up to 30% of the ghosts contain round objects about half the internal diameter of the head. The round objects, called "cores," have the same buoyant density as the coat protein. The core may be a protein spool about which the phage DNA is wound.

Bacteriophage lambda belongs to a large class of viruses which also includes bacteriophages T3 and T7, polyoma virus, rabbit papilloma virus, and human papilloma virus. Members of this class have an isometric head about 500 A in diameter and contain a single molecule of DNA.

A virus particle is a package. The simplest virus particle consists of a protein shell enclosing a single molecule of nucleic acid. I would like to discuss the organization of a nucleic acid molecule in such a package.

Since a nucleic acid molecule is long and thin, it must bend many times to fit inside the virus particle. Phage lambda, for example, contains a DNA molecule 150,000 A long inside a head 500 A in diameter. In spite of the high degree of folding a virus particle can release its nucleic acid molecule without tying it into knots. When a host cell is infected, the nucleic acid must emerge from the protein coat, and serve as a template for its own replication and for transcription leading to the synthesis of viral proteins. In these processes even one unresolvable knot might be disastrous.

Phage lambda has an especially difficult packaging problem because it has a high ratio of nucleic acid to protein. Its buoyant density of 1.5 g cm^{-3} implies equal masses of protein (density 1.3) and DNA (density 1.7). An outside view of phage lambda negatively stained with uranyl acetate is shown in Fig. 1. Lambda has an isometric head which often appears hexagonal in projection, and a tail 1500 A long.

Sometimes in preparations of normal lambda one finds a particle which has lost its DNA. The inside of the head appears black after negative staining be-

cause the absence of DNA allows the stain to fill the head. Several such particles are visible in Fig. 1. Two of them also have a longitudinal channel visible down their tails. A channel is not apparent on particles containing DNA. Similar observations have been reported by Eiserling and Boy de la Tour (1).

Particles of phage lambda can be forced to eject their DNA by heating them to 60 °C or by removing Mg^{++} by dialysis against a chelating agent. Normally,

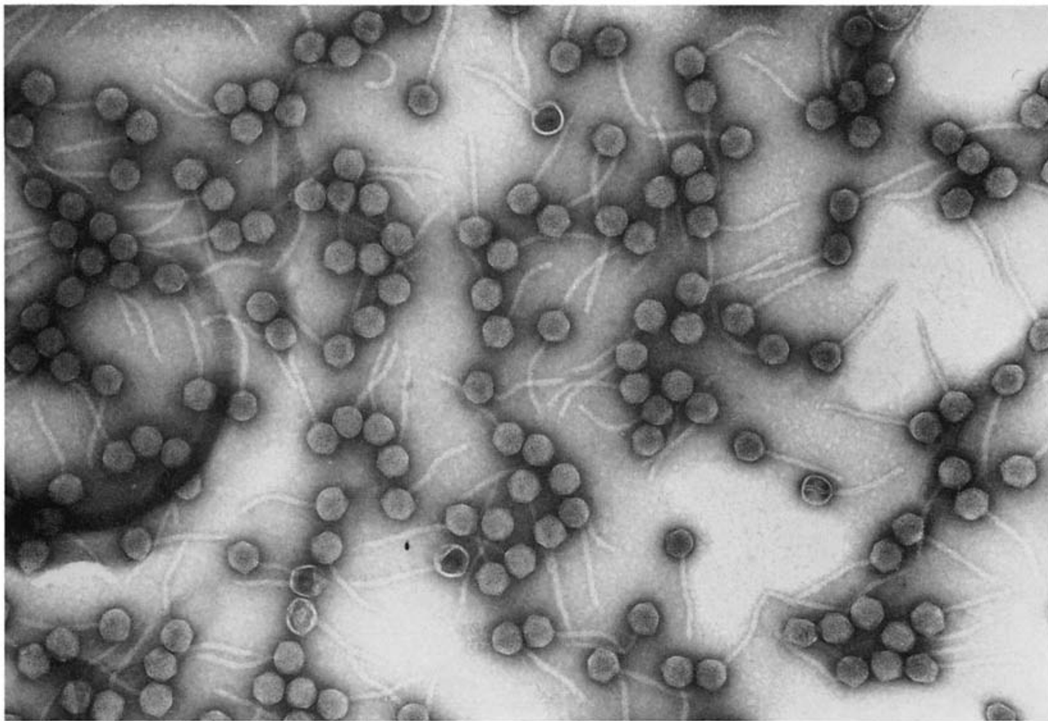

FIG. 1. Bacteriophage lambda, negatively stained with uranyl acetate. × 70,000.

Mg^{++} is added to a preparation to stabilize the virus particles. Fig. 2 is an electron micrograph of particles which have been heated. The loss of DNA does not seem to destroy the structure of the phage coat because the empty heads have approximately the same contour as the normal phage particles. The particles which have ejected their DNA after heating also have a channel in their tails.

Since the phage DNA enters the bacterium by passing through the tail, it is not so surprising that the tails have a channel in them. What is more surprising, perhaps, is that the normal filled phage particles do not have a visible channel. The absence of the channel suggests that it may be filled with part of the DNA molecule in the complete phage particle.

Some of the particles which have ejected their DNA nevertheless contain a solid looking object inside their heads. Approximately 10% of the particles in Fig. 2 contain these objects which I shall call "cores." In other preparations up to 30% of the particles have cores. Fig. 3 shows two particles containing cores, one of which is hexagonal in projection.

These cores are not "petit lambda," small, tail-less particles about 400 A in diameter described by Karamata, Kellenberger, Kellenberger, and Terzi (2). Although lysates of lambda-infected bacteria contain equal numbers of nor-

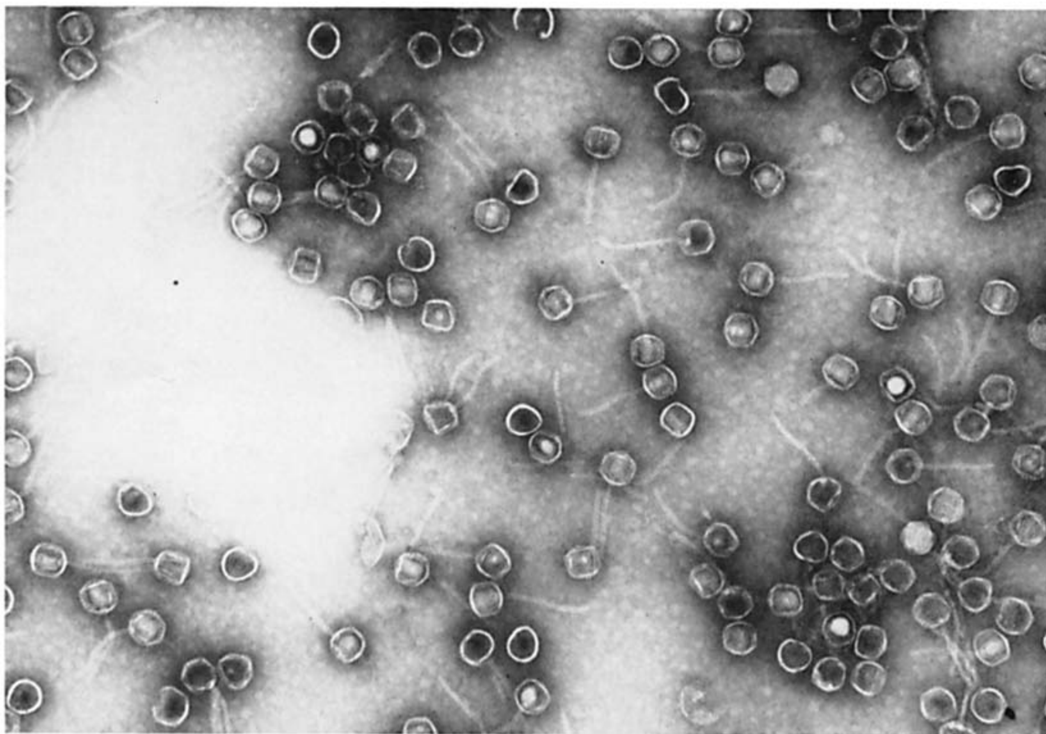

Fig. 2. Bacteriophage lambda, heated 20 min at 61°C and negatively stained with uranyl acetate. × 70,000.

mal and petit lambda, the two can be separated by density gradient centrifugation. The phage preparation used for the experiments reported here was so purified and contained less than 0.5% petit lambda.

It seemed possible that the cores might be a residue of DNA which had not been ejected. If this were the case, then those particles which contain cores should have a higher buoyant density than those which don't. To test this possibility the following experiment was performed. First a mixture of normal particles and ghosts, that is particles which had ejected their DNA, was pre-

pared by dialyzing phage against EDTA at 5 °C. Then the mixture was centrifuged to equilibrium in a cesium chloride gradient.

Two bands were obtained. One band was at density 1.5 g cm^{-3} and was made up almost entirely of normal bacteriophage particles. Fig. 1 is a photograph of material taken from this band. The other band was obtained at density 1.3 g cm^{-3} and contained the ghosts. Seven fractions spanning the region between the two bands were examined microscopically. Few particles of any sort were evident in these fractions.

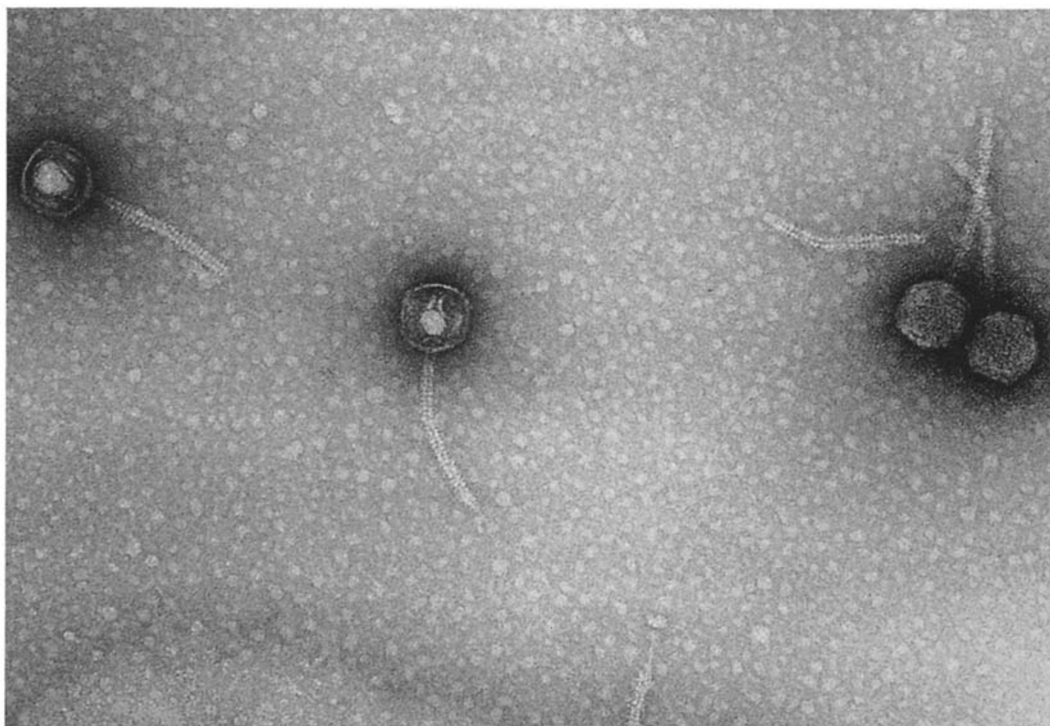

FIG. 3. Bacteriophage lambda, heated 5 min at 61 °C and negatively stained with uranyl acetate. Magnification × 140,000.

Some of the ghosts in the band at 1.3 g cm^{-3} contained cores. To increase the resolution of this experiment, fractions having a density of 1.36 or less were pooled, adjusted to density 1.3 g cm^{-3}, and centrifuged to equilibrium. This time a single band was obtained. Six fractions were collected in the band. Each fraction was examined in the electron microscope and photographs were taken of fields chosen at random in areas where the quality of negative staining was good. All the photographs were counted for the numbers of ghost particles and ghost particles containing cores.

Table I gives the distribution of ghosts and ghosts containing cores in these

fractions. The ghosts formed a band about the density 1.303 g cm^{-3}. The ghosts which contain cores also formed a band which has its peak in the same fraction as the peak of the ghost band. Furthermore, the proportion of ghosts which contained cores seems to be constant across the band of ghosts. That proportion ranges from 7 to 10%. This is to be compared with the proportion of ghosts containing cores in the input to the first CsCl gradient which was 11%.

TABLE I

ELECTRON MICROSCOPICAL ANALYSIS OF CESIUM CHLORIDE DENSITY GRADIENT FRACTIONS

To avoid osmotic shock in the negative staining of particles suspended in concentrated CsCl, it was necessary to lower the salt concentration slowly. This was accomplished as follows. A drop of the phage suspension in CsCl was picked up on a carbon-coated microscope grid. After 1 min, allowed for adsorption to the carbon film, the liquid was washed off with 2 drops each of successively lower concentrations of NaCl: 2 M, 1 M, 0.6 M, 0.2 M, 0.1 M, and finally with 1% uranyl acetate, blotted dry with filter paper, and immediately examined in the microscope.

In order to show that a valid estimate of the proportion of ghosts containing cores could be made by this technique, a reconstruction experiment was performed. Two preparations of ghosts were mixed in various proportions. A preparation in which 9% of the ghosts contained cores was mixed in a ratio of 2:1 with a second preparation in which less than 0.4% contained cores. Microscopic examination of the mixture showed that 6.6% of the ghosts had cores. A second experiment in which the ratio of "high-core" to "low-core" preparations was 1:2 showed 1.7% containing cores.

Fraction No.	Buoyant density GM CM^{-3}	Average No. of particles per EM field		Fraction of ghosts with cores
		Total ghosts	Ghosts with cores	
19	1.310	58	5.1	0.087
20	1.306	157	12.0	0.076
21	1.303	947	67.5	0.071
22	1.300	758	62.5	0.082
23	1.297	146	11.6	0.080
24	1.293	27	2.6	0.097

The average core diameter was measured in a fraction on the high density side of the band and in one on the low density side to see whether there is a correlation between density and core diameter. The average diameter of 36 cores in fraction 19 with a density of 1.310 was 308 A; of 38 cores in fraction 23 with a density of 1.297, the average diameter was 303 A.

The conclusion I wish to draw from this experiment is that ghosts which contain cores have the same buoyant density within ± 0.003 g cm^{-3} as those which do not. The fractionation employed should have been fine enough to detect a core made up of DNA. Approximately 2.7×10^6 daltons of DNA would be required to cover completely the surface of a sphere 300 A in diam-

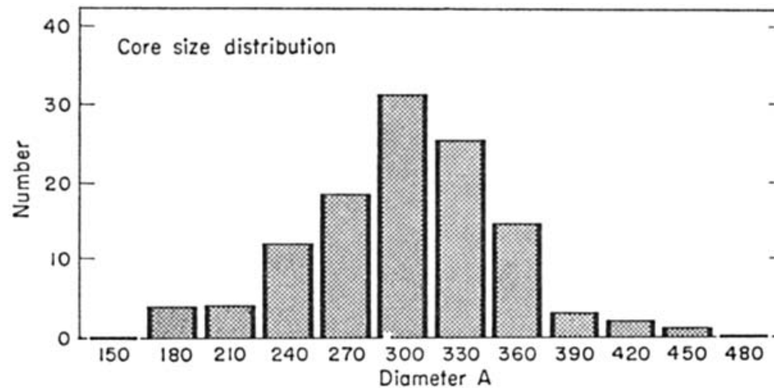

FIG. 4. Distribution of core diameters. One hundred and fourteen cores inside ghosts were measured on the input to the first density gradient centrifugation and fractions 19 and 23 from the second centrifugation. As some cores appear elliptical, the maximum and the minimum diameters were measured and their average tabulated.

eter. The density of a ghost containing this much DNA would be 1.32 g cm^{-3} and would therefore have banded about eight fractions down gradient from the ghost band. This shows that the cores are not DNA. Assuming that the cores are not artifacts of negative staining, it seems likely from their buoyant density contribution that they are made of protein.

Cores are present in other DNA viruses. Klug, Finch, Leberman, and Longley (3) have observed in electron micrographs small spherical objects inside the ghosts (top component) of human wart virus and small particles 380 A in diameter in unfractionated rabbit papilloma virus. They have also found particles both inside and outside ghosts of phage lambda produced by alkaline treatment. These particles may be the cores of the phage.

Since the core is left behind after DNA ejection, the DNA molecule of a normal, filled particle may occupy the cavity between the inside of the head membrane and the core. To see whether that cavity is large enough to ac-

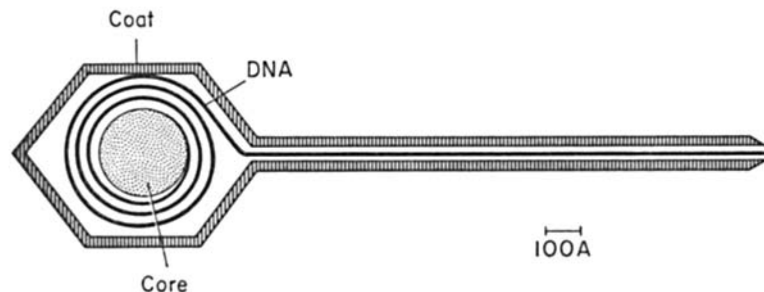

FIG. 5. A hypothetical structure for bacteriophage lambda. For clarity only part of the DNA molecule is shown and its width has been increased out of proportion, otherwise the drawing is approximately to scale.

commodate a molecule of lambda DNA, the outside diameter of the head, the thickness of the head membrane, and the diameter of the core were measured on electron micrographs. The outside diameter of normal particles was found to be 550 $\pm$ 25 A. The thickness of the head membrane, measured on phage ghosts, was found to be 25 $\pm$ 9 A. The inside diameter of the head of a normal phage particle is therefore 500 A.

Fig. 4 is a histogram which gives the distribution of core diameters. The distribution is unimodal and symmetric. The observed spread in the distribution may arise from a variable amount of flattening produced by drying and negative staining because ghosts are slightly larger (570 A in diameter) and more variable than normal particles. The mean core diameter, 300 A, may for the same reason be an overestimate of the diameter of a core in a normal particle. The volume of a spherical shell with an inner diameter of 300 A and an outer diameter of 500 A is 5.1×10^7 A^3. The volume of one molecule of lambda DNA taken as a cylinder 15 microns long (4) and 20 A in diameter is 4.7×10^7 A^3.

The significance of a core is that it might serve as a spherical spool around which a DNA molecule could wind. Such a role is suggested in the hypothetical structure shown in Fig. 5. If the DNA molecule were threaded into the tail, then it ought to be able to unwind systematically from the core without becoming tied in knots. This structure also implies that DNA condensation (5), the process which reduces the volume occupied by the DNA molecule to the size of a completed particle, might be the wrapping of DNA around a protein core. Further experiments are needed to substantiate or to rule out these suggestions.

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Discussion

Dr. Hershey: Well, this is a splendid model that was designed to explain how DNA comes out of the phage head, and lo and behold, it tells us how it goes in. Are there some comments more to the point than mine, or questions about the work?

Dr. Lee Simon: I wondered whether you've done any other work besides negative staining in the microscope to look for these cores.

Dr. Kaiser: We have sectioned whole particles of T4 and of lambda and positively stained the sections. Wanko and Cummings first observed that sections of T4 particles positively stained had a hole in the center, and we have seen the same thing. About a third of our T4 sections have holes in them, and almost all our lambda sections have holes in the middle.

Now, I don't know how seriously to take this observation, because the phage particles were fixed and dehydrated, and a lot could go on during these processes, but I think it does fit in with the existence of a core. There is also radiation evidence, I believe, that there is no DNA in the center of T even phage particles.

Mr. Harvey Cohen: Since this core is present in only a small percentage of the phage, how do you explain the fact that it wasn't found in most of them. Has it escaped just as the DNA has?

Dr. Kaiser: In some preparations approximately 30% of the particles have a visible core. I think that the core may not be apparent in some particles because it has flattened out. Cores can be destroyed by osmotic shock and by heat.

Dr. Donald Ritchie: Have you ever looked for the presence of cores in lambda phage which have infected bacteria? You might expect there to be many more.

Dr. Kaiser: I've tried to do that, but unsuccessfully so far. What you say is true if the structure I have proposed is correct, because injection would seem to be the most natural way of getting the DNA out.